Ann Ig 2019; 31: 130-139 doi:10.7416/ai.2019.2265

Occurrence of Ochratoxin A in breakfast cereals and sweet snacks in Italy: dietary exposure assessment

R. Capei[1], L. Pettini[1], F. Mandò Tacconi[1]

Key words: Ochratoxin A, cereal-based food, dietary exposure
Parole chiave: Ocratossina A, cereali, esposizione alimentare

Abstract

Introduction. *Ochratoxin A, a toxic fungal secondary metabolite, is well known as a nephrotoxic, hepatotoxic, embryotoxic, teratogenic and immunotoxic agent, classified by the International Agency for Research on Cancer as "possibly carcinogenic to humans".*
Objective. *The aim of the study was to determine the occurrence of ochratoxin A in breakfast cereals and sweet snacks in order to estimate the dietary exposure of the Italian population, considering the widespread use of these products for all ages, and in particular for children and teenagers.*
Method. *Ochratoxin A was detected by ELISA technique. The calculation of the estimated exposure was performed by a deterministic approach.*
Results and discussion. *The percentages of contaminated samples tested were 8% for breakfast cereals and 51% for sweet snacks with a range of contamination from 0.5 to 2.1 ng/g. The mean estimated daily intake, depending on age categories, ranged from 2.9% to 8.6% of the latest provisional tolerable daily intake recommended by the European Food Safety Authority (17 ng/kg bw/d) calculated on the total diet.*
Children and teenagers result to be higher in exposure per kg body weight compared to adults.
Conclusions. *The estimate of ochratoxin A exposure levels calculated in the study does not represent a great concern for public health because they are not associated with a significant cancer risk.*

Introduction

Ochratoxin A (OTA) is a toxic fungal secondary metabolite produced by two main fungal genera, *Aspergillus* and *Pennicillum*: *Aspergillus ochraceus*, *A. carbonarius*, and *A. niger* are usually the main producers in warm climates, such as southern Europe, while in cool temperate latitudes, *Pennicillum verrucosum* and *P. nordicum* are commonly found as toxinogenic (1).

OTA is found in a wide variety of foods as a result of fungal contamination in fields during growth, at harvest, in crops, or during storage and transport, depending on climatic and geographical conditions, cultivation technique, packaging, and susceptibility of plants. It is well known that environmental factors such as humidity and temperature can favour the growth of the fungi and consequently the production of the mycotoxins (1).

OTA is a thermostable compound that is not destroyed by the usual techniques of food transformation, processing, cooking, and sterilization (2). A temperature above

[1] *Department of Health Sciences, University of Florence, Italy*

250 °C for several minutes is necessary to reduce its concentration (3).

In Europe, there is a widespread contamination of OTA with highly variable concentrations in the different food commodities. The toxin has been detected in cereals and cereal products, pulses, coffee, cocoa, dried fruits, nuts, spices, grape juice, beer, wine, and also in meat and meat products of animals exposed to contaminated feed (1, 4, 5).

The toxin is absorbed by the gastrointestinal tract, chiefly in the small intestine. It is then distributed through blood, mostly to the kidney, where it accumulates, then to liver, muscle and fat, following the "kidney>liver>muscle>fat" pattern and excreted in urine and faeces (6, 7).

In the blood, OTA is bound to serum proteins in a measure of 99.98%, contributing to its long half-life; in humans the half-life is about 35 days (1, 8), while it varies widely among mammalian species, from 1 to 20 days (6). The concentrations of ochratoxin A in blood samples from healthy persons range between 0.15 and 1.14 ng/mL (6, 9, 10). Detectable amounts have even been found in human milk (11, 12).

OTA is well known as a nephrotoxic, hepatotoxic, embryotoxic, teratogenic, and immunotoxic agent; moreover, the observed effects are dose- and time-dependent (1, 13-16).

The toxin is suspected to be the main agent responsible for human kidney disease, referred to as Balkan Endemic Nephropathy (BEN), and the associated urinary tract tumours (1, 6). The molecular mechanisms involved in OTA toxicity are not clearly defined (1), however, the chemical structure of the toxin is thought to be a key factor (17). OTA ($C_{20}H_{18}ClNO_6$) is a chlorohydro-isocumarin linked through an amide bond to L-phenylalanine (1).

