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Low Occurrence of Mycotoxins in Traditional Peanut Butter is Associated with Risk for Consumers

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Authors' contributions

This work was carried out in collaboration between all authors. Authors ZAB and LTZ designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors MK and RKN managed the analyses of the study. Author LTZ managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The aim of this study was to conduct a quantitative risk assessment linked to Aflatoxin B1 and Ochratoxin A exposure through peanut butter consumption by population of Abidjan District (Côte d'Ivoire).

Study Design: Sampling of peanut butter from markets, analysis of peanut butters samples, consumption survey and quantitative risk assessment.

Place and Duration of Study: University of Nangui Abrogoua, Abidjan (March to August 2014) and University of Ghent, Faculty of Bioscience Engineering, Belgium (September to December 2016).

Methodology: 45 composite samples of peanut butter from 225 total samples were analyzed by HPLC to determine the AFB1 and OTA contents. 450 households were randomly selected and a questionnaire was used to assess the uses of peanut butter, the quantities used for sauce making and the frequency of peanut butter sauce consumption. A probabilistic quantitative risk assessment based on Monte Carlo simulation was carried out to determine the exposure associated with the AFB1 and OTA occurrence.

Results: The values of AFB1 and OTA contents were in the range of 1.522-1.641 ppb. Peanut butter is mainly used for preparation of sauces (84.20 ± 9.38%) and more than half of the

consumers of peanut butter sauce are children (59 to 78 %). The serving size is about 750 g considering a frequency of 2 times consumption per week (55%). For the upper bond scenario, the exposure value for AFB1 was 2.193 ng/kg bw/day while that of OTA was 2.012 ng/kg bw/day for the 99.0th percentile of consumers. Overall, 10.1-15.6% of the population might be exposed to AFB1 chronic toxicity.

Conclusion: This study highlighted a relatively high exposure to AFB1 in peanut butter sauce. Therefore there is a need to implement strategies based on Good Agricultural and Good Hygiene Practices for AFB1 and OTA mitigation along the peanut butter chain in Ivory Coast.

Keywords: Aflatoxin B1; ochratoxin A; peanut butter; consumption survey; risk assessment.

1. INTRODUCTION

Groundnut or peanut (*Arachis hypogaea*) is an oleaginous plant belonging to the family of *Fabaceae* which includes the majority of leguminous seeds. The world production of groundnuts is estimated at 45 million tons with an average productivity of 1.8 ton per hectare in 2013. The main producers are China and India; both are providing more than 60% of the production. Senegal and Sudan are the main producers in Africa with 25% of the production [1].

In West Africa, these dicotyledonous plants serve as the major source of livelihood in terms of nutrients (lipids, proteins) and income generation and they are processed into oil, paste or butter, flour and various derivatives such as snacks and cakes [2]. The traditional processing peanut chain in West Africa and particularly in Ivory Coast is characterized by four main steps: harvesting, stripping, sun-drying, sorting and roasting. Along this short food chain compared to industrial ones, the main hazards are mycotoxins especially Aflatoxin B1 (AFB1) and Ochratoxin A (OTA) produced by toxigenic fungi Aspergillus, Penicillium and Fusarium [3,4]. Indeed. groundnuts are growing from the soil which is the primary reservoir for many toxigenic fungi [5]. Thus, groundnuts could support aflatoxigenic and ochratoxigenic mould growth and OTA and AFB1 production due to many biotic (insects, nematodes) and abiotic (moisture, activity water, temperature) factors in the field and also during storage or transportation [3,6]. Aflatoxin B1 (AFB1), the most toxic of the aflatoxins, is a potent liver carcinogen while OTA is known for confirmed nephrotoxic effect and suspected carcinogenicity [7,8]. In the European Union, maximum limits (MLs) of 2 and 8 ppb for AFB1 in peanuts intended for direct human consumption were adopted. As for Ochratoxin A, the maximum limits set by the European Union are 3 and 5 ppb

