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Occurrence of Ochratoxin A in Cocoa Products and Chocolate

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In this work, the occurrence of ochratoxin A (OTA) in 170 samples of cocoa products of different geographical origins was studied. An immunoaffinity column with HPLC separation was developed to quantify low levels of OTA in cocoa bean, cocoa cake, cocoa mass, cocoa nib, cocoa powder, cocoa shell, cocoa butter, chocolate, and chocolate cream with >80% recoveries. The method was validated by performing replicate analyses of uncontaminated cocoa material spiked at three different levels of OTA (1, 2, and 5 μg/kg). The data obtained were related on the acceptable safe daily exposure for OTA. The highest levels of OTA were detected in roasted cocoa shell and cocoa cake (0.1−23.1 μg/kg) and only at minor levels in the other cocoa products. Twenty-six cocoa and chocolate samples were free from detectable OTA (<0.10 μg/kg). In roasted cocoa powder 38.7% of the samples analyzed contained OTA at levels ranging from 0.1 to 2 μg/kg, and 54.8% was contaminated at >2 μg/kg (and 12 samples at >3 μg/kg). Ochratoxin A was detected in cocoa bean at levels from 0.1 to 3.5 μg/kg, the mean concentration being 0.45 μg/kg; only one sample exceeded 2 μg/kg (4.7%). In contrast, 51.2% of cocoa cake samples contained OTA at levels ≥2 μg/kg, among which 16 exceeded 5 μg/kg (range of 5−9 μg/kg). These results indicate that roasted cocoa powder is not a major source of OTA in the diet.

KEYWORDS: Cocoa; chocolate; ochratoxin; incidences; quality

INTRODUCTION

Mycotoxins are fungal secondary metabolites and are an important economic problem and health hazard. Among mycotoxins, ochratoxin A (OTA) represents one of the most widespread and hazardous substances. The chlorinated isocoumarin compound, ochratoxin A [7-(L-β-phenylalanylcarbonyl)-carboxyl-5-chloro-8-hydroxy-3,4-dihydro-3*R*-methylisocoumarin], is a natural contaminant of foods, drinks, and feeds worldwide. Ochratoxin A is potently nephrotoxic, teratogenic, and immunotoxic, particularly on the renal system. It is produced by a number of fungal species in the genera *Aspergillus*, most notably *Aspergillus carbonarius*, and *Penicillium viridicatum* in cocoa bean at a minimum water activity (A_w) of 0.85 (*1*, *2*). The International Agency for Research on Cancer (IARC) has classified it as a possible human carcinogenic (group 2B) (*3*). Humans and animals can absorb this toxin via the gastrointestinal tract after ingestion of contaminated products, and inhalation of airborne OTA can represent a source of additional exposure. The main dietary sources of OTA are cereals, followed by wine, spices, coffee, musts, grape juice, dairy products, cocoa, beer, dried fruits, and pulses (*4*−*6*). It also has been identified in tissues, and because of its long half-life in mammalian tissues, contamination may also carry over into pork and pig blood of animals fed contaminated feed; it has beeen detected in human blood and breast milk (*7*, *8*). Studer-Rohr et al. (*9*) demonstrated that the terminal half-life of OTA in humans is 10 times longer than in rats, in plasma being 35 days. Urinary monitoring is relatively unexplored due to the lower concentrations involved. A wide range of ochratoxin derivatives (ochratoxins B, C, α, and β) can be isolated, but only ochratoxin A and occasionally ochratoxin B occur naturally in moldy products. Ochratoxin A is moderately stable during food processing, even on heating, in foods and beans. However, the influence of roasting in the reduction of the OTA initially present in green coffee has been reported (*10*).

