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# EFFECT OF INDUSTRIAL EFFLUENTS IN A SMALL RIVER ON FISH AND MOLLUSK DETERMINED FROM MOLECULAR RESPONSES

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Paper industry is considered as one of the main polluters among different kinds of industry in the world. The repeated accidents with mass fish death in the small Ukrainian river (Pryp'yat basin, West Ukraine) were registered during two seasons of 2016 y. The situation was widely discussed in the local periodicals and social network (published only in Ukrainian), and the effluents of cardboard-paper mill (CPM) that is located upstream the river were proposed to be causal factors for these incidents. However, the traditional set of physico-chemical indices of the water was not able to prove this origin. Meanwhile, the expertise of the toxicity of the paper mill effluents is mainly realized by the exposures either *in vivo* or *in vitro* to the extracted effluents during short period [1]. The experience of the analysis of molecular responses in the biota subjected to the effluents in their native surrounding is rather scant. Therefore the aim of this study was to evaluate the validity of molecular biomarkers of stress and exposure to reflect the level and different types of pollution in aquatic animals. This approach is of particular importance in the complex polluted surface waters which contain industrial, agricultural and personal care products at low ppt–ppb concentrations [2, 4].

In this study, the toxicity of the aquatic environment in the small river which is suspected to be polluted by the effluents of the cardboard-paper mill (CPM) was determined. The specimens of fish *Carassius auratus* (Cyprinidae) and mollusk *Unio tumidus* (Unionidae) from the suspected polluted area (SP) and pristine area in

the upper portion of river as control (C) were compared. About 20 individuals of male specimens of fish and mollusks from each site were analyzed. The expertise included a set of biomarkers of general stress and specific effects (neurotoxicity, metal-related toxicity, endocrine disruption) [4]. Some utilised biomarkers were attested only in fish whereas their applying in mollusc is considered as doubtful [3]. The genotoxicity was analysed as DNA stability and nuclear abnormalities. The oxidative injury was assessed from lipid peroxidation level and accumulation of lipofuscin as the end-product of lipid and protein peroxidation in the lysosomes. We determined cholinesterase (ChE) activity in fish as a marker of neurotoxicity, which is oppressed mainly by thiocarbamate and phosphate pesticides, metallothionein concentration (marker of the pollution by toxic metals and stress-related protein), the level of vitellogenin-like protein (Vtg-LP) as a marker of endocrine disruption, which is elevated by environmental oestrogens in male specimens. The suspected endocrine disrupters in CPM effluents are chlorinated organic substances and phenols. The activity of ethoxyresorufin-*O*-deethylase (EROD) as a marker of the microsomal biotransformation of polycyclic hydrocarbons and halogenated aromatic hydrocarbons was measured in the liver of fish. Commonly used for the attesting of water quality chemical parameters were also measured in some points upstream and downstream the location of CPM discharges.

Both fish and mollusk from SP demonstrated plural signs of toxicity and stress: 2.5−3.7 times higher levels of DNA fragmentation and frequency of nuclear abnormalities than in C-groups; low lysosomal membrane stability (in hemocytes of mollusks); higher levels of the lipid peroxidation products (by 2.23 times in the digestive gland of mollusk) and lipofuscin (determined only in fish) in comparison with C-groups. The exposure to the certain xenobiotics was confirmed by *two-* to *threefold increase* of metallothionein levels (response to toxic metals), CYP450-related activity (EROD) and alkaline-labile proteins (responses to endocrine disrupters in male specimens, in particular, to chlorinated organic substances). All these kinds of pollution are typical for the CPM industry. Particularly, even the wastewater treatment can not totally remove this endocrine effect of effluents. Cholinesterase activity in the brain of fish was the same

in the C- and SP-groups proving the low evidence of typical agricultural pollution [van der Oost et al., 2003]. The chemical analysis of water was not so sensitive. These results prove the molecular bioindication to be the most valid approach to assess the toxicity of CPM effluents. Moreover, the comparison with the previous results confirms that this adverse effect is prominently higher than the typical pressure in the agricultural and municipal sites and could be regarded as the consequence of emergency situation [2,3].

To summarize, at the first time, the multi-marker molecular approach of CPM effluents was realized. Only the combination of the indices of stress and specific kinds of pollution allowed distinguishing between CPM effluents and non-pointed sources of pollution typical for the rural area. The further studies of the CPM effluents toxicity must be accented on the environmental monitoring with the elucidation of the specificity of the responses in comparison with the responses to agricultural wastes.

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