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FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

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DISCUSSION PAPER ON OCHRATOXIN A IN COCOA

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 and was presented at the First Session of the Codex Committee on Contaminants (CCCF) held in Beijing, China.

2. At its First Session, CCCF agreed that it is 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 taking into account the comments made at the First Session, for consideration at the Second Session of CCCF. Belgium, Brazil, Côte d'Ivoire, Netherlands, Nigeria, Thailand, Togo, United States of America, European Commission, ECA, ICA and IFT participated in the electronic working group and prepared this discussion paper. A list of participants in the electronic working group is presented in Annex to this document.

INTRODUCTION

3. 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[49]. In cocoa, OTA is mostly associated with cocoa bean shells and fat-free cocoa solids (cocoa powder)[2, 30]. Both fungi and OTA can be present in all stages of the production chain: Harvest, fermentation, drying, storage, food elaboration and transport[18, 26].

4. The name 'Cocoa' is a corruption of the word 'Cacao'[63]. In common usage, 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.

5. Cocoa is a fermented dried 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[58].

6. During industrial processing of cocoa the first steps are roasting and removal of the shell fraction. For technical reasons it is not possible to remove the shell fraction totally. This is acknowledged in the Codex standard (CODEX STAN 141-1983, rev. 1-2001) which specifies the maximum content of shells and germs in cocoa mass and cocoa cake. The maximum level specified in the standard corresponds to about 2% of shells in the nib fraction[33].

7. Around 71% 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)[35]. 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 N. America to be made into cocoa liquor, cocoa butter and cocoa powder (Table 2)[35].

Table 1. World Production of cocoa beans (2004 – 2007) (thousand tonnes)

Country	2004/05		2005/06		2006/07 (forecast)	
Africa	**2381**	**70.4%**	**2644**	**71.2%**	**2427**	**70.7%**
Cameroon	184		167		180	
Cote d'Ivoire	1286		1408		1280	
Ghana	599		740		660	
Nigeria	200		200		180	
Others	112		129		127	
America	**443**	**13.1%**	**445**	**12.0%**	**429**	**12.5%**
Brazil	171		162		140	
Ecuador	116		114		114	
Others	156		169		175	
Asia & Oceania	**560**	**16.5%**	**627**	**16.9%**	**580**	**16.9%**
Indonesia	460		520		470	
Others	100		107		110	
World Total	**3384**	**100.0%**	**3716**	**100.0%**	**3435**	**100.0%**

Source: ICCO Quarterly Bulletin of Cocoa Statistics, Vol. XXXIII, No.3, Cocoa Year 2006/07. Posted: 22 October 2007

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.

8. Cocoa is grown in a 20^{o} belt north and south of the equator. The minimum mean temperature in most cocoa growing regions is 18^{o}C, and the mean maximum temperature 32^{o}C. A high rainfall of 1000-4000 mm/year is required. *Theobroma cacao* (cocoa tree) grows well in a wide range of soils.

CHEMICAL STRUCTURE

9. Ochratoxin A (7-(L-β-phenylalanyl-carbonyl)-carboxyl-5-chloro-8-hydroxy-3,4-dihydro-3R-methyl isocumarin) is a secondary metabolite produced by certain species of *Aspergillus* and *Penicillium*[50], 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[55].

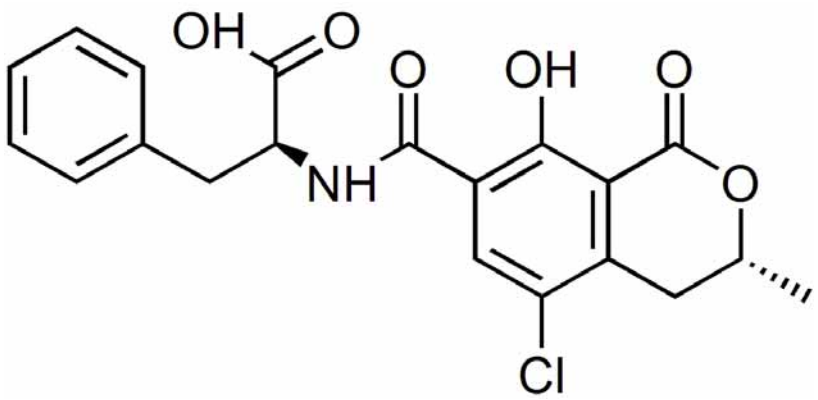

Fig.1. Structure of Ochratoxin A

10. The mammalian enzyme carboxypeptidase A has the ability to cleave OTA into nontoxic products (ochratoxin alpha and phenylalanine)[56].