OTA may, therefore, inhibit several enzymes that use phenylalanine (Phe) as a substrate, especially Phe-tRNA synthetase. It follows that the toxin may cause an inhibition of protein synthesis, mitochondrial harm, and interference with oxidative phosphorylation. There is increasing evidence that renal toxicity caused by OTA is associated with cellular oxidative stress (17).

The IARC (International Agency for Research on Cancer) (18) has classified OTA as possibly carcinogenic to humans (group 2B), based on sufficient evidence in experimental animals, but inadequate evidence for carcinogenicity in humans.

Chronic exposure to low doses of OTA *in vivo* causes renal adenomas and hepatocellular carcinomas in mice and rats (19).

In human lymphocytes, low doses of ochratoxin A induce DNA stable damage and a delay in DNA repair kinetics (20). Although the mechanism of action of OTA genotoxicity and carcinogenicity seems to be related to the formation of DNA adducts, this is still subject to discussion (16, 21).

As stated above, with concern for the potential risk on human public health, the EU set a legal limit of 3 μg/kg of OTA for processed cereals (22). Accordingly, tolerable intakes were also recommended at a weekly range of 112 ng/kg bw (23) on the basis of nephrotoxicity in experimental animals for which the LOEL (Lowest Observed Effect Level) was 8 ng/kg bw per day, and application of a safety factor of 500. Based on further epidemiological studies of nephropathy and genotoxicity, the latest provisional tolerable weekly intake (PTWI) set by the Joint FAO/WHO Expert Committee on Food Additives (6) was 100 ng/kg bw, corresponding to about 14 ng/kg bw per day. However, based on the same effects, in 2006 the European Food Safety Authority (EFSA) proposed a provisional tolerable weekly intake (PTWI) of 120 ng/kg bw/week (1), and confirmed it in 2010 based on new data (24).

According to the annual report of the Rapid Alert System for Food and Feed

Table 1 - EU: notification on mycotoxins in food during 2007-2016. (Data from RASFF portal (26))

Mycotoxin	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	Total
Aflatoxins	695	902	638	649	585	484	341	312	423	461	5490
Ochratoxin A	30	20	27	34	35	32	54	37	42	70	381
Others	29	9	0	5	15	9	10	34	30	20	161
Total	754	931	665	688	635	525	405	383	495	551	6033

(RASFF), in 2016, mycotoxins were in first place among the main hazards in border rejection notification in the European Union (25). Aflatoxins were the primary mycotoxins associated with the notifications, followed by ochratoxin A (Table 1). The notification trend of OTA during 2007 – 2016 shows a significant increase, with a peak of notifications in 2016. (Data from Portal of RASFF) (26).

As the ingestion of contaminated food is the primary route of potential human exposure to ochratoxin A, the aim of the study was to determine the occurrence of ochratoxin A in breakfast cereals and sweet snacks, in order to estimate the dietary exposure of the Italian population.

The choice to analyse these categories of food was due to the widespread use of these products for all ages, and in particular for children and teenagers, and the absence of data about OTA contamination of sweet snacks in Italy. In particular, the consumption of breakfast cereals, considering consumers of all ages and gender, is about 15 g/day, significantly less compared to that of sweet snacks, which is more than twice that amount, about 40 g/day (27). In recent years these products have shown a constant, though slight, increase of consumption (28).

Methods

Samples

A total of 84 samples of breakfast cereals and sweet snacks of the most consumed brands in all of Italy (10 and 12 respectively) were randomly collected, on the basis of the available consumption data (28), in the two major markets in Florence, and tested for ochratoxin A. The samples collected included: 25 breakfast cereals (18 composed only of cereals in different percentages, 7 containing also cocoa) and 59 sweet cakes (36 composed of cereals only, 23 containing also cocoa). It was decided to analyze samples containing also cocoa as they are frequently present in the diet of children and teen-agers.

Ochratoxin A determination

The method used in this study was enzyme-linked immunosorbent assay (ELISA), because it is practical and cost-effective: it is considered also a relatively simple and fast method for screening the occurrence of OTA in cereals, and provides sample extracts generally free of interference (29).

The ELISA test kit (I' screen Ochra- cod. OR 360) was supplied by Tecna, Trieste, Italy.