Undoubtedly, prevention of contamination at the source is the best procedure of controlling the contamination and the most effective public health measure in accordance with the hazard analysis−critical control points (HACCP) system (*11*). Experimental indications show that OTA is formed mainly in the initial stages of sun-drying. The drying period is critical to avoid the appearance of the mycotoxin in cocoa beans, and not during the period of storage (*12*). Treatment with a hypochlorite solution before the initiation of sun-drying decreases the development of toxigenic fungi associated with OTA contamination (*13*). An alternative decontamination procedure is necessary to address this situation in cocoa products. During roasting of alkalinized cocoa powder a stream of air is injected into the roaster, inducing the deamination of the free amino acids and producing ammonia. The principal chemistry of this reaction involves the competition between the Maillard reaction to generate aroma compounds and oxidative deamination (*14*). This observation indicates the existence of crucial factors that influence the efficacy of mycotoxin decontamination by am-

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moniation and holding temperature (*15*). Experimental data are necessary for demonstrating the progress of the OTA decontamination process, by introducing ammoniacal solution during roasting of cocoa beans. Cleaning could also represent an important step in the prevention and reduction of OTA in cocoa beans.

At least 11 countries have now proposed or enacted regulations for OTA. The tolerance levels vary widely with country and commodity (1−50 μg/kg for food and 100−1000 μg/kg for animal feed) (*16*). Consequently, the tolerable intakes have been estimated at 100 ng/kg of body weight (bw)/week (*17*), 1.5−5.7 ng/kg of bw/day (*18*), and no more than 5 ng/kg of bw/day (*19*). The mean dietary intake for humans in the European Union (EU) was found to be in the range of 1−2 ng/kg of bw/day. Ochratoxin A has been regulated only for a few food products in the EU, such as raw cereals (5 μg/kg), cereals intended for human consumption (3 μg/kg), and vine-dried fruits (10 μg/kg) (*20*). According to a working document from the Expert Committee "Agricultural Contaminants" on OTA regulations (*21*), there exist the following proposals for regulations on cocoa and cocoa products: (1) raw materials for manufacturing of foodstuffs (cocoa bean, cocoa nib, cocoa mass, cocoa cake, and cocoa powder, 2 μg/kg) and (2) consumer products (powdered chocolate, chocolate in powder, chocolate, and drinking chocolate, 1 μg/kg).

Several methods have been developed to measure OTA, but few studies have evaluated its presence in cocoa products; these methods vary in complexity and speed of execution. The scarcity of data reflects lack of agreement on which maximum contamination levels is recommended in cocoa bean and derivative products (*4*, *6*). An HPLC method has been developed to allow the determination of patulin, penicillic acid, sterigmatocystin, and zearalenone in samples of cocoa beans (*22*). A simple and sensitive procedure was presented for the analysis of OTA at low parts per billion levels in samples of artificially contaminated cocoa beans (*23*). In 1988, as a result of the introduction of a newly established immunochemical technique for cleanup of OTA, the investigation of occurrence at ≤1 μg/kg in different products was possible (*24*). The discrepancy among the data observed in the literature may be due to differences in the analytical method of detection and quantification applied. Initially, a simple extraction was performed as sample pretreatment; later, the extraction and cleanup procedure was improved in compliance with the forthcoming legal limits. Reliable analytical procedures together with certified reference materials from the Institute for Reference Materials and Measurements (IRMM) of the European Commission [Community Bureau of Reference (BCR), Geel, Belgium] are needed for the establishment and enforcement of tolerance levels of OTA in food. The purpose of our work was (1) to determine incidences and OTA levels in cocoa-derived products (cocoa bean, cocoa nib, cocoa cake, cocoa mass, cocoa powder, cocoa butter, and chocolate) and (2) to measure the potential health risk on the acceptable safe daily exposure for OTA.

MATERIALS AND METHODS

Sampling Plan. Sampling plan designs are determined by specifying sample sizes, sample preparation methods, analytical test methods, and accept/reject criteria (*25*). The 170 samples analyzed from different origins [Ivory Coast (58), Indonesia (38), Guinea (15), Nigeria (14), Cameroon (13), Ghana (8), Malaysia (7), Ecuador (3), Senegal (1), Honduras (1), and Peru (1)] were composed of 21 cocoa beans, 80 cocoa cakes, 8 cocoa mass, 2 cocoa nibs (cotyledon), 31 cocoa powder, 3 raw cocoa shells, 4 expeller and refined cocoa butters, 10 industrial roasted cocoa shell powders, and 11 samples of chocolate and chocolate cream (containing between 30 and 40 g of cocoa powder). Each sample was mixed for preparation of a homogeneous composite. Approximately 500 g of each composite was sent to the analytical laboratory for OTA analysis. Two hundred grams was ground with a Retsch ZM 100 mill (Haan, Germany) to pass a 2 mm screen.