11. OTA keeps its stability during most food processing stages such as cooking, washing, fermenting to an appreciable degree which can be detected in manufactured food products[4]. Boudra[9] has shown that OTA is heat stable and max 20% of OTA in wheat was decomposed by dry heat at 100 ^{o}C for 160 min or 150^{o}C for 32 min. During roasting of cocoa the final bean temperature reaches 100 – 120^{o}C and the duration is 15-70 min[43], therefore roasting is not expected to significantly reduce OTA levels.

TOXICOLOGICAL EVALUATION

12. OTA is classified as a possible human carcinogen (group 2B)[19], [36]. OTA has been reported to be nephrotoxic, immunosuppressive, carcinogenic and teratogenic in animal studies[38, 39, 48, 54, 59]. OTA is thought to be the cause of two chronic diseases, Balkan Endemic Nephropathy and Chronic Intestitial Neprhopathy (in North Africa), and of urothelial tumours in humans[48]. Based on epidemiological associations, the hypothesis on a between OTA exposure early in life and testicular cancer has been put forward[57]. Previous National Toxicology Program (NTP) studies in the United States showed that OTA can induce renal tumours in rodents at high doses[8].

13. Preliminary surveys of OTA in blood conducted in Cote d'Ivoire between 1998 and 2004 have proven the reality of OTA contamination of foodstuffs[69]. The results revealed that 22 out of 63 healthy participants had OTA levels of 0.01 – 5.81µg/L with a mean value of 0.83µg/L compared with 8 out of 39 nephropathy patients undergoing dialysis who had levels of 0.167 – 2.42 µg/L with a mean of 1.05µg/L. Blood concentration of OTA is largely a consequence of eating habits and diets of a people, which in the case of Cote d'Ivoire is skewed by cereals and peanuts rather than by cocoa products.

14. The Scientific Panel on Contaminants in the Food Chain from EFSA (European Food Safety Authority) adopted on scientific opinion related to Ochratoxin A in Food on 4 April 2006[49]. This opinion took into account the outcome of recent research on the toxicology of ochratoxin A[44]. This opinion indicates that the site-specific and renal toxicity as well as the DNA damage and genotoxic effects of OTA, measured in various in vivo and in vitro studies, are most likely attributable to cellular oxidative damage. Thus, on the basis of the lowest observed adverse effect level (LOAEL) of 8µg/kg bw/day for early markers of renal toxicity in pigs, and applying a composite uncertainty factor of 450 for the uncertainties in the extrapolation of experimental data derived from animals to humans as well as for intra-species variability, a TWI of 120ng/kg bw was derived for OTA. Current levels of exposure to OTA in EU member states vary between 15 and 60ng/kg bw/wk[44]. The EFSA panel further recommended that more specific exposure data be collected for certain vulnerable groups, including infants and children and those that consume large amounts of certain regional specialities containing OTA.[49]

15. JECFA at its 68th meeting in June 2007 considered the previous PTWI of 100 ng/kg bw in the light of new data and found no reason to changing that assessment.[68]

SAMPLING

16. Variability in OTA production, combined with the large particle size of some of the foods it contaminates, complicate sampling strategies for detection of ochratoxins[15]. A few, highly contaminated grains can raise the level of a whole shipment above the allowed limit, if the limit is low. However, many sampling strategies are not designed to detect these few, highly contaminated grains[15]. Thus development of low-tech, inexpensive methods for mycotoxin surveillance is a world health imperative[47].

17. Recent data presented by Spanjer and co-workers[70] has indicated that the sample homogenization process is an important factor in OTA determinations in various food matrices. The data indicated that depending on the type of milling procedure, which ultimately determines the particle size distribution, the amounts of OTA that is measured would vary. The relevance of this finding is that erroneous subsample analytical result could lead to lots being wrongly rejected or accepted.