Extraction and determination were performed according to the instructions of the test kit, briefly described as follows: 5g of ground product previously added with 15 mL of 1M HCl was extracted with 30 mL of dichlorometane by shaking for 15 minutes, and then centrifuged at 2200 x *g* for 15 min. A portion of the dichlorometane phase (5 mL) was added with 5 mL of 0.13M $NaHCO_3$ solution (pH=8.1), extracted for 15 min by shaking, and then centrifuged at 2200 x *g* for 15 min; then 150 µL of the aqueous phase was diluted with 350 µL of the 0.13M $NaHCO_3$ solution (dilution factor: 20). 50 µL/well of the diluted solution or

standards were applied in duplicate directly to the ELISA plate, added with 100 μL enzyme conjugate, 50 μL anti-ochratoxin A antibody and incubated for 20 minutes at room temperature. After careful washing, the plates were incubated with 200 μL/well developing solution for 20 min at room temperature. The reaction was terminated by adding 50 μL/well stop solution.

OTA determination

Optical Density (OD) values at 450 nm were measured within one hour by a microplate ELISA reader (Sclavo Reader SR 400). The absorption intensity was inversely proportional to mycotoxin concentration in the samples. All assays were carried out in duplicate, and each standard was measured twice before and after the analysis of samples, obtaining a CV average of standards = 2.1. A calibration curve on a semi-logarithmic scale was made (absorbance % against the corresponding standard concentrations, R^2= 0.99). A Tecna spreadsheet (OR360-361r.4) was also used to obtain OTA values of samples; the concentrations of the standards were 0; 0.05; 0.1; 0.3; 1.0; 2.5 ng/mL. - LOD and LOQ were determined, respectively, 0.5 ng/g and 1 ng/g, corresponding to the concentrations in the curve of 0.025 ng /mL and 0.050 ng /mL (3s and 7s calculated for the average value of absorbance measured B/Bo =100; s = standard deviation). Recovery rate was 85%: it was rated as average of 6 measurements on samples spiked with a known amount of standard.

Exposure assessment

According to the European Food Safety Authority (30), to estimate the toxin exposure, by a deterministic approach, the mean concentration found in the samples was calculated considering: only positive samples, both positive and negative samples, and the highest concentration level found.

For samples containing levels of OTA below the LOD, a value equal to half the LOD was assigned and used for calculation purposes. Contamination values were combined with the food consumption data of the Italian population, divided by the body weight, and according to four age categories (children 3-9.9, teenagers 10-17.9, adults 18-64.9 and elderly ≥ 65). With the exception of the children's age group, gender was also evaluated. Exposure calculations at the 95th percentile of consumption were not performed because, for the Italian population, the data available are highly uncertain and provide only a rough indication of high levels of consumption (27).

For food consumption data, the authors referred to a survey carried out by the Italian Institute for Nutrition (INRAN) (27), which is highly representative of the Italian population for age, gender, geographical area, and the large number of subjects involved. The INRAN-SCAI survey is related to years 2005-2006, but for some food categories, including sweet snacks, data are updated to February 14, 2012.

Results and discussion

Occurrence of Ochratoxin A

The results of the study, corrected for recovery, are shown in Table 2. The results show a high proportion of censored data (mycotoxin that has not been quantified), especially regarding breakfast cereals, which indeed showed values of OTA contamination below the detection limit (92%). Two samples, one of which was composed by several cereals, such as oat, wheat, maize and rice, the other by maize, oat and cocoa, showed a concentration of 1 ng/g and > LOD respectively. This finding was in agreement with data reported by EFSA (1), according to which the average concentration of OTA, derived from a large number of samples available on the EU market, was about 0.20 ng/g, with a high variability. In other surveys, a wide contamination with similar

values of concentration such as not to pose a remarkable risk for public health was found (1, 10, 31).

The OTA frequency distribution in sweet snacks was 51% (30/59), with a mean concentration of 1.34 ng/g. The values of positive samples ranged from ≥ LOD to 2.1 ng/g, were below the legal limit set at 3 μg/kg (22).

Only two brands of 12 showed values

Table 2. Ochratoxin A concentration in breakfast cereals and sweet snacks (ng/g), corrected for recovery.

