

THE EXPOSURE OF GENERAL POPULATION IN CROATIA TO OCHRATOXIN A

IZLOŽENOST HRVATSKOG STANOVNIŠTVA OKRATOKSINU A

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SUMMARY

The exposure of general population in Croatia to ochratoxin A (OTA) was checked in several studies by measuring its concentration either in food samples or in human blood. The concentration of OTA in food and the frequency of OTA-positive samples show high variability from year to year according to the meteorological conditions. Regional variability in OTA contamination of cereals, wine and beans is also significant. However, while higher OTA concentrations and higher number of OTA-positive samples of cereals and beans are found in the northern part of Croatia, wine from the southern part of Croatia contains higher OTA concentrations. These differences are due to the distribution of different moulds, producers of OTA that specifically contaminate various commodities. However, a large-scale study performed on plasma from five Croatian cities collected four times a year showed a higher mean OTA concentration during the summer. The most exposed are citizens of Osijek, because there was no OTA-free sample collected in this town, and the frequency of samples containing the highest OTA concentration was significantly higher than in other cities.

Key words: fumonisin B₁, human blood, maize, mycotoxins, zearalenone, wheat

INTRODUCTION

Ochratoxin A (OTA) is a mycotoxin, secondary metabolite of some species of *Aspergillus* and *Penicillium* moulds. OTA is produced in all climatic zones, and humans are exposed to OTA mostly by ingestion of various contaminated commodities (Speijers and van Egmond, 1993). OTA is frequently found in cereals, cereal products, nuts, coffee, meat, milk, eggs, and other commodities like wine, beer, and cheese. Depending on nutritional habits, the

OTA contamination of these commodities has different impact on human exposure to OTA.

Nephrotoxic and carcinogenic properties of OTA have been demonstrated on a number of experimental animals (IPCS, 2001). The exposure to

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OTA was postulated to be the causative agent of endemic nephropathy (EN) because of histological similarities seen in the kidney of pigs suffering from porcine nephropathy caused by OTA in Scandinavian countries and those seen in the kidney of humans with endemic nephropathy (Krogh, 1974). In humans OTA was found for the first time in blood collected in the endemic region in the western part of Brodska Posavina in Croatia (Hult et al., 1982). In several studies it was found that the occurrence of OTA and its concentration in blood of people from endemic regions was higher than in control regions (Petkova-Bocharova et al., 1991; Radić et al., 1997). However, low concentrations of OTA were frequently found in blood of humans in countries where the endemic nephropathy is not present (Peraica et al., 1999). The toxicological significance of the concentration of OTA in human blood, which is near the detection limit of very sensitive methods, is not clear.

Unfortunately, OTA is not a food contaminant that occurs alone, but usually together with other mycotoxins. Some of them, like citrinin and fumonisin B₁ (FB₁), are also nephrotoxic and have additive and/or synergistic effect in cultured cells and experimental animals (Peraica et al., being printed).

The aim of this review is to present data on human exposure to OTA of healthy population in Croatia. In the presented studies two approaches were applied. In one case the concentration of OTA (alone or with other mycotoxins) was measured in various commodities collected all over Croatia, and in the other OTA concentration in human biological material (plasma and blood) were analyzed. Because of the previously mentioned never completely proved hypothesis of the involvement of OTA in the etiology of EN, most studies in our country were focused on OTA particularly in endemic regions of Croatia. This paper presents the available data on the exposure of general population in Croatia to OTA, and when possible to the other mycotoxins as well.

OTA CONTAMINATION OF VARIOUS COMMODITIES

The analysis of each commodity requires particular method of sample preparation that makes

the estimation of human daily intake rather cumbersome. The other inconvenience of this approach is the uncertainty of the real human exposure to such a contaminated food.

CEREALS

Cereals are considered to be the most important source of OTA and other mycotoxins exposure. In Croatia the first report on OTA in cereals revealed a high percentage of contaminated maize, wheat, wheat bread and barley in endemic region (Pavlović et al., 1979). Although samples were analyzed using thin layer chromatography (TLC) with detection limit of 5 ppb, the number of OTA-contaminated samples was high (15.2, 25.0, 29.4 and 42.9% of samples, respectively), and high variability from year to year was observed.