18. Sampling procedures and performance criteria for the methods of analysis to be used for official control in the EU on the level of OTA in foodstuffs have been provided for by Commission Regulation 401/2006[20]. As there is no EU maximum level for OTA in cocoa, no sampling procedures for the control of OTA in cocoa has been established at the EU level.

ANALYTICAL METHODS

19. To detect OTA occurrence in cocoa products a fast efficient and sensitive analytical method is needed. Cocoa products are food matrices quite different from fresh fruits, cereals, coffee or wine and their extraction step optimization requires specific study[17]. Recently, a rapid antibody-based assay involving sequential clean-up and visual detection of OTA in cocoa powder has been described[71]. The screening test has a cut-off level of 2µg/kg and suitable for use in the field.

20. The validated method for OTA quantification is reversed-phase HPLC protocol with clean-up by immunoaffinity column containing antibodies specific to OTA, and quantified by fluorescence detection11. An interlaboratory study[12] aimed at assessing the performances of 18 laboratories for OTA determination in cocoa powder samples has been described. The study concluded that the results were satisfactory: For example, at low level detection (0.19µg/kg), median level (0.45µg/kg), and high level (1.45µg/kg), satisfactory results were obtained from 10/18, 11/18 and 12/18 participants respectively.

21. When a high number of samples has 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[47].

OCCURRENCE OF OTA AND OTA PRODUCING MOULDS IN COCOA BEANS

22. In an ongoing project[64] in Cote d'Ivoire, cocoa arriving at the ports of Abidjan and San Pedro were evaluated for OTA contamination. Out of 147 samples tested in Abidjan 23 had OTA levels >2.0 µg/kg, and at San Pedro 10 out of 151 samples showed an OTA content >2.0µg/kg. Based on these observations at both ports it was estimated that commercial grade cocoa having OTA content > 2.0 µg/kg constitutes 11% of total cocoa production in Cote d'Ivoire.

23. In 2006 the Federal Agency for the Safety of the Food Chain in Belgium analysed 13 cocoa bean samples and found that 8 were below the LOQ of 0.3µg/kg, and 5 contained OTA levels of 0.4, 0.72, 0.99, 1.7 and 7.7µg/kg.[65]

24. Amezqueta2 detected OTA in 46 cocoa bean samples of different origins and batches. A total of 63% of the cocoa bean samples were contaminated with a level >LOD (0.04µg/kg). The mean and median obtained for the cocoa beans were 1.71 and 1.12µg/kg respectively, within an interval of 0.04 and 14.8µg/kg.

25. A screening of Nigerian ready for sale cocoa beans indicated that out of 59 samples tested 54 were positive for OTA, with concentrations ranging between 1.0 and 277.5µg/kg[72]. An indirect competitive ELISA, much less sensitive than the HPLC method had been used for the determination.

26. Starting in 1999 European industry has analysed samples of imported cocoa beans from different origins. Results show that OTA contaminated cocoa beans are found in all cocoa producing regions. Furthermore levels are found to vary between regions, within regions and between years[29].

27. Several efforts had been made to isolate and identify ochratoxin A producing moulds from cocoa beans. Out of 66 *Aspergillus* strains isolated during fermentation and drying of cocoa beans from Ghana, none 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 *Aspergilli* were found[18].

28. In a Brazilian study, 21 cocoa product samples (cocoa beans, nibs, shells and cocoa powder) were evaluated for presence of moulds with OTA production capacity. Out of 123 *Aspergillus* toxigenic isolates obtained from 42.9% the cocoa samples, 18.2% *A. niger*, 100% *A. carbonarius* and 100% *A. ochraceus* were OTA producers[23].

29. Incidence of OTA in cocoa and cocoa products from various producing countries is shown in Table 3.

Table 3. Incidence of OTA in **cocoa beans** worldwide.