Samples (number)	Positive samples* (%)	Ingredients	ng/g
Breakfast cereals			
Brand 1 (2)	1 (50%)	oats, wheat, maize, rice	1.0
Brand 2 (3)	1 (33%)	maize, oats, cocoa (4%)	>LOD
Sweet snacks			
Brand 1 (4)	2 (50%)	wheat, barley, rice, oats, rye **	1.1
		wheat, cocoa (7%)	1.1
Brand 2 (5)	2 (40%)	wheat, cocoa (4%)	1.3
		wheat, cocoa (45%)	1.4
Brand 3 (4)	2 (50%)	wheat	1.0
		wheat, cocoa (24%)	1.1
Brand 4 (6)	1 (17%)	wheat, cocoa (4%)	1.1
Brand 5 (3)	1 (33%)	wheat, cocoa (20%), hazelnuts (2.8%)	1.3
Brand 6 (4)	2 (50%)	wheat	1.1
		wheat, cocoa (10%)	1.2
Brand 7 (5)	1 (20%)	wheat, cocoa (5%)	1.6
Brand 8 (5)	2 (40%)	wheat, cocoa (7%)	2.1
		wheat	1.6
Brand 9 (4)	2 (50%)	wheat	1.5
		wheat, cocoa (4%)	1.6
Brand 10 (5)	1 (20%)	wheat	1.3
Brand 11 (6)	6 (100%)	wheat	
		wheat	
		wheat	
		wheat	
		wheat, cocoa (5%)	
		wheat, cocoa (4%)	
Brand 12 (8)	8 (100%)	wheat	>LOD
		wheat	
		wheat	
		wheat	
		wheat	
		wheat	
		wheat	
		wheat, cocoa (4%)	

* Samples with concentration ≥ LOD

**The first is the cereal present in the largest quantity

below LOQ, but more than LOD in each sample tested, in a total of 14 snacks (6 and 8 respectively) of which 79% (11/14) consisted only of cereals, and 21% (3/14) also with cocoa. In all the other brands, the frequency of positivity ranged from 17% (relative to the products of brand 4) to 50% (regarding those of brands 1, 3, 6 and 9). This variability among the different brands is probably due to the origin of the ingredients, which unfortunately is impossible to verify.

The highest value (2.1 ng/g) was found in one sample of brand 8 composed by wheat and cocoa; brand 8 also showed a high value of contamination in the other sample (1.6 ng/g) composed by only wheat.

OTA was found in 17 out of 36 (48%) snacks composed by cereals only, and in 13 out of 23 (57%) snacks containing also cocoa. Despite the higher percentage of positive samples with cocoa, the mean value of contamination was quite similar to those composed by cereals only (1.27 ng/g and 1.38 ng/g respectively) while the standard deviation (0.24 vs 0.31) showed a high variability. This higher frequency of positivity and the slightly higher value of concentration could be also due to the contribution of cocoa rather than that of cereals. Unfortunately it is impossible to compare these results with others, because at this time similar researches refer only to individual foods and not to foods made up of different ingredients. On the other hand, cocoa often results contaminated with the toxin, as reported in many international studies (1, 17, 32) and, in Italy, by Brera et al. (33). OTA is the most common mycotoxin found in cocoa, mainly in cocoa powder, with a frequency of more than 90%, but the levels of contamination are usually below the legal limit. In 2003, the Italian Ministry of Health, claiming a precautionary principle, imposed a legal maximum limit for cocoa powder (0.5 μg/kg) and chocolate products (2 μg/kg) (34).

Compared to the research carried out in Europe and in other countries, our results showed similar values. The contamination of OTA is indeed widespread, but with a low level of concentration (1, 10), which at that time represented, in principle, a limited risk to health. Higher concentrations of OTA in cereal-based foods are found often in countries such as Morocco (5), Pakistan (4) and Lebanon (35), where the climatic conditions are probably a key factor for the growth of the fungi and the formation of the toxin.

On the other hand, mycotoxins are unavoidable, and although developing countries are at the centre of attention most of the time, developed countries are also at risk of exposure due to contaminated food imports. Despite the fact that regulatory agencies do their best to carry out controls on the permissible concentrations of mycotoxins, the introduction of contaminated imported foods can sometimes occur.

Exposure assessment

Breakfast cereals were not included in the calculations of estimated toxin exposure by consumers, since the frequency of positivity was very low (8%).