Cocoa Products Definition. *Cocoa beans* are oval beans similar in size to almonds. The beans have a thin shell. A major part of the OTA in cocoa beans is found in the shells. The shells are physically removed at an early stage of processing (25−50% of the OTA originally present in the bean is removed with the cell fraction).

The cocoa nib is the kernel of the bean left after removal of the shells.

Cocoa mass (also called *cocoa liquor*) consists of finely ground cocoa nibs and has the same composition as nibs. Grinding has no effect on OTA.

Cocoa Cake. Cocoa mass contains ∼50% fat. Processing of cocoa mass by pressing gives cocoa cake, a press cake with ∼10−20% fat, and cocoa fat (butter). Ochratoxin A is associated with the fat-free cocoa solids and is therefore concentrated in the cake compared to cocoa mass.

Expeller cocoa butter is the fat obtained by the expeller process from cocoa beans or from cocoa beans combined with cocoa nib, cocoa mass, cocoa press cake, fat-reduced cocoa press cake, or any combination of two or more of these [containing not more than 0.5% unsaponifiable matter determined using petroleum ether and not more than 1.75% acidity expressed as oleic acid (FFA)].

Cocoa Powder: Finely Ground Cocoa Cake. Cocoa powder is not consumed as such. It is used for the preparation of drinking chocolate, sweetened cocoa, and sweetened cocoa powder. Cocoa powder is also used as an ingredient in cookies and cakes, puddings, etc. Due to its strong taste the content of cocoa powder in the products as consumed is low, normally below 5%.

Cocoa Shells. Cocoa beans are divided anatomically into three parts, the cotyledon, shell, and germ, in proportions ranging between 87 and 88, between 11 and 12, and between 0.7 and 1, respectively. Main crop West African cocoas normally have shell contents of 11−12%, a norm against which other cocoas are judged. Cocoa shells have been regarded as a potential alternative to conventional sources of dietary fiber.

Powdered chocolate, chocolate in powder, drinking chocolate, sweetened cocoa, and sweetened cocoa powder are mixtures of cocoa powder with sugar and in some cases milk powder. The mimimum cocoa powder content as defined by the Chocolate Directive is in the range of 25−32%.

Roasting. The 31 samples of cocoa powder were collected from processed (fermented, dried, cut into nibs, and roasted) cocoa beans of different origins. Before roasting, the cocoa beans were processed to separate the shell from the nibs, broken, and then screened. The nibs were roasted in batches of 3 tons. The roaster consisted of a rotating cylinder (Barth, Germany), the envelope of which was heated by the combustion gas burner, in three basic steps: (1) cocoa nibs were mixed with water in natural cocoa (the term "natural" is often used in connection with nonalkalized cocoa powders, pH 5.60−5.90) ("natural process") or with an aqueous solution of alkali, plus incorporated air in the case of alkalinized cocoa (pH 7.20−7.92) extracts ("Dutch process") and heated to 85 °C by steam injection into the roaster; (2) progressive heating took place until the selected temperature (125 °C) was reached; and (3) the selected temperature was maintained for 3 min in the roaster (total roasting process time of 47 min) (*14*). The same processing was applied to the cocoa shell material. Samples were preserved at room temperature and analyzed as soon they arrived at the laboratory. Cocoa bean samples analyzed were free of cocoa shell material and separated into a cocoa bean and a shell fraction.

Reagents and Reference Standards. Solvents were of analytical (Panreac, Barcelona, Spain), Fluka Chemika (Buchs, Switzerland), and HLPC (Merck, Darmstadt, Germany) grades. Ultrapure water (Milli-Q, Millipore Corp., Bedford, MA) was prepared for chromatographic use. OTA (from *Aspergillus ochraceus*) was obtained from Sigma Chemical Co. (St. Louis, MO) and OTA standard solution (1000 ng/mL) from R-Biopharm Rhône Ltd. (Glasgow, U.K.).

