Monitoring the mycotoxins in food and their biomarkers in the Czech Republic

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Testing of the presence of toxigenic microfungi and mycotoxins in foodstuffs in the food chain is an important part of the food safety strategy in The Czech Republic. At the national level, control of their presence in the entire food chain is assured by Public Health Protection Agencies, by the Veterinary Administration and by the Czech Agriculture and Food Inspection Authority. This article summarizes surveillance activities of Public Health Protection Agencies and mycotoxins findings in dietary raw materials and foodstuffs from the 1990s to 2004 in the Czech Republic. At present, the health risk from the mycotoxins exposure from foodstuffs is assessed to be relatively low in the Czech Republic, especially as far as the foodstuffs of the Czech origin are concerned. It may result in late toxic effects (*e.g.*, carcinogenic risk) following a single or repeated ingestion of low mycotoxins doses from foodstuffs. Nevertheless, the overall situation may change due to the globalization of the food market. In order to minimize the risk associated with mycotoxins and eliminate their impact on Czech public health, continuous monitoring of the presence of toxigenic moulds, mycotoxins, and their biomarkers is necessary, in conjunction with strict respect to European Union legislation.

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1 Introduction

Mycotoxins are naturally occurring secondary metabolites of several toxigenic microfungi that contaminate the whole food chain, from the agricultural cultures to the plate of consumers. The occurrence of mycotoxins may differ from year to year. The toxic effects of mycotoxins on human (and animal) health are acute (with a rapid onset and an obvious toxic response) or chronic (characterized by mycotoxins low-dose exposure over a long-time period). Mycotoxins can induce various adverse biological effects, *e.g.*, hemorrhagic, hepatotoxic, nephrotoxic, neurotoxic, estrogenic, teratogenic, mutagenic, and carcinogenic. Mycotoxins are associated with various diseases (mycotoxicoses) in humans throughout the world: *e.g.*, ergotism, alimentary toxic aleukia, aflatoxicosis. Therefore, control of the presence of toxigenic microfungi and mycotoxins in the whole food chain is necessary [1]. Also the attention of public health authorities and state surveillance agencies in the Czech Republic has focused on systematic control of the presence of mycotoxins in foodstuffs [2].

Human diseases caused by mycotoxins has been known since the time immemorial. In the Czech lands, *e.g.*, a great wave of ergotism was described by Professor Jan Antonin Scrinci in the 18th century [1]. Epidemic of ergotism caused by ergot alkaloids from *Claviceps purpurea* (due to the consumption of bread made of contaminated wheat flour) affected region of Mimon in Bohemia in 1736 – 1737 and approximately 500 cases of illness and 100 cases of death were recorded [1].

In Czechoslovakia the era of modern research on mycotoxins started in the 1970s [3, 4]. Nowadays, in the Czech

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Abbreviations: AF M_1, aflatoxin M_1; **IPH,** Institute of Public Health; **MH,** Ministry of Health; **NIPH,** National Institute of Public Health; **NRC,** National Reference Centre; **NRL,** National Reference Laboratory; **OTA,** ochratoxin A

Republic the control of mycotoxins and toxigenic microfungi in the entire food chain – ranging from the primary production till the points of sale – is assured by local institutes of Public Health Protection under the responsibility of Ministry of Health (MH) and by different agencies under the responsibility of Ministry of Agriculture (MAg): the Czech Agriculture and Food Inspection Authority (CAFIA), the Veterinary Administration, and to a certain degree also by the State Phytosanitary Administration and by the Central Institute for Control and Testing in Agriculture [1, 2]. In addition, some private laboratories analyze feedstuffs, raw materials, and foodstuffs.

The systematic research and monitoring of toxigenic microfungi, and mycotoxins in foodstuffs of food chain is carried out under the responsibility of MH by the National Institute of Public Health (NIPH) Prague, Centre for the Hygiene of Food Chain (CHFCH), National Reference Centre (NRC) for Microfungi and Mycotoxins in Food Chains in Brno. The hygienic surveillance of mycotoxins within the scope of reference and expertise activity is assured by the Institute of Public Health (IPH) Hradec Kralove, National Reference Laboratory (NRL) for Biomarkers of Mycotoxins and Mycotoxins in Food in Hradec Kralove. The privilege and the advantage of these laboratories under the authority of MH is to monitor selected mycotoxins biomarkers (*e.g.*, ochratoxin A (OTA) in serums, aflatoxin M_1 (AF M_1) in urines) [1, 5, 6].