for cereals [9]. Toxicological reference values have been defined for AFB1 and OTA as follow: Provisional Maximum Tolerable Daily Intake (PMTDI) of 1 ng/kg bw/day was considered for AFB1 and Provisional Tolerable Daily Intake (PTDI) of 17.1 ng/kg bw/day for OTA [10,11]. Furthermore, the physico-chemical properties of AFB1 and OTA revealed that they are guite stable during heat processing operations leading to their presence in peanuts products as peanut butter that is traditionally made by grinding or crushing dried and roasted groundnuts [12,13]. In fact, many recent reports have revealed a cooccurrence of AFB1 and OTA in peanut products [13–15]. This co-occurrence may result in some cases to synergistic action in humans and animals based on toxicological effects of AFB1 and OTA [16].

Except for some qualitative studies on urine biomarkers of AFB1 and OTA in humans linked to the consumption of raw and processed peanut in Ivory Coast [15], there is a lack of extensive mycotoxins risk assessment of processed peanut as peanut butter that is purchased in retail markets and mixed with different ingredients for cooking in order to make sauce that accompany starchy foods. This study was therefore undertaken to implement a quantitative risk assessment linked to AFB1 and OTA exposure through peanut butter sauce consumption by Ivorian population. The results of this study could serve as starting point for providing sustainable solutions in order to reduce or alleviate mycotoxins health related problems in Ivory Coast. Indeed, It's important recalling that the final uses of risk assessment outputs are: the characterization of the most important factors influencing the risk of hazards identified in the food chain, the identification of risk strategies for mitigation and the establishment of guidelines for ranking priorities to be addressed in public health and food safety programs.

2. MATERIALS AND METHODS

2.1 Samples Collection and HPLC Analysis

In order to establish mycotoxins (AFB1 and OTA) exposure being linked to the consumption of peanut butter in the District of Abidjan (Ivory Coast), 225 samples were aseptically collected (sterile sampling material) from 9 main markets and transported in icebox (1-4°C) to the National Laboratory of Animal and Agricultural for AFB1 and OTA determination. For this, 45 composite samples were used to determine the AFB1 and OTA contents by using HPLC method after extraction and purification through immunoaffinity column as described in previous published work [13]. The HPLC operating conditions are described in the Table 1.

2.2 Consumption Survey

450 households (50 per commune) were randomly selected for the consumption survey. This investigation was carried out by using a questionnaire in order to collect the following information: number of people living in the households, categories of consumers based on age and gender, uses of peanut butter, quantities of peanut butter bought on markets for the preparation of sauces and frequency of peanut butter sauce consumption. The respondents were housewives.

2.3 Risk Assessment

2.3.1 Exposure assessment

A probabilistic model based on Monte Carlo simulation was carried out to determine the

exposure associated with the mycotoxins (AFB1 and OTA) in peanut butter. A modular process risk model (MPRM) was used to define the distribution of inputs and outputs (Table 2). Three simulations (5000 iterations) were performed with the add-in @risk software for Microsoft Excel version 7 (Palisade Corporation, USA).

2.3.2 Risk characterization

The calculated exposure values were compared with tolerable daily intakes (TDIs). For AFB1 a Provisional Maximum Tolerable Daily Intake (PMTDI) of 1 ng/kg bw/day was considered for adults and for children without hepatitis B [10]. For OTA, a Provisional Tolerable Daily Intake (PTDI) of 17.1 ng/kg bw/day set by the European Food Safety Authority [11] was used in this study. Additionally, the Bench Mark Dose Lower limit for 10% increased cancer risk (BMDL₁₀) was combined with the AFB1 or OTA exposure to estimate the Margin of Exposure (MOE) used to quantify the increase of cancer risk. For AFB1 the value of BMDL₁₀ was 170 ng/kg bw/day [10] while that of OTA was chosen at 16.1 µg/kg bw/day or 16100 ng/kg bw/day [17,18].