Similar variability of OTA-positive samples was found in maize collected in 1996 (N=45) and 1997 (N=54) in Croatia (10 and 20 %) using more sensitive high performance liquid chromatography method (HPLC) with detection limit of 0.2 ppb (Jurjević et al., 1999). The mean OTA concentration in positive samples was 62.7 and 20.2 ppb, respectively. Further research on maize collected in different Croatian regions showed regional variability in OTA contamination, with the significantly higher OTA concentration ($P < 0.001$) in samples collected in Slavonski Brod than in other regions (Puntarić et al., 2001). The mean OTA concentrations in samples collected in Slavonski Brod, Osijek, Hrvatsko Zagorje and Istria were 20.00, 0.81, 0.42 and 0.40 ppb, respectively. In our study on maize (N=49) collected in all regions where maize is grown (Table 1), OTA was present in 39 % of samples and the mean OTA concentration in positive samples was 1.5 ppb (Domijan et al., 2005c). Such low mycotoxin concentrations are not a significant source of exposure to humans, but they may contribute to exposure from other commodities. It is interesting that all maize samples contained FB₁, and most of the samples (84 %) zearalenone (ZEA). In another study on maize (N=15) similar results of the co-occurrence of OTA with other mycotoxins were obtained (Domijan et al., 2005b) showing the importance of the research on experimental animals with simultaneous application of different mycotoxins.

Table 1. Mycotoxins concentration in maize collected in all Croatian maize-producing regions (N=49)**Tablica 1. Koncentracije mikotoksina u kukuruzu sakupljenom u svim hrvatskim županijama u kojima se uzgaja (N=49)**

	Concentration in positive samples (mean±SD, µg/kg) Koncentracija u pozitivnim uzorcima (srednja vrijednost±SD, µg/kg)	Range - Raspon (µg/kg)	Number of positive samples Broj pozitivnih uzoraka
FB ₁	459.8 ± 310.7	142.2 – 1377.6	49/49
FB ₂	68.4, 109.2, 3084.0	68.4 – 3084.0	3/49
ZEA	3.84 ± 6.68	0.43 – 39.12	41/49
OTA	1.47 ± 0.38	0.9 – 2.54	19/49

Domijan et al. *Food Addit Contam* 2005c;22:677-680.

Table 2. Distribution of OTA-positive bean samples in Croatia (N=45)**Tablica 2. Raspodjela uzoraka graha koji sadrže OTA (N=45)**

Part of Croatia - Hrvatske regije	Number of samples (positive/analyzed) Broj uzoraka (pozitivni/analizirani)	Mean concentration ± SD (µg/kg) Srednja koncentracija ± SD (µg/kg)
Northern - Sjeverne	5/7	0.30±0.06
Eastern - Istočne	6/13	0.37±0.17
Central - Središnje	6/20	0.54±0.26
Southern - Južne	0/5	0
Total - Ukupno	17/45	0.41±0.21

Domijan et al. *Food Chem Toxicol* 2005d;43: 427-432.

The analysis of wheat confirmed the regional variability in OTA contamination (Puntarić et al., 2001) with significantly higher ($P < 0.001$) mean OTA concentrations found in wheat samples from Slavonski Brod than in Osijek, Hrvatsko Zagorje and Istria (38.78, 8.71, 2.07 and 1.33 ppb, respectively).

and that all OTA-containing samples were contaminated either with *Penicillium* or *Aspergillus* spp. (Domijan et al., 2005d). OTA was found in 42 % of all samples, but there was no OTA in beans collected in the southern part of Croatia (Adriatic coast).

BEANS

Beans are a commodity frequently consumed in Croatia, particularly in winter. Sporadically analyzed beans collected in endemic areas of Croatia proved that beans might be contaminated with OTA (Pleština et al., 1990; Radić et al., 1997). Our study on beans collected all over Croatia showed that the concentration of OTA in beans from endemic regions in general was not high (0.25-0.92 ppb) (Table 2),

GRAPES AND WINE

OTA is a frequent contaminant of wine (Ottender et al., 2000) and wine contamination with OTA is considered an important contributor to human exposure to OTA as Zimmerli and Dick proved that higher OTA exposure in men was related to the consumption of red wine (Zimmerli and Dick, 1995). Visconti et al. estimated that 15 % of daily intake of OTA was due to wine consumption (Visconti et al., 1999).