Food safety strategy was and is one of the priorities of the Czech government especially with regard to the accession of the Czech Republic to the European Union [2]. Currently, the content of mycotoxins in foodstuffs in the Czech Republic is regulated by the existing legislation of the European Union for aflatoxins B_1, B_2, G_1, G_2, M_1, OTA, and newly for deoxynivalenol, zearalenone, and fumonisins (Commission Regulation/EC/No. 856/2005). Moreover, the recommendations for exposure limits of Joint Food and Agriculture Organization (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA) and Scientific Committee on Food of the European Union are followed [1].

Since the beginning of the 1990s series of experimental research projects were carried out in order to determine the levels of selected mycotoxins (aflatoxins, cyclopiazonic acid, OTA, patulin, DON, fumonisin B_1, T-2 toxin, zearalenone, sterigmatocystin, and alternaria toxins) in samples of various foodstuffs and raw materials [7–12]. The research of the presence of mycotoxins in foodstuffs and biological materials including blood serum, urine, or kidneys has become a part of the project called "Monitoring the Health State of the Population in Relation to the Environment" launched on the ground of the resolution No. 369/1991 of the Government of the Czech Republic under the auspices of the National Institute of Public Health. At present, within this project, there are two specific programs focusing on mycotoxins – MYCOMON (moulds and mycotoxins in foodstuffs) and Human Biomonitoring (mycotoxins in biological material). Apart from this project, a certain number of samples were tested in the course of the reference and expertise activities of public health laboratories [13–18]. Up to now, the principal aim of research has been to gather data on the level of the immediate mycotoxins contamination of foodstuffs, which would subsequently enable to determine the dietary exposure and to assess the potential health risk resulting from the intake of mycotoxins. It is generally known that the dietary exposure of humans to mycotoxins can be estimated either by analyzing mycotoxins in food using the known amount of the consumed diet or by analyzing levels of mycotoxins in a biological material (serums, urines, kidneys, *etc.*) [6, 13, 16, 19–21]. Hence, both types of samples were monitored.

2 Materials and methods

Systematic surveillance activities needed the development of a scale of routine analytical methods for determination of mycotoxins.

2.1 Sample characterization

Samples of different raw materials, foodstuffs, human blood serum, and urine were obtained from research studies [9, 11, 12, 19, 22, 23] within the project Monitoring the Health State of the Population in Relation to the Environment (MYCOMON and Human Biomonitoring) [24, 25] but also from reference and expertise activity of the public health laboratories [13, 14, 17, 18].

Samples of foodstuffs for purposes of MYCOMON program were purchased from retailers in 12 different locations in the Czech Republic. In total, 300 randomly and not probabilistically collected samples of food were included in the study [2]. Program MYCOMON included i.a. the determination and identification of toxigenic microfungi and OTA in raisins in 1999–2002 [26].

Samples of blood serum and urine for purposes of Human Biomonitoring were obtained from four different areas (Benesov, Plzen, Usti nad Labem, and Zdar nad Sazavou) [16].

2.2 Samples preparation

After the extractions of mycotoxins from the samples by means of organic solutions (*e.g.*, methanol/water, ACN/

water, chloroform), the analytes were cleaned up. The cleanup of mycotoxins was carried out using immunoaffinity and solid-phase chromatography or exceptionally using the liquid–liquid extraction (only for OTA extraction from serum) [2, 18, 21, 26].