The Ochraprep immmunoaffinity columns were purchased from Rhône Diagnostic Technologies. These columns contain an anti-

ochratoxin A antibody immobilized onto a gel material and are filled with preserved buffer solution. The specifications for these columns are that they will recover >80% of OTA standard added at 10 μg/kg in cocoa sample.

Instrumentation. The HPLC equipment consisted of Waters 2795 separation modules and a 2475 multiwavelength fluorescence detector (Waters Chromatography Division, Milford, MA). The system was equipped with Waters Millennium software, version 32, for data processing.

Standard Preparation. A stock solution of OTA at 1 mg/mL in benzene/acetic acid (99:1, v/v) was prepared and assayed according to an AOAC method (*26*). The standard solution was diluted to a concentration of 100 ng/mL using methanol. This solution was diluted to 0.05, 0.1, 0.25, 0.5, 1, 3, and 5 ng/mL in Milli-Q water.

Sample Preparation. Finely ground cocoa and cocoa products (10 g) or cocoa shells (50 g) were weighed and extracted in a mixture of 200 mL of methanol/3% aqueous $NaHCO_3$ solution (50:50, v/v) for cocoa products or 200 mL of acetonitrile/water (60:40, v/v) for cocoa shells. The suspension was blended for 3 min at medium speed (13000 rpm) using an Ultra-Turrax T25 homogenizer (IKA-Werke, Staufen, Germany). This homogenized solution was filtered through a Whatman no. 4 filter paper. Then 20 mL (cocoa products) or 4 mL (cocoa shells) of filtrate, equivalent to 1 g of sample, was diluted with 20 mL of phosphate-buffered saline (PBS) solution (cocoa products) or with 44 mL of PBS solution (cocoa shells) (*27*).

Affinity Cleanup. The diluted extracts were applied to an Ochraprep immunoaffinity column at a flow rate of 2−3 mL/min. After the column had been washed with 20 mL of distilled water (cocoa products) or with 20 mL of PBS solution (cocoa shells) at a flow rate of ~5 mL/min, OTA was eluted with 1.5 mL of acidified methanol [acetic acid/methanol (2:98, v/v)] and 1.5 mL of distilled water. To ensure complete removal of the bound toxin, the acidified methanol was left in contact with the column for 3 min. This was achieved by reversing the flow of methanol (backflushing) two or three times to ensure complete elution of OTA and collection in an amber vial to give a 3 mL total volume.

HPLC System. The resulting solutions were analyzed by reverse-phase HPLC with fluorescence detection. A 250 × 4.6 mm i.d., 5 μm, Tracer Extrasil ODS2 column with a 10 × 5 mm, 5 μm, Tracer ODS guard column (Teknokroma, Barcelona, Spain) was used along with a mobile phase of acetonitrile/water/acetic acid (51:47:2, v/v/v). This elution was performed at 40 °C at a flow rate of 1 mL/min. The injection volume was 50 μL for both standard solutions and sample extracts. The fluorescence detector was operated at an excitation wavelength of 333 nm and at an emission wavelength of 443 nm. Ochratoxin A concentrations in cocoa extracts were determined from the standard curve, using peak area and height for quantitation.

Chemical Confirmation. The methanol extract was evaporated to dryness at 40 °C under a gentle stream of nitrogen, and the residue was redissolved in 300 μL of boron trifluoride/methanol complex (10% solution in methanol). This solution was heated for 10 min in a heating block and allowed to cool to room temperature. The identity of OTA was confirmed by forming the methyl ester that gave a retention time (RT) of ~17 min.

Statistical Analyses. Analysis of variance (ANOVA) was used to determine significant differences among geographical origins of the samples analyzed. A Student−Newman−Keuls test was used to perform a multiple comparison of means [$Q = (\bar{x}_{max} - \bar{x}_{min})/s_{\bar{x}}$]. The SAS statistical package was used (*28*). Group differences were considered to be statistically significant at a level of $P < 0.05$. For each parameter the mean of three replicates was taken as the variation limit.