In a pilot study on grapes, it was found that grapes in Croatia were also contaminated with OTA, the mean OTA concentration being 10 ppb (not published). A study on commercial wines revealed high frequency of OTA contamination (Domijan and Peraica, 2005a). The OTA concentration in all red wines was above the detection limit (10 ng/L), while three white wines (out of seven) were OTA-free (Table 3). Three OTA-free white wines were from the north of Croatia (inland), while all wines produced in

Split, Varaždin and Zagreb) were analyzed for the OTA concentration using HPLC (Table 4 and Table 5). Our results showed that the highest OTA exposure occurred during summer when higher temperatures favor mould growth and mycotoxins production on previous year cereals crop. The highest mean OTA concentrations in human plasma, as well as the highest number (100 %) of OTA-positive samples were found in Osijek. These data are in accordance with the highest OTA concen-

Table 3. OTA concentration in commercial wines in Croatia

Tablica 3. Koncentracija OTA u hrvatskim vinima

Samples of wine Uzorci vina	Positive/total Pozitivni/ukupno	OTA concentration - Koncentracija OTA (ng/l)			
		Minimal* Najniža*	Maximal Najviša	Median Medijan	Mean ± SD Srednja vrijednost ± SD
Red - Crno	7/7	12	47	22	22 ± 11
White - Bijelo	4/7	15	22	15	10 ± 9
Part of Croatia - Dio Hrvatske					
South - Jug	5/5	15	47	22	25 ± 12
North - Sjever	6/9	12	22	15	11 ± 8

Domijan and Peraica. *Arh Hig Rada Toksikol* 2005a;56:17-20.

* Concentration in OTA-positive samples

the south (Adriatic coast) were OTA-positive. The highest concentrations found in our study are lower than in other studies in Europe, which makes Croatian wines more suitable for consumers (Ottender et al., 2000; Visconti et al., 1999).

OTA IN HUMAN BLOOD

The real proof of human exposure to OTA is its finding in blood. In contrast to the OTA analysis in food, the advantage of such approach is that only one analytical method should be applied. However, blood sampling is an invasive method and unpleasant to subjects. It is well known that low concentrations of OTA could be found in blood of humans from endemic regions where EN is not known (Peraica et al., 1999). We have performed a single research on exposure to OTA of healthy population in Croatia (Peraica et al., 2001). About 50 plasma samples from blood bank collected four times in a year in five Croatian cities (Osijek, Rijeka,

trations in food collected in the same region (Jurjević et al., 1999, Puntarić et al., 2001).

In our study, the mean DI, calculated from the mean OTA concentration in all collected samples (0.30 ng/ml) was 0.40 ng/kg. b.w./day, which is below the mean DI in European countries (0.90 ng/kg b.w./day) (Hohler 1998). We used the method of Breitholtz et al. (1991) to calculate the daily intake (DI) from the mean concentration of OTA in human blood. This method is not perfect because it works on certain assumptions such as the plasma clearance (taking into consideration only glomerular filtration, therefore underestimating the intake) and bioavailability (taken from the experiments on non-human primates). The OTA contamination of commodities such as cereals, wine, coffee, and pork has never been systematically investigated in our country in such fashion as to enable calculation of exposure. However, a large-scale investigation involving 13 European countries (Jorgensen and Bilde, 1996) confirmed the value of evaluation of OTA exposure from findings in human blood.

Table 4. The frequency of OTA occurrence and the mean concentration in plasma samples collected over a year. Estimated daily intake was calculated from the mean OTA concentration of all samples**Tablica 4. Učestalost nalaza OTA i srednja koncentracija u uzorcima plazme sakupljenim tijekom godine dana. Procijenjeni dnevni unos izračunat je iz srednje koncentracije OTA u svim uzorcima**

Collecting period Vrijeme sakupljanja	Number of samples Broj uzoraka	OTA (ng/ml)				Estimated daily intake (ng/kg b.w./day) Procijenjeni dnevni unos (ng/kg t.j.t./dan)
		< 0.2	0.2 - 1.0	> 1.0	Mean concentration Srednja koncentracija	
March Ožujak	242	112	109	21	0.36	0.48
June Lipanj	249	101 ^b	135 ^b	13	0.39	0.52
September Rujan	242	141	94 ^c	7	0.25	0.34
December Prosinac	250	161 ^c	85	4 ^c	0.19	0.25
Total Ukupno	983	515	423	45	0.30	0.40

Peraica et al. Arch Toxicol 2001;75:410-414.