2.3 The determination of mycotoxins

The methods employed for the quantification of aflatoxins (B_1, B_2, G_1, G_2, M_1), OTA, patulin, deoxynivalenol, sterigmatocystin, fumonisin B_1, cyclopiazonic acid, and altenuene in food, feed, and biological materials included RP HPLC with UV and fluorescence detection, HPTLC and ELISA methods [1, 2, 6, 19, 21, 26, 27]. The combination of immunoaffinity chromatography and HPLC, HPTLC, ELISA increased the selectivity and the sensitivity of these methods [2, 27]. The cleanup of mycotoxins in the samples of foodstuffs was carried out using standard, commercially available immunoaffinity columns [2, 27]. The analysis of OTA in samples of raisins was carried out by HPTLC after IAC cleanup too [26]. The employed methods were evaluated by analyzing various samples of food, feed, human blood serum, urine, animal, and human kidneys [2, 6, 19, 20, 27]. Validations of the methods were performed in accordance with the protocol approved by the Association Official of Analytical Chemists (AOAC) and following the principles of the International Conference on Harmonization (ICH) Guidelines for planar chromatography [2, 21, 27]. Where available, the certified reference materials of Community Bureau of Reference (*e.g.*, BCR, CRM No. 263, 284, 379, 472) were used for validation of the methods, the other methods were validated by spiked samples (this was the case especially of mycotoxin biomarkers in human serum and kidneys where certified reference materials are not available yet) [2, 21, 27]. Laboratories successfully participated in FAPAS (proficiency testing) [2]. The methods employed for determination of mycotoxins were accredited to CSN EN ISO/IEC 17 025 [2, 21, 26, 27].

3 Results

3.1 Findings of mycotoxins in foodstuffs

The levels of aflatoxins B_1, B_2, G_1, G_2, and aflatoxin M_1, OTA, patulin, and deoxynivalenol in samples of different raw materials and foodstuffs were analyzed during the reference and expertise activities of the IPH in Hradec Kralove from 2000 to 2004 (see Tables 1–5) [2]. The basic sta-

Table 1. Level of aflatoxins B_1, B_2, G_1, G_2 in raw materials and foodstuffs in 2000–2004 (5 years) (results of IPH, NRL Hradec Kralove)

Foodstuffs	*n*	*n*+ (%)	Arithmetic mean (μg/kg)	Median (μg/kg)	Percentile 90% (μg/kg)	Minimum (μg/kg)	Maximum (μg/kg)
Peanuts	893	72 (8.1)	(6.8) 11.3	(0) 4.0	(0.18) 4.0	4.0	540.0
Pistachio	30	2 (6.7)	(20.9) 24.6	(0) 4.0	(0) 4.0	4.0	62.2
Almonds	26	0	–	–	–	–	<4.0
Cereals	72	0	–	–	–	–	<0.4
Malt	148	3 (4.2)	(0.005) 0.4	(0) 0.4	(0) 0.4	0.4	0.8
Cereal porridges	75	0	–	–	–	–	<0.4
Wheat flour	77	2 (2.6)	(0.03) 0.4	(0) 0.4	(0) 0.4	0.9	1.4
Dried milk	140	2 (1.4)	(0.01) 0.4	(0) 0.4	(0) 0.4	0.6	0.9
Baby food	210	0	–	–	–	–	<0.4

a) Tables – cited after: Ostry, V., Skarkova, J., Malir, F., Sykorova, S., Advances on the occurrence of toxigenic fungi and mycotoxins in The Czech Republic, in: Logrieco, A., Visconti, A., sub [2].
b) *n*, numbers of samples; *n*+, numbers of positive samples;%, percentage of positive samples.
c) Only few of the obtained results were positive (see the column percentage in the tables). Since more than 60% of the results obtained were under the LOQ, the arithmetic mean, median, and percentile are expressed in accordance GEMS/Food – Euro as follows. First result in the brackets is counted with substitution of zero; the second result (without the brackets) is counted with substitution of the LOQ.

Table 2. Level of AF M_1 in foodstuffs in 2000–2004 (5 years) (results of IPH, NRL Hradec Kralove)

Foodstuffs	*n*	*n*+ (%)	Arithmetic mean (μg/kg)	Median (μg/kg)	Percentile 90% (μg/kg)	Minimum (μg/kg)	Maximum (μg/kg)
Milk	118	1 (0.9)	(0.004) 0.02	(0) 0.02	(0) 0.02	–	0.5
Milk porridge	52	0	–	–	–	–	<0.02

a); b); c); see legend under Table 1.