2.4 Statistical Analysis

All analyses were carried out in triplicates and data expressed as means \pm standard deviation. One way analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) were carried out to assess significant differences between means (p<0.05) using STATISTICA 7.1 (StatSoft). The add-in @risk® for Microsoft Excel version 7 (Palisade Corporation, USA) was used for the probabilistic risk assessment modeling.

 Table 1. Operating conditions for HPLC analysis of Aflatoxin B1 and Ochratoxin A in peanut butter samples

Chromatograph	Operating condition	ns
	AFB1	ΟΤΑ
Column	Shim-pack CLCG-ODS C18,	Shim-pack CLCG-ODS C18,
	(4 µm, 150 mm x 4,6 mm)	(4 µm, 150 mm x 4,6 mm)
Mobile phase	MetOH/ACN (50:50; v/v)	AcetOH/H ₂ O/ACN (1/50/49; v/v/v)
Flow	0,5 mL/min	0,5 mL/min
Column temperature	40°C	40°C
Injected volume	20 μL	100 μL
Detector	(λex: 365 nm; λem: 435 nm)	(λex: 330 nm ; λem : 460 nm)
Analysis time	15 min	10 min

Cell	Variable	Description	Units	Distribution/Values
A3	Ns	Total samples	-	= 45
A4	N _{nd}	Non detected samples	-	= 10 ^(a)
				= 3 ^(b)
A5	F _{nd}	Non detected fraction	-	= A4/A3
A6	Cd	Concentration detected	µg/kg	=Risk Beta General
				(1,6133;2,5612;0;5; Risk Name
				("AFB1 LB")) ^(a)
				=Risk Triang (0;1,97;4;Risk
				Name ("OTA LB")) ^(b)
A7	Cr	Concentration retail	µg/kg	= IF(RAND()>A5, A6, <i>ND value</i>)
A8	Cr	Concentration retail	ng/kg	= A7*1000
A10	D _f	Dilution factor	-	= Risk Uniform (0.33, 0.50)
A11	P _f	Processing factor	-	= 1
A12	C _c	Concentration cooking	ng/kg	= A8*A10*A11
A14	Ps	Portion size	g	= Risk Pert (600, 750, 900)
A15	Fc	Frequency	-	= Risk Discrete
		consumption/week		({1,2,3};{0.4,0.55,0.05})
A16	Fs	Family size	-	= 5
A17	Bw	Body weight	kg	= Risk Discrete ({35,65};{3,2})
A18	Co	Consumption	g/kg bw/day	= (A14*A15)/(A16*A17*7)
A19	Co	Consumption	kg/kg bw/day	= A18/1000
A21	Exp	Exposure	ng/kg bw/day	= A12*A19

Table 2. Variables of modular process risk model for Aflatoxin B1 and Ochratoxin A exposure

^(a): Value or distribution for AFB1. Best fit distributions were determined for the quantified concentration data using the Chi-square statistics (mean, skewness, 50, 90 and 95 percentiles)

^(b): Value or distribution for OTA. Best fit distributions were determined for the quantified concentration data using the Chi-square statistics (mean, skewness, 50, 90 and 95 percentiles)

ND value: Non detected value = 0 for lower bound; = LOD/2 for medium bound; = LOD for upper bound [17]. The distribution for dilution factor, portion size, frequency consumption, family size and body weight was based on survey results

3. RESULTS AND DISCUSSION

3.1 Occurrence of AFB1 and OTA

The chromatogram of AFB1 and OTA after extraction is depicted in Fig. 1. The results of HPLC analysis of AFB1 and OTA in peanut butter samples are presented in Table 3. The maximum value for AFB1 was 4.38 ppb while that of OTA was 3.78 ppb. The mean value of AFB1 was in the range of 1.522-1.524 ppb and that of OTA was in the range of 1.638-1.641 ppb with no significant difference (p>0.05). The presence of AFB1 and OTA in analyzed peanut butter samples may be linked to the intrinsic characteristics of groundnuts that favor fungal growth and also the poor drying and storage methods along the traditional processing chain [4]. Indeed, groundnuts are pulled up at harvest (4-5 months after planting). At this stage peanut contains 25 to 50% moisture and contamination with Aspergillus and Penicillium moulds is associated with drought stress and insect damages. Drought stress can also cause pod