The estimated daily intake of OTA based on the concentration found in sweet snacks is reported in Table 3. As with international studies, the mean level of contamination was used because, in general, about 50% of the samples analysed was below the LOD, while the use of median value would be problematic (6).

The resulting dietary exposure estimate ranged from 2.9% to 8.6% of PTDI or PTWI: 0.49-1.46 ng/kg bw per day, corresponding to 3.43 - 10.22 ng/kg bw per week regarding overall Italian consumers and the mean OTA concentration of positive samples only.

If we consider all the samples, positive and negative, by substituting the results lower than LOD with LOD/2, the estimated daily intake is clearly lower, representing the 1.82 - 5.41% of the PTDI set for OTA (0.31 to 0.92 ng/kg bw per day, therefore 2.17 - 6.44 ng/kg bw per week).

Table 3 - Exposure assessment of Ochratoxin A for Italian consumers of sweet snacks.

			Positive samples [a]						Positive and negative samples					
Age/sex (years)	Body weight (Kg) [b]	Mean consumption (g) [b]	Contamination (ng/g)		Exposure (ng/Kg bw/day)		% of PTDI [d]		Contamination (ng/g)		Exposure (ng/Kg bw/day)		% of PTDI [d]	
			Mean	Range	Mean	Min-Max	Mean	Min-Max	Mean[c]	Range	Mean	Min-Max	Mean	Min-Max
3 – 9.9 M/F	26.1	40.2			1.46	0.77 – 3.2	8.60	4.53-18.82			0.92	0.39 – 3.2	5.41	2.29-18.82
10-17.9 M	57.1	44.1			0.73	0.39 – 1.6	4.30	2.30-9.41			0.46	0.20 – 1.6	2.70	1.17-9.41
10-17.9 F	49.1	39.7			0.77	0.40 – 1.6	4.50	2.35-9.41			0.49	0.21 – 1.6	2.90	1.23-9.41
18 – 64.9 M	78.4	40.4	0.95	0.5 – 2.1	0.49	0.26 – 1.0	2. 90	1.53-5.88	0.60	0.25 – 2.1	0.31	0.13 – 1.0	1.82	0.76-5.88
18 – 64.9 F	62.2	37.2			0.57	0.30 – 1.2	3.35	1.76-7.05			0.36	0.15 – 1.2	2.12	0.88-7.05
≥ 65 M	78.1	51.6			0.63	0.33 – 1.3	3.70	1.94-7.64			0.40	0.17 – 1.3	2.35	1.0-7.64
≥ 65 F	65.0	36.1			0.53	0.28 – 1.1	3.11	1.65-6.47			0.33	0.14– 1.1	1.94	0.82-6.47

[a] Samples with concentration ≥ LOD (0.5 ng/g). The mean value is obtained attributing LOQ/2; value to all the samples reported to be lower than LOQ

[b] Data reported by Leclercq et al. (27)

[c] The mean value is obtained attributing LOD/2 value to all the samples reported to be lower than LOD

[d] PTDI set at 17 ng/Kg bw/day by EFSA (24)

Children, whose body weight is lower compared to adults, result in a higher exposure per kg body weight considering both the mean (1.46 ng/Kg bw/d) and the minimum and maximum values (0.77 – 3.2 ng/kg bw/d), which are 8.6, 4.53 and 18.82% of PTDI, respectively. Teenagers had a mean exposure level of 0.73 ng/Kg bw/d for males (4.3% of PTDI) and 0.77 for females (4.5% of PTDI) with a range of 0.39 - 1.6 ng/Kg bw/d and 0.40 - 1.6 ng/Kg bw/d, respectively.

It is well known that children and/or teenagers represent the main risk groups and the most vulnerable population. Nevertheless, in these two age categories, which are the major sweet snack consumers, the exposure remained below the health-based guidance value.

Dietary exposure for Italian consumers of sweet snacks was generally in agreement with those reported by EFSA(1) and JFCA (6). In other European studies, the sampling plans were different, and food groups were composed by different foods. Therefore, comparisons of exposure levels for ochratoxin A in these studies should be made cautiously. However, in general, our findings are similar to those of studies carried out in Spain (36), The Netherlands (37), France (2) and Canada (38). In each of these studies, the estimated exposure ranged from 1 to 3 ng/Kg bw/d.