RESULTS AND DISCUSSION

Analytical Procedure. A preliminary study was carried out to establish the optimal procedure for the recovery of OTA. The applied method was based on a modified procedure originally described by Biopharm Rhône Ltd. (*27*). The extraction of OTA with a mixture of methanol/3% aqueous $NaHCO_3$ solution (50:50, v/v) followed by high-speed homogenization yielded higher OTA levels (by 15−30%) compared with a 1% aqueous $NaHCO_3$ solution (*27*) or with a methanol/1% aqueous $NaHCO_3$ solution (50:50, v/v). No significant changes in the OTA retention time were observed from day to day with freshly prepared mobile phase, when the separation was performed at 40 °C at a flow rate of 1 mL/min. The retention time was ~8 min for OTA. The stability of the OTA stock solution was tested regularly by injecting a standard working solution into the HPLC system. The OTA peak appeared to be excellently resolved (**Figure 1**), with good results in terms of recovery and repeatability. The main advantage of immunoaffinity columns in the cleanup step is that the matrix interference can be removed nearly completely. Ochratoxin A was identified by coelution with a spike of standard, working with negative samples and enrichment samples, and chemical confirmation. The fluorescence emission had the advantages of greater sensitivity and selectivity than UV absorption. Ochratoxin A standards were analyzed at various excitation and emission wavelengths to obtain maximum response conditions. The emission wavelengths were then scanned at the optimum excitation wavelength.

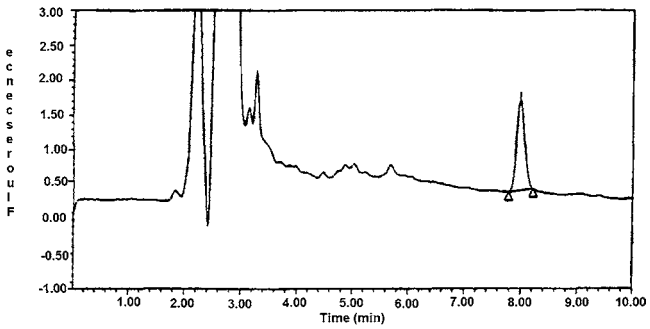

Figure 1. Chromatographic separation of ochratoxin A in cocoa powder.

Table 1. Method Recovery, Precision, and Accuracy for Determination of Ochratoxin A in Cocoa Powder and Roasted Cocoa Shell

OTA added, μg/kg	OTA found, μg/kg	recovery,%	accuracy,%	precision,%
		Cocoa Powder		
1.02	0.83 ± 0.074	81.4	18.6	8.9
2.16	1.81 ± 0.081	83.4	16.2	4.48
5.17	4.37 ± 0.20	84.5	15.5	4.58
		Roasted Cocoa Shell		
1.02	0.82 ± 0.080	80.4	19.6	9.76
2.16	1.78 ± 0.088	82.4	17.6	4.94
5.17	4.33 ± 0.21	83.8	13.9	4.85

Detection sensitivities were evaluated by serial dilutions. Linearity checks were performed using standards solutions and cocoa beans containing various predetermined levels of added OTA. The variance in homogeneity was investigated and confirmed using the Cochran test. The response of the HPLC system was linear over the concentration range of 0−10 μg/kg (correlation coefficient > 0.999). The limits of detection (LOD) and quantification (LOQ) of an analytical procedure are determined by analyzing a number of low-concentration samples. The concentration of these samples should span the lowest fourth of the range established during the linearity study (*29*). The detection limit (signal-to-noise ratio ≥ 3) of the HPLC method was 0.1 μg/kg, which is comparable to that reported by Pittet et al. (*30*), and the quantification limit was 0.30 μg/kg, sufficiently low for quality control purposes. Good recoveries of spiked samples demonstrated that the method had no significant matrix interference except for raw cocoa shells. Precision and accuracy were determined in roasted cocoa powder and roasted cocoa shell samples spiked with OTA at three