splitting in the soil, leaving the kernels exposed to the soil microflora [5]. After harvesting and stripping, the collected groundnuts pods are shelled and then sun-dried. Contamination by toxigenic moulds will occur at this steps if the recommended moisture level (10% or less) is not achieved within a short period of time. Indeed, *Aspergillus* and *Penicillium* can easily grow and produce AFB1 and OTA at Aw > 0.85 [19]. However, the mean value for AFB1 was low compared to the limit of 2 ppb set by the European Union. The non-detected analytical results were replaced by zero or the limit of detection (LOD) to produce an upper and lower boundary [10].

3.2 Consumption Survey

The consumption data of the present study is based on a survey conducted in 450 households of Abidjan district. On average, 5 people live in each of the 450 visited households. The various usage of the peanut butter in the households is presented in Table 4. The peanut butter is mainly used for the preparation of sauces in the households (84.20 ± 9.38) to be followed by cakes (8.06 ± 2.23), caramels (7.14 ± 3.79) and others (0.60 ± 0.11). For sauce making, women mixed peanut butter and water generating a dilution factor of 0.33 to 0.5. The domestic temperatures used for cooking ($80-100^{\circ}$ C) have no reduction effect on AFB1 and OTA concentrations as underlined by some authors [20]. The different categories of consumers are presented in Fig. 2. More than half of the consumers of peanut butter sauce are children

(59 to 78 %) followed by adults (7 to 31 %) and infants (5 to 15 %). After cooking, the serving size is 600 g for minimum, 750 g for the most likely and 900 g of sauce for the maximum, considering a frequency of 1 consumption per week (40%), 2 times consumption per week (55%) and 3 times consumption per week (5%) by a family of 5 members (3 children and 2 adults). It was assumed that the consumption was equally shared among the family members [21]. The considered body weight was 35 kg for children and 65 kg for adults.

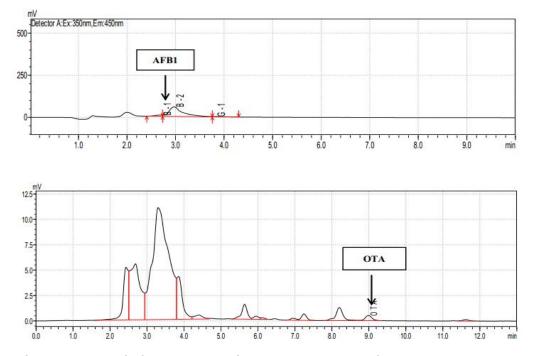


Fig. 1. Chromatogram of Aflatoxin B1 and Ochratoxin A extracted from peanut butter samples