As far as we know, in Italy there is little data about the level of OTA dietary exposure by cereal-based food consumers. To compare our results, we can refer to the research carried out by Miraglia et al. (39) in which the average dietary exposure was 1.13 ng/Kg bw/d, and as a general trend, intakes for body weight were higher in the young than in the adult population. More recently, Solfrizzo et al. (40) found, by urinary biomarker determination, a very high probable daily intake (PDI) of 0.139 µg/Kg bw/d. However, the urinary excretion rate of OTA was determined in piglets, which, most

likely, is completely different from that in humans. In addition, the food diet approach did not cover all sources of OTA exposure, due to the fact that this mycotoxin occurs in a very high number of different foods and beverages.

Conclusions

The estimates of ochratoxin A exposure levels calculated in the study, similar to those found in other European studies, are well below the provisional tolerable daily intake recommended by EFSA and JFCA. Therefore, at this time, they do not represent a great concern for public health, because they are not associated with a significant cancer risk, based on the lowest adverse effect limit observed in animals. This positive result is probably related to the efficiency of the European risk management measures concerning the maximal levels of ochratoxin A in foods. However, one concern is the fact that these products are widely consumed by children and teenagers who are more sensitive than adults to the effects of mycotoxins.

Although human exposure cannot be completely prevented, it would be prudent to reduce exposure to OTA as much as possible. Therefore, it is important to carry out constant monitoring and also to find new ways to prevent contamination in cereal-based food production.

Riassunto

Presenza di Ocratossina A in cereali da colazione e snack dolci in Italia: valutazione dell'esposizione attraverso la dieta

Introduzione. L'ocratossina A, un metabolita secondario tossico fungino, è un noto agente nefrotossico, epatotossico, embriotossico, teratogeno e immunotossico, classificato dall'Agenzia Internazionale per la Ricerca sul Cancro come potenzialmente cancerogeno per l'uomo.

Obiettivo. Lo scopo dello studio era determinare la presenza di ocratossina A nei cereali per la colazione e negli snack dolci, al fine di stimarne l'esposizione alimentare della popolazione italiana, considerando l'uso diffuso di questi prodotti per tutte le età, in particolare per i bambini e gli adolescenti.

Metodo. L'ocratossina A è stata dosata con tecnica ELISA. Il calcolo dell'esposizione stimata è stato effettuato con un approccio deterministico.

Risultati e discussione. Le percentuali dei campioni contaminati testati sono state dell'8% per i cereali da colazione e del 51% per gli snack dolci con un *range* di contaminazione compreso fra 0,5 e 2,1 ng/g. L'assunzione giornaliera media stimata, in base alle fasce d'età, variava da 2,9% a 8,6% del valore di assunzione giornaliera provvisoria tollerabile raccomandata dall'Autorità Europea per la Sicurezza Alimentare (17 ng/kg di peso corporeo/giorno) calcolata sulla dieta totale. I bambini e gli adolescenti risultano essere maggiormente esposti rispetto agli adulti.

Conclusioni. I livelli di esposizione all'ocratossina A calcolati nello studio, che al momento non sono associabili ad un significativo rischio di cancro, non destano preoccupazione per la salute pubblica.

References

1. European Food Safety Authority (EFSA). Opinion of the Scientific Panel on Contaminants in the Food Chain on a request from the Commission related to ochratoxin A in food. EFSA J 2006; **365:** 1-56. Available on: http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2006.365/epdf [Last accessed: 2017 Dec 12].
2. Sirot V, Fremy JM, Leblanc JC. Dietary exposure to mycotoxins and health risk assessment in the second French total diet study. Food Chem Toxicol 2013; **52**: 1-11.
3. Boudra H, Le Bars P, Le Bars J. Thermostability of ochratoxin A in wheat under two moisture conditions. Appl Environ Microbiol 1995; **61**: 1156-8.
4. Iqbal SZ, Rabbani T, Asi MR, et al. Assessment of aflatoxins, ochratoxin A and zearalenone in breakfast cereals. Food Chem 2014; **157**: 257-62.
5. Copetti MV, Iamanaka BT, Nester MA, et al. Occurrence of ochratoxin A in cocoa by-products and determination of its reduction during chocolate manufacture. Food Chem 2013; **136**; 100-4.
6. Joint FAO/WHO Expert Committee of Food Additives [JECFA]. Evaluation of certain food