Table 3. Level of OTA in foodstuffs in 2000–2004 (5 years). (Results of IPH, NRL Hradec Kralove)

Foodstuff	*n*	*n*+ (%)	Arithmetic mean (μg/kg)	Median (μg/kg)	Percentile 90% (μg/kg)	Minimum (μg/kg)	Maximum (μg/kg)
Cereals	114	10 (8.8)	(0.5) 0.7	(0) 0.28	(0.2) 0.29	0.4	37.0
Malt	119	8 (6.7)	(0.1) 0.4	(0) 0.35	(0) 0.35	0.4	13.4
Flour	24	4 (16.7)	(0.2) 0.5	(0) 0.35	(0.38) 0.63	0.5	1.8

a); b); c); see legend under Table 1.

Table 4. Level of patulin in foodstuffs in 2000–2004 (5 years) (results of IPH, NRL Hradec Kralove)

Foodstuffs	*n*	*n*+ (%)	Arithmetic mean (μg/kg)	Median (μg/kg)	Percentile 90% (μg/kg)	Minimum (μg/kg)	Maximum (μg/kg)
Baby fruit food	310	3 (1)	(0.14) 10	(0) 10	(0) 10	10	20
Fruit foodstuffs	219	16 (7.3)	(18.3) 27.6	(0) 10	(3.4) 10	10	3150*)
Fruit drink	294	42 (14.3)	(4.3) 12.9	(0) 10	(0) 17	10	81
Malt	70	9 (12.9)	(4.3) 13.0	(0) 10	(8.4) 14.8	14	90

a); b); c); see legend under Table 1.
d) *) One sample of blueberries extremely contaminated by patulin.

Table 5. Level of deoxynivalenol in foodstuffs in 2000–2004 (5 years). (Results of IPH, NRL Hradec Kralove)

Foodstuffs	*n*	*n*+(%)	Arithmeticmean (μg/kg)	Median (μg/kg)	Percentile 90%(μg/kg)	Minimum (μg/kg)	Maximum (μg/kg)
Barley	156	28 (18)	(145.6) 264.9	(0) 151	(247.1) 275.4	100	6010
Others Cereals	17	3 (17.7)	(65.9) 107.1	(0) 58.8	(235.9) 235.9	100	500
Cereal porridge	10	1 (10)	(20.0) 100	(0) 100	(20) 100	–	200
Malt	120	24 (20)	(61.3) 159.6	(0) 121	(183.1) 199.8	100	1350
Flour	29	10 (34.5)	(100) 141.4	(0)139.7	(210.8) 210.8	100	470

a); b); c); see legend under Table 1.

tistical processing of data was carried out according to the report presented at the workshop in the framework of the Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme (GEMS/Food)-Euro [28].

Determination and identification of toxigenic moulds and OTA in raisins was carried out in 1999–2002. The analyses of 12 samples of raisins available on the Czech market showed that 4, 4, 7, and 3 samples in 19992000 and 2002, respectively, were OTA positive at levels higher than the quantification limit (1 μg/kg). In general, OTA levels ranged from 1.9 to 63.6 μg/kg. The average value (mean) for all samples was 4.6 μg/kg, the median 0.5 μg/kg, and the percentile 90% was 8.4 μg/kg. In the calculations of the average value, median, and 90% percentile of all samples in a set, those samples that were below the LOQ were assigned a value of half the LOQ (*i.e.*, 0.5 μg/kg). The raisins with high levels of OTA were imported from Turkey and these raisins were contaminated by *Aspergillus* section *Nigri* [26].

In addition, as a response to the European Union (EU) project "WINE-OCHRA RISK" (initiated in May 2001), a pilot study called "The Czech contribution to project WINE-OCHRA RISK" was started in the Czech Republic. The aim of this pilot study was to monitor the mycobiota of wine grapes, occurrence of ochratoxigenic microfungi in wine grapes and OTA and alternaria mycotoxins in grape fresh juice, must, and wine from domestic crop in the year 2004. Five vineyards (Hosteradice, Miroslav, Podmoli, Dobsice, and Horni Dunajovice) with 22 wine grape varieties were selected for the pilot study in the southeast part of Moravia, the Znojmo wine region. A sensitive HPTLC method for quantification of OTA in grape fresh juice, must, and wine was developed. Grape fresh juice, must, and wine samples were purified on commercial immunoaffinity columns. The OTA detection limit was 4 ng/L, the LOQ was 8 ng/L of grape fresh juice, must, and wine. The mycological examination norm CSN ISO 7954 (1994) for calculation of moulds in foodstuffs was applied. Classical mycological methods were used for identification of specific moulds. Ochratoxigenic microfungi (*Aspergillus carbonarius*, other *Aspergillus* section *Nigri*, *Aspergillus ochraceus*,

