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Official control of ochratoxin A in food of animal origin in Poland between 2003 and 2012

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Abstract

Between 2003 and 2012, 1413 samples of kidneys, liver, and muscles from swine, cattle, sheep, horses, chickens, turkeys, geese, ducks, and fish were examined for the presence of ochratoxin A. The examination was performed in the framework of "The National Residue Control Programme for Chemical, Biological, and Drug Residue in Animal Tissues and in the Food of Animal Origin". The mycotoxin was determined by liquid chromatography with fluorescence detection after immunoaffinity column clean up. The limit of quantification was 0.2 µg/kg. Ochratoxin A was found only in swine kidney samples (n = 1092). It was detected in 28.8% of the kidney samples at the concentrations from 0.2 to 29.2 µg/kg. The most of the samples (25.5%) contained OTA at the concentration ranging from 0.2 to 5 µg/kg, which is below the provisional action level for OTA in kidneys, established in Poland at the concentration of 5 µg/kg. Furthermore, 24 (2.2%) samples had mycotoxin concentrations between 5 and 10 µg/kg and 13 (1.2%) samples above 10 µg/kg.

Key words: animal tissues, ochratoxin A, residues, national monitoring, Poland.

Introduction

According to the available literature, ochratoxin A (OTA) is the most often occurring fungal toxin in the cool temperature regions in the world such as Canada, eastern and northern Europe, including Poland (7, 8, 10, 14). It occurs naturally in a wide variety of raw agricultural commodities. OTA is found predominantly in cereal and cereal products as a result of fungal infection in crops, in the field during growth, at harvest, in storage and in shipment under favourable environmental conditions. The mycotoxin was evaluated in 2004 by the Scientific Committee on Food, which concluded that OTA may be nephrotoxic, neurotoxic, immuno-toxic, teratogenic, and cancerogenic to a wide range of organisms (10). Swine is one of the most sensitive species to the adverse effects of the mycotoxin. OTA may lead to pathological changes in the swine kidneys, such as tubular atrophy, interstitial fibrosis, and hyalinised glomeruli. The main target site of OTA toxicity is the renal proximal tubule. Chronic effects may be apparent when low levels of mycotoxin are ingested by the animals, and clinical symptoms include decreased weight gain, feed conversion rate, retardation of growth and increased disease susceptibility (1). As OTA has a high affinity to blood proteins, it can likely accumulate in different organs of animals. Since the 1970s, the presence of OTA has been documented in muscles, fat, liver, and kidneys of pigs fed diets contaminated with OTA (12, 13, 17). Another problem is the food safety issue due to the transfer of the toxin to humans through the food chain (carry-over) (20). In parts of Bulgaria, Romania, and the former Yugoslavia, dietary exposure to OTA, *via* plant products and *via* animal derived food products, may have association with the Balkan Endemic Nephropathy, a chronic progressive kidney disease in humans (11, 21, 22).

Testing the presence of OTA in the food chain is an important part of the food safety and was included in "The National Residue Control Programme (NRCP) for Chemical, Biological, and Drug Residue in Animal Tissues and in the Food of Animal Origin" in all countries of the European Union (EU) according to the Annex I of the Council Directive 96/23/EC of 29 April (6). The Directive requires Member States to adopt and implement a national residue monitoring plan for specific groups of residues. Member States must assign the task of co-ordinating the activities of the central and regional departments responsible for monitoring the

various residues, collecting the data, and sending the results of the surveys undertaken to the European Commission each year. The Directive lays down specific sampling levels and frequencies, as well as the groups of substances to be monitored for each food commodity.

In Poland, the presence of residues, including OTA, in tissues and muscles of different animal species is monitored by the Veterinary Inspection in accordance with the Directive of the Minister of Agriculture and Rural Development (9, 24).

The role of NRCP is to compile significant information on chemical and biological residues in food of animal origin, and it is an important part of any strategy to minimise unwanted residues in food, and help in ensuring consumer confidence in product quality and safety. The monitoring data may be essential for taking, if necessary, further legislative measures.

The objective of the paper is to present the results of the official control of OTA in food of animal origin in Poland between 2003 and 2012.