			Number of samples			%
Origin	**Year**	**Ref**	**Total**	**>LOQ**	**>2 µg/kg**	**>2 µg/kg**
Abidian	2005	64	147		23	16
San Pedro	2005	64	151		10	7
Cocoa beans		65	13	5	1	8
Cote d'Ivoire		2	33	24	5	15
Camroon		2	7	3	1	14
Equitorial Guinea		2	6	2	0	0
Cote d'Ivoire	1999-2005	29	1014		193	19
West Africa ex CdI	1999-2005	29	347		10	3
Asia	1999-2005	29	136		2	1
America	1999-2005	29	210		1	0
Africa		7	21	16	1	5

EFFECT OF MANUFACTURING ON OTA CONTENT OF PRODUCTS

30. OTA was analysed in 15 pairs of cocoa shell and nib samples[29]. The samples were industrially processed. The results showed that on average 48% (range 25-72%) of OTA in the beans was removed with the shell fraction. The calculated OTA concentration in the beans was 0.3-3.0µg/kg

31. In a study where cocoa shells were removed by hand, Amezqueta[1] 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%.

32. 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 other cocoa products[7].

33. 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 contamination varied widely between 3.37 and 46.15µg/kg with an average of 24.0µg/kg. Shells of unroasted beans were the most heavily contaminated with a mean value of 91.0µg/kg. Chocolates contained 1.86µg/kg on the average, and butter was free of OTA (see Fig. 2). On the average about 70% of the OTA was removed with the shell fraction.

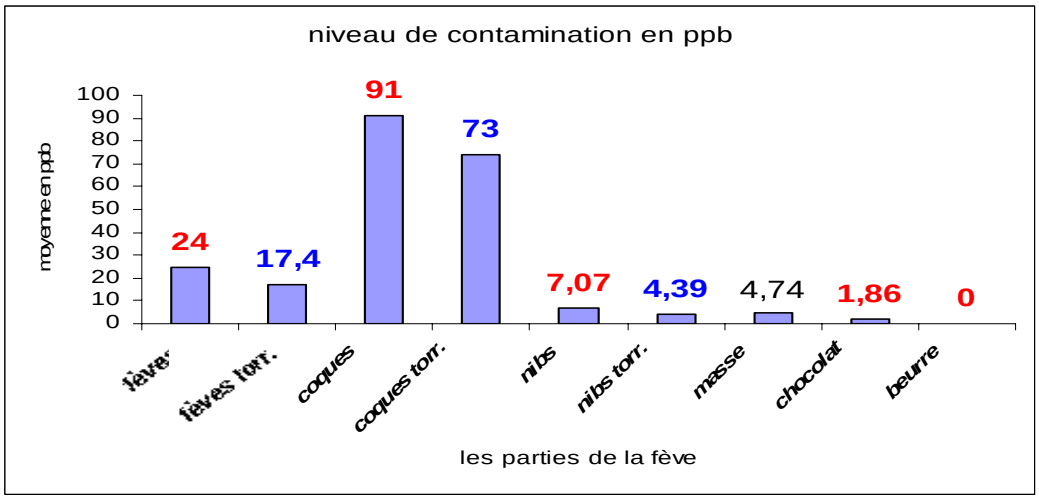

Fig. 2. Mean level of contamination of different components of cocoa beans during processing of contaminated beans [Legend: *niveau de contamination en ppb* = level of contamination in ppb (µg/kg); *moyens en ppb* = means in ppb; *les parties de la fève* = parts of the bean; *fèves* = beans; *fèves torr*. = roasted beans; *cocques* = shells; *cocques torr* = roasted shells; *nibs torr*. = roasted nibs; *masse* = mass; *chocolat* = chocolate; *beure* = butter. Data from Cote d'Ivoire[64])].

OCCURRENCE OF OTA IN COCOA PRODUCTS

34. The Belgian Federal Agency for the Safety of the Food Chain, in 2005, analysed 10 cocoa powder and 9 chocolate samples from the open Belgium market. The results showed that 5 cocoa powder samples were below the LOQ (0.3µg/kg) and the remaining 5 samples contained the following OTA levels: 0.60, 0.72, 0.76, 0.78, and 0.81µg/kg. All the 9 chocolates samples contained OTA levels below the LOQ.[65]

35. In a 2005 study[42] involving 41 retail chocolates in Japan, all samples showed some level of contamination with OTA. Levels in 14/41 were below the LOQ (0.10 µg/kg), 7/41 were below 0.20 µg/kg and the rest (20/41) contained levels above 0.20 µg/kg, all in the range of <0.10 to 0.94µg/kg.

