Příloha II.

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<u>Kinetics of biomarkers, bioaccumulation and elimination of peptide toxins</u> <u>microcystins in different freshwater fish species</u>

<u>Ondřej Adamovský</u>¹, Jana Hlávková², Radovan Kopp², Klára Hilscherová¹, Pavel Babica¹, Miroslava Palíková³, Veronika Pašková¹, Stanislav Navrátil³, Luděk Bláha¹

¹Masaryk University & Institute of Botany CAS, Brno, C.R.

² Mendel University of Agriculture and Forestry, Brno, C.R.,

³ University of Veterinary and Pharmaceutical Sciences, Brno, C.R.

Microcystins are highly toxic peptide toxins produced in toxic cyanobacterial water blooms developing in freshwaters due to anthropogenic contamination by nutrients. In a series of studies, different freshwater fish species (common carp, silver carp, tilapia) were exposed to Microcystis spp. dominated natural cyanobacterial water bloom for up to two months, and microcystin kinetics and elimination as well as modulation of biochemical markers were studied. Toxins accumulated up to 1.4 to 29 ng/g fresh weight and 3.3 to 19 ng/g in the muscle of silver carp and common carp, respectively (determined by anti-microcystin ELISA). Higher concentrations were detected in hepatopancreas (up to 226 ng/g in silver carp) with a peak after initial four weeks, and bioconcentration factors ranged 0.6 to 1.7 for muscle and 7.3 to 13.3 for hepatopancreas. Microcystins were completely eliminated within 1 to 2 weeks after the transfer of fish to clean water. Mean estimated elimination half lives (t1/2) ranged from 0.7-8.4 days. Our study also showed significant modulations (and inter-specific variability) in biochemical markers measured in hepatopancreas. Levels of glutathione (GSH), and catalytic activities of glutathione-S-transferase (GST) and glutathione reductase (GR) were the most sensitive parametrs in fish indicating oxidative stress and enhanced detoxification processes. Calculation of hazard indexes using conservative US EPA methodology indicated rather low risks of MCs accumulated in edible fish. Supported by the Czech Ministry of Education (MSM 6215712402), and Ministry of Agriculture (NAZV QH71015).

Keywords: microcystins, bioaccumulation, toxicokinetics, biomarkers

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Analysis of microcystins in different matrices ; Microcystin kinetics (bioaccumulation, elimination) and biochemical responses in common carp and silver carp exposed to toxic cyanobacterial blooms.

<u>Ondřej Adamovský</u>¹, Jana Hlávková², Radovan Kopp², Klára Hilscherová¹, Pavel Babica¹, Miroslava Palíková³, Veronika Pašková¹, Stanislav Navrátil³, Luděk Bláha¹

¹Masaryk University & Institute of Botany CAS, Brno, C.R.

² Mendel University of Agriculture and Forestry, Brno, C.R.,

³ University of Veterinary and Pharmaceutical Sciences, Brno, C.R.

Cyanotoxins, particularly microcystins (MCs), have been shown to be a hazard to human health. MCs accumulate in aquatic organisms probably as a result of irreversible binding to liver protein phosphatases. The aim of this study was to evaluate the methods for detection of MCs in fish tissue (liver) using various detection methods. MC-LR, -RR, -YR was used as a representative congeners. Livers and raw water were obtained from Novovesky pond, where cyanobacteria is commonly present. The homogenates of liver were extracted by a water/ methanol/ hexan mixture subsequently analyzed via the LC-MS, anti-MC-LR ELISA and anti-Adda ELISA. It was shown, that ELISA systems can overestimate the real amount of MCs in samples or create false-possitive samples due to unspecific matrix influence with comparing to LC-MS method. On the other hand, Anti-Adda ELISA can determine congeners of microcystins and their fragments to which other analytical methods are insensitive. In less difficult matrices, such as raw water, Anti-Adda ELISA is well applicable, without matrix-associated affects. The pilot results from the monitoring of microcystins confirmed us, that the estimated concentration in waters and related health risks can be underestimated due to used methods.

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