Material and Methods

Sampling. The sampling was performed in the framework of the NRCP according with an internal Instruction of the Chief Veterinary Officer for each year. The sampling plan procedure was based on the Council Directive 96/23/EC of 29 April. (6). As a part of the NRCP, the presence of OTA is determined annually in approx. 140 samples of kidneys, liver, and muscles from different animal species (Table 1). The samples are collected by the authorised personnel - veterinary inspectors, at slaughter-houses all over Poland.

About 0.2 kg of muscles or whole kidneys from swine, cattle, sheep, horses, fish, and whole liver from chickens, turkeys, geese, and ducks were sampled. Each sample was identified accordingly by a sampling document in which the date and place of the sampling were indicated, with other information that could be helpful for the specialist. The whole sample was homogenised and stored at -18°C prior to analysis. Subsamples of 50 g were analysed. From January 2003 to December 2012, a total of 1413 samples, including 1092 samples of kidneys from swine, were collected (Table 1).

Reagents and chemicals. All chemicals were of analytical grade. Organic solvents used for the mobile phase were of HPLC grade. They were purchased from J.T. Baker (Germany). Water (>14 MΩ × cm) was obtained from a MilliQAdvantage system (Millipore, USA). The immunoaffinity columns OchraTest™ were purchased from Vicam Inc (USA). Analytical standard of OTA (CAS: 303-47-9, $C_{20}H_{18}O_6NCl$, molecular weight: 403.82 g/mol) was obtained from Sigma-Aldrich Chemical Company (Germany). Stock solution was prepared in toluene-acetic acid (99:1, v/v) at a final concentration of 1 mg/mL. The OTA working solutions were prepared by diluting the stock solution.

Procedure for determination of OTA. The studies were carried out with a standard analytical procedure developed for OTA determination by liquid chromatography (LC) in tissues and muscles of animal origin. The method was validated in-house according to the requirements of the Commission Regulation and the Commission Decision (2, 4). Recoveries for fortified samples at the concentrations 0.2; 2.5; 5; and 7.5 µg/kg were at the ranges 65.2%–72.5% and 67.3%–98.3% for liver and kidneys, respectively. Repeatability and within-laboratory reproducibility, indicating the precision of the method, were at the ranges 3.9%–9.5% and 7.7%–12.2% for liver, 4.5%–17.2% and 7.1%–17.8% for kidneys. The limit of quantification was 0.2 µg/kg. The method fulfilled international analytical criteria.

Extraction and clean-up. A 10 g sample was extracted with 50 mL of chloroform after adding 0.1 M phosphoric acid (5 mL). The mixture was homogenised for 3 min at 10 000 rpm. The sample was then centrifuged at 3 500 rpm at 4°C for 15 min. The supernatant was filtered through a folder filter. Then, 25 mL of the eluate (=5 g sample equivalent) was evaporated to dryness by rotary evaporator in water bath at 50°C, under reduced pressure. The residue was re-dissolved in 4 mL of methanol. Then, the sample was mixed by vortex for 1 min, diluted with 21 mL of 1% sodium chloride, and filtered through a glass microfiber filter. A 5 mL of the extract (=1 g sample equivalent) was applied to column OchraTest ™WB.

Table 1. Samples obtained in the framework of the National Residue Control Programme, 2003–2012

Year	Samples											Total
	Kidneys				Liver				Muscles			
	Swine	Cattle	Sheep	Horses	Chickens	Turkeys	Geese	Ducks	Swine	Chickens	Fish	
2003	91	8	2	3	18	2	3	2	–	–	10	139
2004	101	10	1	2	17	2	2	–	4	1	5	145
2005	109	7	2	2	10	3	2	4	–	–	7	146
2006	116	6	2	2	5	2	2	2	–	–	6	143
2007	119	6	2	2	8	2	2	1	–	–	6	148
2008	128	6	2	2	6	2	1	2	–	–	4	153
2009	119	6	2	2	8	2	2	1	–	–	6	148
2010	102	5	2	2	6	2	2	3	–	–	6	130
2011	105	4	2	1	6	3	2	2	–	–	6	131
2012	102	5	2	4	6	3	2	2	–	–	4	130
Total	1092	63	19	22	90	23	20	19	4	1	60	1413