36. In a study[7] of 170 cocoa and cocoa product samples from various geographical origins, out of the 136 which were cocoa products or chocolates, OTA was not detected in 21 whilst OTA in the remaining samples ranged between 0.1µg/kg and 9.0µg/kg.

37. The report on the Task for Scientific Cooperation 3.2.7[44] showed that 81.3% of the cocoa-derived products analysed were contaminated with OTA. This means out of the 547 cocoa product samples analysed 445 were positive. The contamination level varied from 0.01 to 3.8µg/kg, with 0.23µg/kg average[44].

38. Tafuri[58] analysed 18 samples of cocoa in powder (sold in Italy) for OTA presence. Nine samples were below the limit of detection of 0.01µg/kg; the other nine had the toxin from 0.22 to 0.77µg/kg, with a mean of 0.43µg/kg. The analytical method used here had only about 20% recovery.

39. MAFF[41] presented data in 1997 and 1998 on cocoa powder samples which indicated that 19/20 samples analysed in 1997 contained OTA to a maximum level of 2.4µg/kg with a mean of 0.68 µg/kg and 20/20 samples analysed in 1998 yielded a mean of 1.67µg/kg. Out of 40 chocolate samples, 30 contained OTA at levels less than 0.6µg/kg.

40. CAOBISCO/ECA/FCC[29] launched a research project in 1999 "to specify as precisely as possible occurrence of OTA and the conditions favouring OTA formation in cocoa and actions believed to minimize formation". Analysis of cocoa products on the European market, under this project, confirmed that only low levels of OTA are present in cocoa containing products as consumed. The mean OTA levels found in the various products were as follows (samples have been analysed by different laboratories with LODs of 0.1, 0.2, or 0.5µg/kg): milk chocolate (228 samples), 0.16µg/kg; dark chocolate (536 samples), 0.26µg/kg; cocoa drink powder (247 samples), 0.20µg/kg; cocoa powder (1189 samples), 1.0µg/kg. Averages were calculated using LOD/2 for samples that have OTA levels below the detection limit.

41. In a similar study[14] conducted in Spain, 13 European countries, Argentina and Japan, 296 samples of different types of chocolate and cocoa powder were analysed for their OTA content. Results indicated that although 99.7% of the samples were contaminated with OTA, consumption of chocolate and cocoa products under normal conditions contributed only a minor fraction of the TDI of OTA.

42. An overview of levels of OTA encountered in various cocoa products is presented in Table 4.

Table 4. OTA content of various **cocoa products**

		Number of samples			µg/kg			
	Ref	**Total**	**>LOQ**	**>2µg/kg**	**Max**	**Median**	**Mean**	**Comments**
Cocoa mass	7	8	4	2	3.5		1.07	
Cocoa mass, Netherlands	44	1	0		<0.25			
Roasted nibs	7	2	0	0	<0.1			
Cocoa butter	7	4	0		<0.1			
Cocoa butter, Netherlands	44	6	0		<0.25			
Cocoa cake	7	80	74	41	9		2.79	
Cocoa powder	7	31	29	17	4.4		2.41	
Cocoa powder, Spain	14	21				0.24		
Cocoa powder, not Spain	14	5				0.17		
Cocoa powder	29	1189	1094	143			1	
Cocoa powder	65	10	5				0.4	
Cocoa powder	58	18	9		0.77		0.43	Low recovery, about 20%
Cocoa powder	41	20	19		2.4		0.68	
Cocoa powder	41	20	20				1.67	
Cocoa powder, Germany	44	96	91		1.8		0.38	
Cocoa powder, UK	44	40	39		2.4		1.2	
Cocoa powder, Netherlands	44	6	0		<0.25			
Cocoa drink powder	29	247	101	0			0.2	
White chocolate, Spain	14	5				0.03		
White chocolate, not Spain	14	9				0.03		
Milk chocolate, Spain	14	47				0.12		
Milk chocolate, not Spain	14	122				0.1		
Milk chocolate	29	228	52				0.16	
Dark chocolate, Spain	14	35				0.25		
Dark chocolate, not Spain	14	52				0.27		
Dark chocolate	29	536	300				0.26	
Chocolate	65	9	0				<0.3	
Chocolate	42	41	27		0.94			
Chocolate	41	40						30 samples <0.6 µg/kg
Chocolate, Germany	44	352	297		3.6		0.12	
Chocolate, UK	44	40	18		0.6		0.38	
Chocolate, Netherlands	44	8	0		<0.25			
Chocolate / chocolate cream	14	11	9		1.59		0.63	