Table 2. Levels of Ochratoxin A Found in Cocoa Product Samples[a]

sample	geographical origin	incidence	mean OTA level,[a] μg/kg	
			concn range	mean value
cocoa bean	Ivory Coast (9), Guinea (5), Cameroon (3), Nigeria (3), and Senegal (1)	21/16	0.1–3.5	0.45
cocoa cake	Indonesia (38), Ivory Coast (17), Ghana (8), Malaysia (7), Nigeria (5), Ecuador (3), Honduras (1), and Peru (1)	80/74	0.1–9	2.79
cocoa mass	Ivory Coast (6) and Cameroon (2)	8/4	0.1–3.5	1.07
raw cocoa shell	Ivory Coast (1), Cameroon (1), and Nigeria (1)	3/3	1.8–3.4	2.60
roasted cocoa shell	Ivory Coast (7) and Cameroon (3)	10/10	2.90–23.1	11
roasted nibs	Ivory Coast (1) and Nigeria (1)	2/0	<0.1	<0.1
cocoa butter	Ivory Coast (3) and Cameroon (1)	4/0	<0.1	<0.1
cocoa powder	Ivory Coast (14), Guinea (10), Nigeria (4), and Cameroon (3)	31/29	0.1–4.4	2.41
chocolate and chocolate cream		11/8	0.1–1.59	0.63

[a] Mean values were calculated by assuming that samples < LOD contained OTA at the LOD level of 0.1 μg/kg.

concentrations, 1, 2, and 5 μg/kg ($n = 3$ for each concentration). The following equations were used: accuracy, % = (measured concentration − actual concentration/actual concentration) × 100; precision, % = (standard deviation/mean concentration) × 100. The results are shown in **Table 1**. For roasted cocoa powder, recoveries ranged from 81.4 to 84.5% (mean = 83.1%) with relative standard deviations (RSD) between 4.48 and 8.9%, meeting requirements of the AOAC Peer-Verified Methods Program (*31*). For raw cocoa shell the results indicated a great variability (ranging from 40 to 85%) and a high relative standard deviation (>10%). In contrast, the recoveries varied from 80.4 to 83.8% (mean = 82.2%) for industrially roasted cocoa shell powder, with RSDs between 4.85 and 9.76%. A possible explanation for this discrepancy may be associated with significant differences in water-holding capacity (WHC) values between raw cocoa shell and roasted cocoa shells (*32*). Because of the high WHC value in the raw cocoa shell, the volume of OTA extract collected from the sample was not constant.

Analysis of Cocoa Samples. Ochratoxin A concentration levels found in cocoa product samples are summarized in **Table 2**. Of the 170 samples analyzed, 26 samples did not contain OTA (<0.10 μg/kg). Ochratoxin A was detected at levels ranging from 0.1 to 23.1 μg/kg. The determined OTA contaminations were not corrected for recoveries. In roasted cocoa powder OTA was not detected in 2 samples (i.e., samples contained <0.10 μg/kg) (6.5%), and a total of 12 samples contained OTA at levels ranging from 0.1 to 2 μg/kg (38.7%). Seventeen samples were found to contain >2 μg/kg (54.8%), among which 12 contained >3 μg/kg. In cocoa cake OTA was not detected in 6 samples (7.5%), 33 samples were contaminated with OTA at ≤2 μg/kg (41.3%), and 41 samples contained OTA at levels ≥2 μg/kg (51.2%), among which 16 exceeded 5 μg/kg (range of 5−9 μg/kg). The average level of OTA in the cocoa beans was 0.45 μg/kg (range of 0.1−3.5 μg/kg), only 1 sample exceeded 2 μg/kg (4.7%), and no OTA was detected in 5 samples. Ochratoxin A content ranged from 0.1 to 3.5 μg/kg for cocoa mass (mean = 1.07 μg/kg), 2 samples (25%) containing levels >2 μg/kg. Average OTA was 11 μg/kg (range of 2.9−23.1 μg/kg) (100% positive samples) for the roasted cocoa shells and 2.60 μg/kg in raw cocoa shells. This low-level contamination probably could be influenced by the reduced recoverability obtained and can be considered as a discordant value in relation to the high levels of OTA detected in roasted cocoa shell. In general, the concentrations of OTA in cocoa bean and cocoa cake were very variable, and elevated values were detected in cocoa shell. Ochratoxin A was not detected in cocoa butter or in roasted cocoa nib samples (<0.10 μg/kg) (**Figure 2**). On the basis of 11 samples OTA exhibited a low