Table 2. Incidence and levels of ochratoxin A in swine kidneys, 2003–2012

Survey year	Number of samples analysed	Number of positive samples	Number of kidneys with different levels of OTA residues, µg/kg, (x ± SD)		
			≥0.2–5	≥5–10	≥10
2003	91	33	31 (0.8 ± 0.6)	1 (5.6)	1 (29.2)
2004	101	31	21 (2.2 ± 1.1)	6 (7.1 ± 1.1)	4 (13.1 ± 2.1)
2005	109	24	21 (1.8 ± 1.2)	3 (6.9 ± 1.6)	nd
2006	116	27	22 (1.2 ± 0.8)	3 (7.5 ± 1.4)	2 (11.8 ± 1.6)
2007	119	31	27 (2.1 ± 1.1)	3 (7.2 ± 0.6)	1 (18.2)
2008	128	28	23 (1.9 ± 1.3)	2 (6.1 ± 0.8)	3 (15.4 ± 3.3)
2009	119	29	24 (1.4 ± 1.0)	4 (7.3 ± 1.4)	1 (18.5)
2010	102	37	37 (1.2 ± 0.9)	nd	nd
2011	105	41	40 (1.4 ± 1.2)	nd	1 (11.7)
2012	102	34	32 (1.2 ± 0.6)	2 (6.5 ± 1.6)	nd
Total	1092	315	278 (1.5 ± 1.1)	24 (7.0 ± 1.1)	13 (15.0 ± 5.2)

nd – not detected

Subsequently, the column was washed with 10 mL of a wash solution (25 g of sodium chloride + 5 g of sodium bicarbonate + 0.1 mL of Tween 20, made up to 1 L of water). OTA was eluted by passing 1.5 mL of methanol and then the eluate was evaporated to dryness under a gentle stream of nitrogen at 35°C. The residue was re-dissolved in 0.3 mL of mobile phase before LC analysis.

Conditions of determination of OTA. Analyses of OTA were performed on liquid chromatograph Agilent 1100 HPLC (Agilent Technologies) with: valve injector system (0.9 mL loop), mobile phase pump (isocratic), and fluorescence detector. The software used to manage the chromatographic data was Agilent ver. ChemStation Rev. A.10.01. The chromatographic separation was performed at a wave length of λ_{ex} = 340 nm and λ_{em} = 470 nm, on an analytical column reversed – phase (Inertsil 5 µm ODS-3 100 Å, 150 × 4.6 mm, Phenomenex) coupled with octadecyl guard column (4 × 2 mm, Phenomenex). The mobile phase consisted of acetonitrile + water + acetate acid glacial (49.5:49.5:1, v/v/v).

Results

The results of the NRCP are shown in Table 2. Residues of OTA were found only in swine kidney samples. The mycotoxin was detected in 28.8% of samples at concentrations from 0.2 to 29.2 µg/kg. Most of the samples (25.5%) contained OTA at the concentration range from 0.2 to 5 µg/kg, which is below the provisional action level for OTA in kidneys, established in Poland at the concentration of 5 µg/kg. Furthermore, 24 (2.2%) samples had the mycotoxin concentrations between 5 and 10 µg/kg, whereas only 13 out of 1092 (1.2%) analysed samples had toxin amounts above 10 µg/kg: from 10.7 to 29.2 µg/kg. The incidence of positive samples was from 24 to 41 in different years. In the successive years, the percentage of non-compliant samples (≥5 µg/kg) varied from 1% in 2011 to 9.9% in 2004. Only in 2010, there were no non-compliant samples.