FACTORS AFFECTING THE PRESENCE OF OTA IN COCOA

43. In an ongoing OTA programme[64] 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 2.8µg/kg, however, partially rotten pods showed a contamination level of 0.3 to 0.74µg/kg whilst beans from good pods had 0.22 to 0.37µg/kg OTA content.

44. Only one major study[6] has been conducted 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 study was done by European chocolate and cocoa industry and trade comprising Caobisco, ECA and FCC between 1999 and 2004 in West Africa.

45. Experiments in large commercial farms in Cote d'Ivoire indicated that very little OTA was produced during well controlled fermentations in big boxes followed by sun drying.[6]

46. Data from smallholder farms in Cote d'Ivoire indicated higher levels of OTA incidence. For example, out of 62 bean samples, 24 had levels below 0.5µg/kg and 11 had levels above 2µg/kg; from a total of 168 samples taken the following cocoa season 63 had OTA content above 0.5µg/kg, 28 had levels greater than 2µg/kg, 7 samples had levels above 10µg/kg with 48 samples free of OTA[6].

47. The data from these studies[6] also show that OTA levels vary within the cocoa season (Fig.3) and levels also relate with the extent of damage to the cocoa pod damage (Fig.4). For example, mean OTA distribution by pod phytosanitary condition was as follows: healthy (2.3µg/kg), pest damaged (4.2µg/kg), wounded (19.8µg/kg), rotten (7.2µg/kg) and mummified (3.4µg/kg).