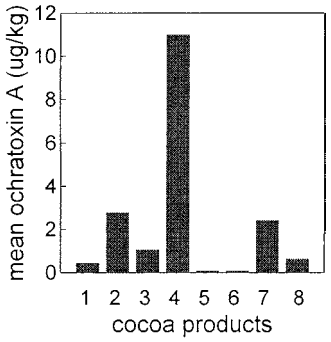

Figure 2. Ochratoxin A contamination in cocoa products: 1, cocoa bean; 2, cocoa cake; 3, cocoa mass; 4, roasted cocoa shells; 5, roasted nibs; 6, cocoa butter; 7, cocoa powder; 8, chocolate.

concentration in chocolate and chocolate cream samples, between 0.1 and 1.59 μg/kg (mean = 0.63 μg/kg), and the majority of samples (81.8%) contained OTA at <1 μg/kg. All samples of cocoa cake from Indonesia and Malaysia contained OTA at a level of >0.7 μg/kg, with an average of 2.97 μg/kg ($n = 50$) and a range of 0.7−9 μg/kg. Seventeen samples (34.7%) exceeded 3 μg/kg of OTA. Cocoa cake samples from other geographical origins contained OTA at variable levels (average = 2.52 μg/kg) ($n = 30$) but, in general, lower than 2 μg/kg (56.7%). No significant differences ($P < 0.05$) were noted in the OTA levels between cocoa cake samples and location origins. According to the proposed regulations for OTA in cocoa and cocoa products (2 μg/kg), if this standard is used as a criterion, it could be catastrophic for the cocoa market. At least 40% of the cocoa cakes would be rejected once they arrived at European ports.

Toxicological Aspects. On the basis of the kidney toxicity of OTA in pigs, and using a 500-fold margin of safety, the Joint FAO/WHO Expert Committee on Food Additives (*33*) has allocated a provisional tolerable weekly intake for OTA of 112 ng/kg of bw. In 1995, the Joint Expert Committee on Food Additives (JECFA) of the World Health Organization (WHO) set a Provisional Tolerable Daily Intake (PTDI) of 16 ng/kg of bw/day (*17*). The European Commission's Scientific Committee for Food (SCF) has recently recommended that it would be prudent to reduce exposure to OTA as much possible, ensuring that exposures are toward the lower end of the range of tolerable daily intakes (TDIs) of 1.2−14 ng/kg of bw/day, which have been estimated by other bodies (e.g., <5 ng/kg of bw/day) (*19*). This tolerance agrees with the values (0.4−2.4 ng/kg of bw/day), calculated for a lifetime risk level for cancer using the Knudson−Mooglavkar model, which assumes that OTA does not have initiating properties (*34*). Compared with the PTDI

proposed by the WHO, the average OTA intake in Europe seems to be rather low. These differences are also reflected in risk management measures that have been implemented or proposed with different maximum contamination levels being applied to different commodities and to the same commodity in different countries. Considering that the roasted cocoa powder and chocolate were contaminated at average OTA levels of 2.41 and 0.63 μg/kg, respectively, and based on a mean consumption of 3.14 kg of chocolate and cacao products per year in Spain (*35*), cocoa should contribute only a minor fraction to the OTA (TDI). These values are similar to the OTA concentrations found in different types of chocolates and cacao powders commercially available in the European market (*4*, *6*). Unfortunately, due to many possible sources of uncertainties (e.g., consumption data) the main food sources of OTA intake for humans are still in debate. However, from a recent survey, the European Commission−SCOOP-EU task 3.2.7, aimed at the evaluation of OTA exposure assessement by the EU consumer, cocoa products' contribution to the exposure is not excessive (4% of the total contribution) (*36*). The selection and development of tolerances and adequate guidelines to ensure that the distributed cocoa products are safe is necessary. Obviously it is tremendously important that coordinated rules be established among different countries to regulate the maximum permitted levels of this toxin in cocoa and cocoa products, using as a basis the TDI established as a function of the immediate and chronic toxicity in experimental animals, compared with the average cocoa product consumption of the population.

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