Discussion

Regulations for mycotoxins concentrations have been established in the EU countries in order to protect consumers from the harmful effects of these compounds and to keep contaminants at levels, which are toxicologically acceptable. In regard to OTA, the maximum levels for foodstuffs of plant origin are as follows: cereals - 5 µg/kg, cereal products - 3 µg/kg, dried vine fruit - 10 µg/kg, roasted coffee - 5 µg/kg, wine - 2 µg/kg, grape juice - 2 µg/kg, and processed cereal-based foods for infants and children - 0.5 µg/kg (5). However, OTA levels were not established in meat and meat-based products. The national legislation for OTA in products of animal origin exists in three countries. The Italian Ministry of Health established OTA maximum level of 1 µg/kg for pig meat and derived products (15). Estonia accepted 10 µg/kg for pig liver. In Denmark, if OTA in kidneys is higher than 25 µg/kg, the whole carcass is rejected, because the meat is supposed to be highly contaminated; if OTA is between 10 and 25 µg/kg, edible offals are eliminated;

and if the mycotoxin in kidney tissue is lower than 10 µg/kg, only kidneys are discarded. The most countries of the EU adopted the action level, which is 5 µg/kg (10).

The results presented in this paper suggest that the percentage of positive samples containing OTA in kidneys was rather high, because it varied from 22% to 39% depending on the year of evaluation. It is important to emphasise that the limit of quantification of OTA is very low compared with the action level (25 times lower), and it was the cause of such situation. On the other hand, the amount of non-compliant samples for OTA in swine kidneys was relatively low (1% - 4.3%), especially in 2004 when 9.9% of the kidney samples were contaminated by OTA at concentrations higher than 5 µg/kg. Data for the occurrence and contamination level of OTA in food of animal origin, published in 2003-2012 by the Commission of the European Communities, was generally similar (3). For example, in the successive years, non-compliant samples for OTA from swine tissues and muscles were found as follows: in 2003 - Finland (two samples) and Estonia (six samples); in 2004 - Austria (one sample): in 2005 - Cyprus (one sample) and Finland (one sample); in 2007 - Finland (one sample) and Cyprus (seven samples); in 2008 - Austria (two samples); in 2009 - Austria (one sample) and Greece (four samples); in 2011 - Finland (one sample). No non-compliant samples for OTA were found in 2006, 2007, 2010 (3).

In Serbia, the investigations of OTA contamination of the Serbian pork and chicken meat were carried out in naturally exposed animals, which are part of production chain (18, 19). A total of 540 samples of pig's blood, kidneys, liver, and chicken gizzard were randomly selected from slaughtered pigs (n=90) and chickens (n=90). In porcine tissue samples, 26.6% of the 90 liver samples contained OTA at the range of 0.22–14.5 µg/kg. The prevalence of OTA in serum and kidneys were very similar (31% and 33.3%), with a maximum concentration of 220.8 µg/L, and 52.5 µg/kg, respectively. In the majority of chicken tissue samples, measurable amounts of OTA were not detected. OTA was also determined in 90 samples of pork ham obtained from nine different farms in Italy (23). Sixty percent of the samples were positive, and the average concentration was 0.125 µg/kg. Only three of the samples showed contamination exceeding 1 µg/kg. In Denmark, paired samples of kidneys and meat were collected from 300 healthy pigs and analysed for OTA. The concentrations of mycotoxin in kidneys ranged from 0 to 15 µg/kg (mean 0.5 µg/kg, median 0.18 µg/kg) and in meat from 0 to 2.9 µg/kg (mean 0.12 µg/kg, median 0.03 µg/kg) (16).

Data on the presence of OTA in food of animal origin is limited, but it seems that the occurrence and contamination levels of OTA in Poland do not represent a particular situation, and it is generally not different from other European countries.

It has been estimated that within the EU, the overall contribution of products of animal origin containing OTA residues, to which humans are exposed, does not exceed 3% of the total ingested OTA; however, it may reach 10% in certain regions with distinct preferences for traditional meat products (10).

The results of survey of residues of OTA in animal tissues and muscles indicate that the concentrations of the mycotoxin in the examined samples are far below the values posing risk to the health of consumers. However, to protect consumer's health, residue levels of OTA in products of animal origin should be monitored and controlled because it is important to keep OTA consumption as low as possible. The results of national monitoring have an impact on the decisions of local veterinary inspection and central veterinary authorities responsible for the NRCP. There are special investigation rules for each non-compliant case, which are performed by Veterinary Inspection authorities.

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