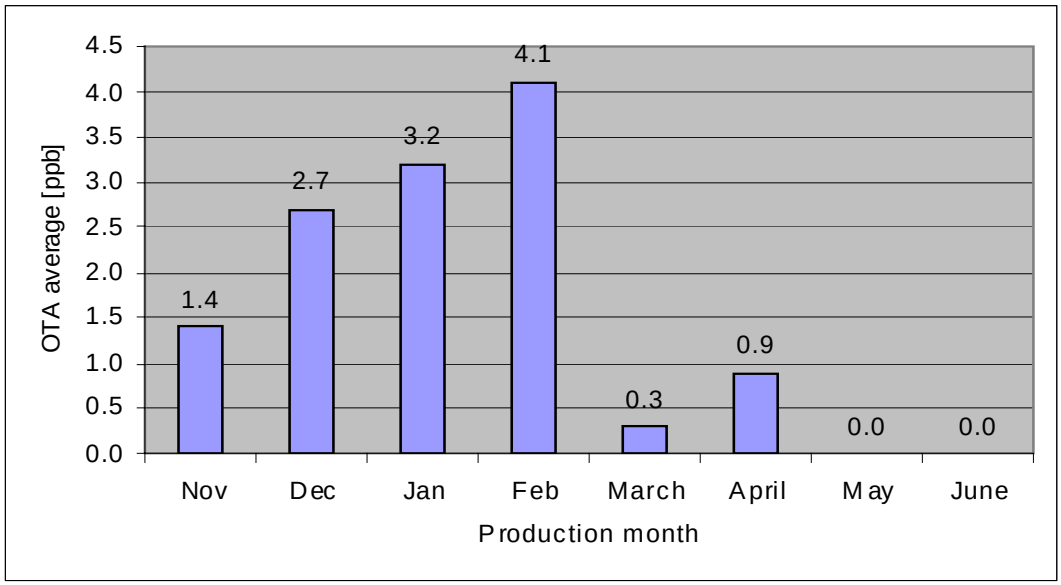

Fig. 3. Variation of OTA levels as a function of month of harvest

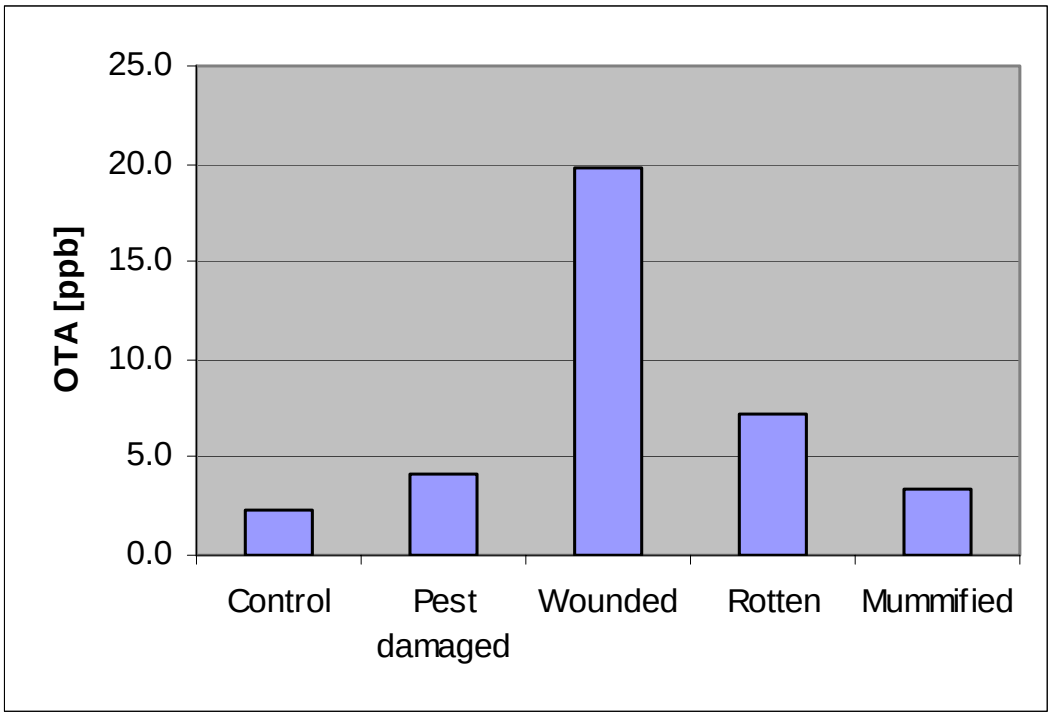

Fig. 4. Effect of phytosanitary condition of the cocoa pods on levels of OTA found in the dried cocoa beans

48. OTA was found to be present in beans which have just been fermented and the levels increased during drying.

49. Drying conditions alone were not found to be responsible for the OTA levels, but there are interactions between harvesting, fermentation and drying conditions.

50. 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-former, *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.[18]

51. Studies using cocoa media demonstrate the potential of *A. ochraceus* and other moulds that can be isolated from cocoa, e.g., *A. carbonarius*, *A. niger*, *A. tubingensis*, to grow and produce OTA. OTA production was shown to strongly depend on environmental factors such as temperature, pH and water activity of the substrate. For instance, a water activity of 0.97 was found optimal for OTA biosynthesis. OTA production was also shown to be species-specific. For example, model OTA producers, *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.[18] [BFE = Bundesforschungsanstalt fur Emaehrung, i.e. Institute for Hygiene and Toxicology – Federal Research Centre for Nutrition, Karlsruhe, Germany].

52. Sixty five lactic acid bacteria isolates of cocoa origin were tested using a spot method for their ability to inhibit growth of 12 ochratoxin A producing moulds. Most tested *L. fermentum* and *L. plantarium* strains inhibited mould growth[18].