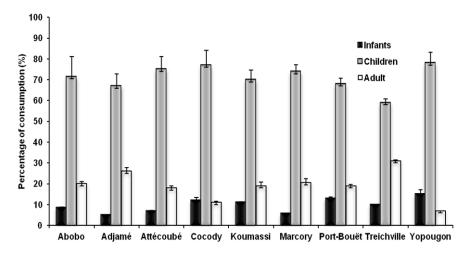


Fig. 2. Categories of peanut butter sauce consumers

Communes	AFB1 (ppb), LOD = 0.01 ppb		OTA (ppb) LOD = 0.05 ppb	
	LB	UB	LB	UB
	1.93	1.93	1.98	1.98
	1.64	1.64	2.32	2.32
Abobo	1.94	1.94	2.02	2.02
	2.48	2.48	2.45	2.45
	2.11	2.11	1.88	1.88
	1.90	1.90	2.13	2.13
	2.08	2.08	2.09	2.09
Adjamé	3.02	3.02	3.78	3.78
· j · · ·	1.15	1.15	2.17	2.17
	0.00	0.01	0.98	0.98
	4.38	4.38	1.23	1.23
	3.02	3.02	2.09	2.09
Attécoubé	0.00	0.01	2.37	2.37
	3.45	3.45	1.97	1.97
	1.60	1.60	2.94	2.94
	1.68	1.68	1.99	1.99
	1.98	1.98	0.00	0.05
Cocody	1.22	1.22	1.83	1.83
coocay	3.47	3.47	1.92	1.92
	1.20	1.20	2.01	2.01
	1.14	1.14	1.97	1.97
	0.00	0.01	2.34	2.34
Koumassi	0.00	0.01	1.16	1.16
Roumassi	3.51	3.51	2.17	2.17
	0.00	0.01	1.96	1.96
	1.94	1.94	2.47	2.47
	0.00	0.01	1.13	1.13
Marcory		3.11		2.06
Ivial COLY	3.11		2.06	
	0.00	0.01	1.17	1.17
	0.00	0.01	1.97	1.97
	1.16	1.16	0.88	0.88
Davit haven	1.54	1.54	1.01	1.01
Port-bouet	0.64	0.64	0.24	0.24
	2.06	2.06	0.45	0.45
	0.00	0.01	0.07	0.07
	0.00	0.01	0.61	0.61
- · · ···	0.31	0.31	1.17	1.17
Treichville	0.07	0.07	0.00	0.05
	0.47	0.47	2.02	2.02
	0.30	0.30	0.00	0.05
	1.66	1.66	1.04	1.04
	3.02	3.02	1.33	1.33
Yopougon	1.19	1.19	2.20	2.20
	2.16	2.16	2.15	2.15
	2.97	2.97	1.98	1.98
n	45	45	45	45
Min	0.000	0.010	0.000	0.050
Max	4.38	4.38	3.78	3.78
Mean ± SD	1.52 ± 1.24 ^a	1.52 ± 1.24 ^ª	1.64 ± 0.83 ^a	1.64± 0.82

Table 3. Aflatoxin B1 and Ochratoxin A contents in peanut butter samples based on upper and				
lower boundary (LB and UB) scenarios				

LB: Lower boundary; UB: Upper boundary; LOD: Limit of detection. Means within each row for each parameter with different superscripts are significantly (p<0.05) different

Communes	Uses (%)			
	Sauces	Caramels	Cakes	Other
Abobo	86.70±4.20 ^c	7.80±3.54 ^b	5.50±1.73 ^b	0.00±0 ^a
Adjamé	92.70±6.15 [°]	1.30±1.07 ^ª	3.40±1.96 ^b	2.60±2.06 ^c
Attécoubé	89.30±2.96 ^c	5.20±1.31 ^{ab}	5.50±2.51 ^b	0.00±0.00 ^a
Cocody	72.60±3.14 ^{ab}	12.80±2.50 ^c	13.50±0.87 ^c	1.10±0.26 [▶]
Koumassi	79.10±5.43 ^b	9.20±1.71 ^{bc}	11.70±4.94 [°]	0.00±0.00 ^a
Marcory	89.50±5.41°	6.40±4.47 ^{ab}	4.10±0.98 ^b	0.00±0.00 ^a
Port-Bouët	67.30±1.39 ^ª	9.20±2.71 ^{bc}	23.50±3.89 ^d	0.00±0.00 ^a
Treichville	87.50±1.80 [°]	7.20±2.71 ^b	5.30±1.84 ^b	0.00±0.00 ^a
Yopougon	93.10±1.01°	5.20±1.11 ^{ab}	0.00±0.00 ^ª	1.70±0.36 ^{bc}
Average	84.20±9.38	7.14±3.79	8.06±2.23	0.60±0.11

Table 4. Consumption uses of peanut butter in the investigated households

Means in column with different superscripts are significantly (p<0.05) different

3.3 Risk Assessment

The distribution of consumption data (Fig. 3) revealed that the population consumed 0.315-1.363 g peanut butter sauce/kg body weight/day with 90% of probability. Fig. 4 shows the probabilistic distributions of concentration (AFB1 and OTA), for lower boundary (LB) and upper boundary (UB) by using @Risk software. For the scenario of lower boundary (non detected values = 0), the concentration of AFB1 in peanut butter sauce ranged between 0 and 1.450 ppb for 90% of probability. For OTA at lower boundary, the concentration ranged between 0 and 1.288 ppb for 90% of probability.