additives and contaminants. Sixty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva: WHO, 2007 (WHO Technical Report Series no. 947). Available on: http://apps.who.int/iris/bitstream/10665/43870/1/9789241209472_eng.pdf [Last accessed: 2017 Dec 12].
7. Duarte SC, Alves MR, Pena A, et al. Determinants of ochratoxin A exposure-A one year follow-up study of urine levels. Int J Hyg Environ Health 2012; **215**: 360-7.
8. Hagelberg S, Hult K, Fuchs R. Toxicokinetics of ochratoxin A in several species and its plasma-binding properties. J Appl Toxicol 1989; **9**: 91-6.
9. Palli D, Miraglia M, Saieva C, et al. Serum levels of ochratoxin A in healthy adults in Tuscany: correlation with individual characteristics and between repeat measurements. Cancer Epidemiol Biomarkers Prev 1999; **8**: 265-9.
10. Nguyen KTN, Ryu D. Concentration of ochratoxin A in breakfast cereals and snacks consumed in the United States. Food Control 2014; **40**: 140-4.
11. Turconi G, Guarcello M, Livieri C, et al. Evaluation of xenobiotics in human milk and ingestion by the newborn–an epidemiological survey in Lombardy (Northern Italy). Eur J Nutr 2004; **43**: 191-7.
12. Biasucci G, Calabrese G, Di Giuseppe R, et al. The presence of ochratoxin A in cord serum and in human milk and its correspondence with maternal dietary habits. Eur J Nutr 2011; **50**: 211-8.
13. Chopra M, Link P, Michels C, et al. Characterization of ochratoxin A-induced apoptosis in primary rat hepatocytes. Cell Biol Toxicol 2010; **26**: 239-54.
14. Wangikar PB, Dwivedi P, Sinha N, et al. Teratogenic effects in rabbits of simultaneous exposure to ochratoxin A and aflatoxin B(1) with special reference to microscopic effects. Toxicology 2005; **215**: 37-47.
15. Muller G, Burkert B, Moller U, et al. Ochratoxin A and some of its derivatives modulate radical formation of porcine blood monocytes and granulocytes. Toxicology 2004; **199**: 251-9.
16. Pfohl-Leszkowicz A, Manderville RA. An update on direct genotoxicity as a molecular mechanism of ochratoxin a carcinogenicity. Chem Res Toxicol 2012; **25**: 252-62.
17. Marin S, Ramos AJ, Cano-Sancho G, et al. Mycotoxins: Occurrence, toxicology, and exposure assessment. Food Chem Toxicol 2013; **60**: 218-37.
18. International Agency for Research on Cancer (IARC) Ochratoxin A. In: IARC monographs on the evaluation of carcinogenic risks to humans. Some naturally occurring substances: food items and constituents, heterociclyc aromatic amines and mycotoxin. 1993; **56**: 489-521. Lyon, France. Available on: http://monographs.iarc.fr/ENG/Monographs/vol56/mono56.pdf [Last accessed: 2017 Dec 18].
19. Atroshi F, Biese I, Saloniemi H, et al. Significance of apoptosis and its relationship to antioxidants after Ochratoxin A administration in mice. J Pharm Pharmaceut Sci 2000; **3**: 281-91.
20. Gonzàlez-Arias CA, Benitez-Trinidad AB, Sordo M, et al. Low doses of ochratoxin A induce micronucleus formation and delay DNA repair in human lymphocytes. Food Chem Toxicol 2014; **74**: 249-54.
21. Mally A. Ochratoxin A and mitotic disruption: mode of action analysis of renal tumor formation by ochratoxin A. Toxicol Sci 2012; **127**: 315-30.
22. European Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. EU O J L 364, 5-24. Available on: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:364:0005:0024:EN:PDF [Last accessed: 2017 Dec 19].
23. Joint FAO/WHO Expert Committee of Food Additives (JECFA). Evaluation of certain mycotoxins in food. Fifty-sixth report of the Joint FAO/WHO. WHO Technical Report Series no. 906. Geneva, 2001. Available on: http://whqlibdoc.who.int/trs/WHO_TRS_906.pdf?ua=1 [Last accessed: 2017 Dec 19].
24. European Food Safety Authority (EFSA). EFSA Panel on Contaminants in the Food Chain; Statement on recent scientific information on the toxicity of Ochratoxin A. EFSA J 2010; **8**: 1626. [7 pp.]. doi:10.2903/j.efsa.2010.1626. Available on: www.efsa.europa.eu [Last accessed: 2017 Dec 19].
25. Rapid Alert System for Food and Feed (RASFF). Preliminary Annual Report 2016. European Commission. Available on: https://ec.europa.eu/food/safety/rasff_en [Last accessed: 2017 Dec 19].
26. RASFF Portal. Available on: https://webgate.ec.europa.eu/rasff-window/portal/?event=SearchForm&cleanSearch=1 [Last accessed: 2017 Dec 19].