DIETARY INTAKE

53. The Scientific Panel on Contaminants in the Food Chain from EFSA adopted on scientific opinion related to dietary exposure of adult European consumers to OTA on 4 April 2006[49]. This opinion indicates that at present the weekly exposure ranges from 15 to 60 ng per kg OTA bodyweight per week, including high consumers of foods containing OTA. 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 CONTAM Panel concluded that more data would be needed to assess exposure rates of this segment of consumers, taking into account their dietary preferences.

54. The SCOOP Task 3.2.2 presented data which indicated that the daily intake of cocoa was 31g/day/person corresponding to OTA intake of 21ng/kg/wk/person. This report also noted that this amount of cocoa contributed 5% of total OTA intake compared to cereals which contributed the bulk of 55%. The follow up SCOOP Task 3.2.7[44] indicated that cereal was still the main contributor at 50% with cocoa at 4% of total intake.

55. To estimate the dietary exposure to OTA, the Food and Environmental Hygiene Department (FEHD)[27] of Hong Kong completed a study in February of 2006 which covered 8 major food groups including chocolate and cocoa products. Of the 287 food samples which tested for OTA, it was found that dietary exposure to OTA was 4 and 9ng/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.

REGULATORY STATUS

56. In the EU, Commission Regulation (EC) No. 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs[21], establishes maximum levels for Ochratoxin A 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.

57. 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 recent EFSA opinion*".

58. The opinion of the Scientific Panel on Contaminants in the Food Chain from EFSA related to Ochratoxin A in Food has been adopted on 4 April 2006[49]. The European Community is currently considering the appropriateness of establishing a maximum level for OTA in food commodities other than those for which already a maximum level exists at EU level.

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

PREVENTION AND REDUCTION OF OTA IN COCOA

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

61. Recent research backed by the European chocolate and cocoa industry in some producing countries has shown that OTA can be found on beans from most producing countries and that the practice during early processing steps at the cocoa farm is critical. This means interventions have to be made at the farm level for a significant reduction of OTA contamination to happen.[29]

62. In a related study[6] to find sources of ochratoxin A contamination and development in cocoa beans, results acquired in smallholder 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. Thus, it should be possible to reduce OTA levels using post-harvest practices that could be implemented by cocoa smallholders.

63. 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[1]. The Codex standard for cocoa (cacao) mass (cocoa/chocolate liquor) and cocoa cake (CODEX STAN 141-1983, rev. 1-2001)[33] contains maximum levels for cocoa shell and germ and these maximum levels should at least be respected.

64. No quality management systems have ever been implemented in the primary processing of cocoa. Dahl[24], 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.

65. 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.

66. A proposal for a methodology to reduce OTA contamination in cocoa by working with cocoa farmers has been discussed[29].

67. Recent data[66] 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.

68. An insect parasitoid, *Prorops nasuta*, found in many coffee-producing countries, has been found to be infected with the OTA-producing *Aspergillus westerdijkiae* in a recent study[67]. This finding has raised the possibility that an insect parasitoid might be disseminating an ochratoxin-producing fungus in coffee plantations. This observation emphasizes the need for a better understanding of insect-mould-target plant interactions. This finding could have similar implications for cocoa.

CONCLUSIONS AND RECOMMENDATIONS

69. The present Discussion Paper on OTA in Cocoa leads to the following broad conclusions and recommendations for consideration at the Second Session of CCCF:

    (a) New data from Cote d'Ivoire indicate that if cocoa beans are not properly handled in order to minimize ochratoxigenic fungi colonization of cocoa beans, extremely high levels of OTA could develop in cocoa.

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

    (c) Cocoa is a minor component of the human diet and has a small contribution to total dietary intake of OTA (5 % of total intake).

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

    (e) Recommendations:

        (i) Codex member states as well as the cocoa processing industry should be encouraged to monitor levels of OTA in cocoa and cocoa products over a period of several years.

        (ii) Research on methods to prevent and/or reduce contamination of cocoa in the field, during the primary processing and storage of cocoa should be encouraged and continued. There is need for a better understanding of the insect-mould-cocoa interactions in symptomatic and asymptomatic infections of cocoa in the field. This also includes studies to identify stages of the cocoa production chain which are critical for fungi contamination and possible OTA production as well as identification of OTA producing fungi species found wherever cocoa is cultivated.

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