The values of exposure to AFB1 and OTA in peanut butter sauce for the 2 scenarios (LB, UB) are presented in the Table 5. Considering the

high level of consumers population (99.0th percentile) the exposure values for AFB1 were 2.072 and 2.193 ng/kg bw/day for lower bound and upper bound, respectively. Compared to the AFB1 exposures values, those of OTA were slightly lower (1.832 and 2.012 ng/kg bw/day) for the 99.0th percentile of consumer's population. The calculated exposure values for AFB1 in this study were lower than aflatoxins exposure in Ghana (9.9-99.2 ng/kg bw/day) measured from peanut consumption. Nevertheless, these values were higher than the estimated AFB1 exposure (0.63-0.66ng/kg bw/day) for urban Lebanese adults [22]. The main sources of uncertainty in the exposure distribution values are: the non detected values for the concentration (left censored values) and the number of repetition for the tested samples by using the HPLC method.

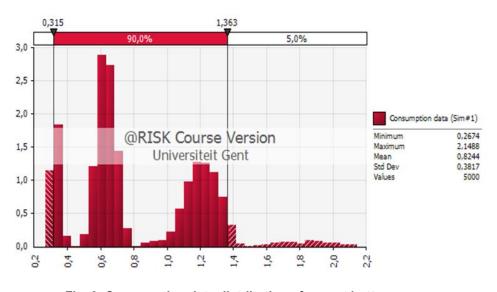


Fig. 3. Consumption data distribution of peanut butter sauce

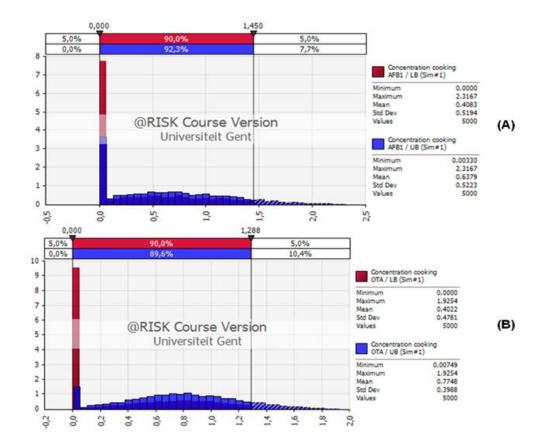


Fig. 4. Concentration data distribution of Aflatoxin B1 (A) and Ochratoxin A (B) in peanut butter sauce

The use of PMTDI value of AFB1 (1 ng/kg bw/day) as reference, indicated that 10.1-15.6% of the population might be exposed to a chronic toxicity due to AFB1 by peanut butter consumption. For OTA, there is no risk of chronic toxicity for all the population consuming peanut butter sauce by using the PTDI of 17.1 ng/kg bw/day as reference value. In order to evaluate the carcinogenic effects due to chronic toxicity, the values of margin of exposure (MOE) were calculated (Table 6). The MOE values varied from 77 to 3195 for AFB1 while those related to OTA varied from 8001 to 203539. The calculated MOEs for AFB1 were all below 10.000 and those of OTA were above 10,000 except for P99. According to EFSA [23], a MOE larger than 10,000 based on the BMDL₁₀ could be considered as low health concern [24]. In our study, the consumption of peanut butter by Ivorian population should be considered as a public health concern with regard to AFB1 contamination. Indeed, many studies have demonstrated the association between the ingestion of aflatoxin-contaminated foods and the