27. Leclercq C, Arcella D, Piccinelli R, et al. on behalf of the INRAN-SCAI 2005-06 Study Group (2009). The Italian food consumption survey INRAN-SCAI 2005–06: main results in terms of food consumption. Public Health Nutr 2009; **12**: 2504-32. Available on: http://nut.entecra.it/710/I_consumi_alimentari__INRAN-SCAI_2005-06.html [Last accessed: 2017 Dec 19].
28. Arkios Italy Annual Report 2013. Available on: http://www.arkios.it [Last accessed: 2017 Dec 19].
29. Fujii S, Ono EYS, Ribeiro RMR, et al. A comparison between enzyme immunoassay and HPLC for ochratoxin A detection in green, roasted and instant coffee. Braz Arch Biol Technol 2007; **50**: 349-59.
30. European Food Safety Authority (EFSA). Overview of the procedures currently used at EFSA For the 175 assessment of dietary exposure to different chemical substances. EFSA J 2011; **9**: 2490. doi:10.2903/j.efsa.2011.2490.
31. Araguás C, González-Peñas E, López de Cerain A. Study on ochratoxin A in cereal-derived products from Spain. Food Chem 2005; **92**: 459-64.
32. Mounjouenpou P, Gueule D, Fontana-Tachon A, et al. Filamentous fungi producing ochratoxin A during cocoa processing in Cameroon. Int J Food Microbiol 2008; **128**: 234-41.
33. Brera C, Debegnach F, De Santis B, et al. Ochratoxin A in cocoa and chocolate products from the Italian market: Occurrence and exposure assessment. Food Control 2011; **22**: 1663-7.
34. Ministero della Salute. Circolare del 28 novembre 2003, n. 6. Valori massimi ammissibili di ocratossina A nel cacao. GU n. 286, del 10.12.2003. Available on: http://www.gazzettaufficiale.it/eli/id/2003/12/10/03A13359/sg [Last accessed: 2017 Dec 19].
35. Raad F, Nasreddine L, Hilan C, et al. Dietary exposure to aflatoxins, ochratoxin A and deoxynivalenol from a total diet study in an adult urban Lebanese population. Food Chem Toxicol 2014; **73**: 35-43.
36. Coronel MB, Marin S, Cano-Sancho G, et al. Exposure assessment to ochratoxin A in Catalonia (Spain) based on the consumption of cereals, nuts, coffee, wine, and beer. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 2012; **29**: 979-93.
37. Sizoo EA, Van Egmond HP. Analysis of duplicate 24-hour diet samples for aflatoxin B1, aflatoxin M1 and ochratoxin A. Food Addit Contam 2005; **22**: 163-72.
38. Kuiper-Goodman T, Hilts C, Billiard SM, et al. Health risk assessment of ochratoxin A for all age-sex strata in a market economy. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 2010; **27**: 212-40.
39. Miraglia M, Brera C. Assessment of dietary intake of ochratoxin A by the population of EU Member States. Reports on tasks for scientific cooperation. Report of experts participating in Task 3.2.7 Publisher: SCOOP Directorate-General Health and Consumer Protection, 2002.
40. Solfrizzo M, Gambacorta L, Visconti A. Assessment of Multi-Mycotoxin Exposure in Southern Italy by Urinary Multi-Biomarker Determination. Toxins 2014; **6**: 523-38. doi:10.3390/toxins6020523.

Corresponding author: Dr. Francesco Mandò Tacconi, Department of Health Sciences, University of Florence, V.le Morgagni 48, 50134 Florence, Italy
e-mail: francesco.mandotacconi@unifi.it