^b P<0.001; ^c P<0.05

Table 5. The frequency of OTA occurrence and the mean concentration in plasma samples collected in five cities. Estimated daily intake was calculated from the mean OTA concentration of all samples**Tablica 5. Učestalost nalaza OTA i srednja koncentracija u plazmi sakupljenoj u pet gradova. Procijenjeni dnevni unos izračunat je iz srednje koncentracije OTA u svim uzorcima**

City - Grad	Number of samples Broj uzoraka	OTA (ng/ml)				Estimated daily intake (ng/kg b.w./day) Procijenjeni dnevni unos (ng/kg t.j.t./dan)
		< 0.2	0.2 - 1.0	> 1.0	Mean concentration Srednja koncentracija	
Osijek	196	37 ^a	131 ^a	28 ^a	0.56	0.75
Rijeka	198	153 ^a	45 ^a	0	0.13	0.17
Split	191	96	86	9	0.31	0.42
Varaždin	200	119 ^a	75	6 ^b	0.31	0.42
Zagreb	198	110 ^a	86	2 ^b	0.19	0.25
Total - Ukupno	983	515	423	45	0.30	0.40

Peraica et al. Arch Toxicol 2001;75:410-414.

^a P<0.001; ^b P<0.01

LEGISLATION REGARDING MYCOTOXIN CONTAMINATION

In Croatia, maximal tolerable level for OTA in maize and in wine is 5 µg/kg while for beans it is not established (NN 16/2005). Maximal tolerable level for FB₁+FB₂+FB₃ and ZEA in maize is 4000 and 200 µg/kg, respectively. However, fumonisins and ZEA levels are provisional until final decision of the EU.

The EU defined this limit for OTA contamination of raw cereal grains (5 µg/kg), and wine (2.0 µg/kg). Maximal level for ZEA for unprocessed maize is settled at 350 µg/kg. The mean concentration of mycotoxins found in our studies never exceeds the above limits, although some samples containing high concentration of FB₁ and FB₂, as well as human blood samples with very high OTA concentration show that strict control of food is needed.

CONCLUSION

The exposure to OTA in Croatia is rather frequent, particularly in Slavonsko-brodsko county. Although the OTA concentrations found in blood of inhabitants of five Croatian cities are not high, strict control of food commodities is very important not only because of sporadic very high concentrations either in human blood or in cereals found in our investigations, but also because of the co-occurrence of various mycotoxins. Nowadays, little is known about possible additive or synergistic toxic or carcinogenic effects of various mycotoxins not only in humans, but also in experimental animals.

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SAŽETAK

Izloženost hrvatskog stanovništva mikotoksinu okratoksinu A (OTA) ispitivana je u nekoliko navrata mjerenjem njegove koncentracije u uzorcima hrane ili u ljudskoj krvi. Koncentracija mikotoksinu OTA u hrani i učestalost pozitivnih uzoraka različita je u uzorcima sakupljenim u različitim godinama, što ovisi o meteorološkim uvjetima. U žitaricama, vinu i grahu nađene su značajno različite koncentracije OTA s obzirom na mjesto uzorkovanja. Raspodjela uzoraka koji sadrže veću koncentraciju OTA različita je u različitim prehrambenim namirnicama. Značajno veća koncentracija OTA nađena je u uzorcima žitarica i graha sakupljenih u sjevernim dijelovima Hrvatske, dok je vino iz istog područja sadržavalo manju koncentraciju OTA negoli vino iz južnih krajeva. Te se razlike mogu objasniti time što u različitim namirnicama različite gljivice proizvode OTA. U opsežnom istraživanju izloženosti OTA hrvatskog stanovništva, sakupljeni su uzorci krvi u pet hrvatskih gradova (Osijek, Rijeka, Split, Varaždin i Zagreb) četiri puta tijekom godine. Najviša je srednja koncentracija u svim uzorcima nađena u uzorcima sakupljenim u ljeti, a stanovnici Osijeka najviše su izloženi ovom mikotoksinu. Svi uzorci sakupljeni u Osijeku sadržavali su OTA, a učestalost uzoraka koji su sadržavali veće koncentracije OTA bila je značajno viša negoli u drugim gradovima.

Ključne riječi: fumonisin B₁, ljudska krv, kukuruz, mikotoksini, pšenica