Table 6. OTA in human blood serum (results of IPH HK)

Year 1994 – 2002	Incidence of OTA		Range (μg/L)		Mean[b)] (μg/L)	Median[b)] (μg/L)	90% percentile[b)] (μg/L)
	n	*n*+[a)] (%)	Minimum	Maximum			
Total	2.206	2.077 (94.2)	0.1	13.7	0.28	0.2	0.5

a) *n*, numbers of samples; *n*+, numbers of positive samples%, percentage of positive samples.
b) [a)], OTA contamination ≥ 0.1 μg/L.
c) [b)], Level of OTA < 0.1 μg/L considered as 1/2 LOQ = 0.05 μg/L (convention).

Table 7. Level of AF M_1 (pg/g creatinine) in human urine (results of NIPH, NRC Brno)

Year 1997 – 1998	Incidence of AFM_1		Range (pg/g)		Mean[b)] (pg/g)	Median[b)] (pg/g)	90% percentile[b)] (pg/g)
	n	*n*+[a)] (%)	Minimum	Maximum			
Total	205	118 (57.6)	19.0	19.219	390.5	127.0	585.0

a) *n*, numbers of samples; *n*+, numbers of positive samples; %, percentage of positive samples.
b) [a)], AFM_1 contamination ≥ 125 pg/L urine.
c) [b)], Level of AFM_1 < 125 pg/L considered as 1/2 LOQ = 62.5 pg/L (convention).

Penicillium verrucosum and Penicillium nordicum) were not found in the grape samples. The presence of OTA (in grape fresh juice, must, and wine) was not confirmed. *Alternaria* microfungi were isolated from the grape samples; however, alternaria mycotoxins were not discovered in grape fresh juice, must, and wine [29].

3.2 Findings of some biomarkers of exposure to mycotoxins in biological fluids

IPH-NRL Hradec Kralove and NIPH-NRC Brno also analyzed selected mycotoxins biomarkers in the Czech population, in particular OTA in human blood serum and AF M_1 in urine (see Tables 6, 7) [21].

Monitoring the OTA biomarker in human serum from some other regions (Ostrava, Zlin, Liberec, and Praha) of the Czech Republic has been restarted in the year 2005.

4 Discussion

4.1 Exposure to mycotoxins

Species of *Fusaria* and *Alternaria* moulds are known to occur most commonly in the Czech Republic. Findings of not only OTA and aflatoxin B_1 but also of other monitored mycotoxins in raw materials and foodstuffs of Czech origin are not very frequent and not too high (from this point of view, Czech foodstuffs are competitive). Neither are the findings of the biomarkers of exposure to important mycotoxins in biological fluids in the Czech population (*e.g.*, OTA in blood serum and AF M_1 in urine of the blood donors). Nevertheless, the overall situation may change because of the globalization of the food market (see, *e.g.*, the findings of aflatoxins in peanuts, of OTA in raisins imported from Turkey, the findings of patulin in one sample of blueberries imported from Ukraine *via* Germany). The current risk resulting from the dietary exposure mycotoxins is relatively low but it can increase due to new food sources. In the light of the foregoing, monitoring the presence of toxinogenic moulds and mycotoxins in food in the Czech Republic is clearly necessary.

4.2 Conclusions

At present, the levels of mycotoxins in foodstuffs are relatively low especially as far as foodstuffs of the Czech origin are concerned. Nevertheless, the overall situation in the Czech Republic may change because of the globalization of the food market. Thus, it seems desirable to continue monitoring of the presence of toxigenic moulds, mycotoxins, and their biomarkers. At the same time, the strict respect of EU legislative food safety standards and their further development (see, *e.g.*, Regulation No. 856/2005) and also of the recommendations of JECFA FAO/WHO and European Union – Scientific Committee on Food (EU SCF)) is necessary.

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