important recalling that 4.6-28.2% of all global HCC cases may be attributable to AFB1 exposure. Moreover, for individuals exposed to chronic hepatitis B virus (HBV) and AFB1 together, cancer risk is increasing 30 times greater [26]. Only the high percentiles (P99) for OTA give rather low MOE values, indicating a potential health concern. Moreover, the magnitude of the MOE can be used by risk managers for priority setting and for advising the exposures to be reduced to as low as reasonably achievable [27]. In addition, the co-occurrence of AFB1 and OTA may explain a synergistic action exposing the consumers (children and adults) to serious health problems on a regularly basis of consumption of sauces made by using peanut butter. In order to reduce the risk due to exposure of AFB1 and OTA, a food safety management system (FSMS) of the peanut butter must be implemented. For this, the developed FSMS must focus on Good Agricultural Practices (GAP) at the farm level such as: selection of resistant varieties of

risk of hepatocellular carcinoma (HCC) [25]. It's

	Exposure AFE	Exposure AFB1 (ng/kg bw/day)		A (ng/kg bw/day)
	AFB1	% PMTDI	ΟΤΑ	% PTDI
Lower boun	d (LB)			
Mean	0.331	33.1	0.341	1.99
P50	0.053	5.32	0.079	0.46
P75	0.536	53.6	0.581	3.39
P90	1.001	100.1	1.001	5.85
P95	1.376	137.6	1.291	7.54
P99	2.072	207.2	1.832	10.71
Upper bound	d (UB)			
Mean	0.510	51.0	0.638	3.73
P50	0.379	37.9	0.546	3.19
P75	0.764	76.4	0.879	5.14
P90	1.242	124.2	1.280	7.48
P95	1.600	160.0	1.537	8.98
P99	2.193	219.3	2.012	11.76

Table 5. Dietary exposure (ng/kg bw/day) of Aflatoxin B1 and Ochratoxin A associated with the				
consumption of peanut butter sauce				

PMTDI: Provisional maximal tolerable daily intake = 1 ng/kg bw/day for AFB1 PTDI: Provisional tolerable daily intake = 17.1 ng/kg bw/day for OTA The values in bold are greater than PMTDI or PTDI

Table 6. Margin of exposure (MOE) estimation based on dietary exposure to Aflatoxin B1 and Ochratoxin A in peanut butter sauce

	MOE AFB1	MOE OTA			
Lower bou	Lower bound (LB)				
Mean	513	47214			
P50	3195	203539			
P75	317	27710			
P90	169	16083			
P95	123	12470			
P99	82	8788			
Upper bou	Upper bound (UB)				
Mean	333	25235			
P50	448	29487			
P75	222	18316			
P90	136	12578			
P95	106	10474			
P99	77	8001			
The values in	hold are holew 10.00	00 based on PMDL			

The values in bold are below 10,000 based on BMDL₁₀ MOE: Margin of exposure

groundnuts, use of appropriate fertilizers and pesticides, crop rotation, irrigation management, early harvesting, proper sun-drying and storage [28,29]. At the country level, authorities must enforce regulation on mycotoxins in commodities and react to new challenges such as climate change to develop technologies for AFB1 and OTA control.

4. CONCLUSION

The results of this study highlighted a relatively high exposure to AFB1 in peanut butter compared to the tolerable daily intake. Thus, the regular consumption of peanut butter by the Ivorian population constitutes a public health problem due the carcinogenic and nephrotoxic effects of AFB1 and OTA respectively. Therefore there is a need to implement strategies for mitigation of AFB1 and OTA occurrence along the peanut butter chain in Ivory Coast. These applying strategies should consist of GAP/GMP/GHP at farm level and HACCP at industrial level to ensure a sustainable food safety management system. To be effective in the long term, AFB1 and OTA reduction in the food chain must be directed at (i) educating families, farmers, stakeholders along the value chain as well as governments about the health risks associated with mycotoxins; (ii) investing in local capacity to support further activities both to reduce mycotoxins in agricultural products and to monitor mycotoxin levels in crops.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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