Monitoring of drinking water quality at Ferrara city: study of metabolic perturbation in *Cyprinus Carpio*

F. Vivarelli[1], D. Canistro[1], A. Sapone[1], M. Paolini[1]

[1] Dept. of Pharmacy and Biotechnologies – FaBiT, Molecular Toxicology Unit, ALMA MATER STUDIORUM University of Bologna

In the global pollution scenario, a latent, and therefore insidious danger is the presence of toxic chemicals in drinking water, derived, paradoxically, from the disinfection process which is compulsory to protect public health from diseases. In most countries, the surface water is disinfected by chloro-derivatives which react with the dissolved organic matter (humic and fulvic acids), giving rise to the formation of carcinogenic organo-chlorinated by-products. Many of them, however, such as dichloroacetic acid and chloroform are not mutagens and act through epigenetic mechanisms. It is not to be underestimated the risk of gastrointestinal and urogenital tumors related to chronic exposure to these compounds, emerged from epidemiological investigations. This study is aimed to evaluate the possible effects on xenobiotic metabolism of fish (*Cyprinus carpio*) exposed to Ferrara city drinking water. It is known that the up-regulation of cytochrome P450 (CYP450) superfamily is associated with non-genotoxic (epigenetic) outcomes such as co-carcinogenesis (increasing bioactivation) and promotion (overgeneration of reactive free radicals). *C. carpio*, widely distributed in rivers and lakes, was recently considered an effective biomarker recommended by the EU to assess the toxic effects of chemicals in the aquatic environment. Animals were treated daily (3 or 6 consecutive days) intraperitoneally with concentrated drinking water collected from the distribution networks of the city, at 3L/eq dosage. The evaluation of metabolic perturbation has been performed in the hepatopancreas subcellular preparation. Four sampling points were selected: F-raw river water, A-water after treatment, R1 and R2-water from two points chosen along the network. After 3 day treatment, a marked and significant ($p<0.01$) increase in the hydroxylation of p-nitrophenol (p-NFI, up to 330%), O-deethylation of ethoxyresorufin (EROD, up to 400%), at R2 point, as well as O-demethylation of methoxyresorufin (MROD, up to 440%) at the network point A, were recorded. After 6 day administrations, several inactivations were found; in particular, MROD was down-regulated at the network point A up to 68%, $p <0.01$. Significant increases were also observed at R1 and R2 points for p-NFI and O-dealkylation of pentoxyresorufin (PROD), up to 120 and 200%, respectively, $p<0.01$. Using the testosterone as multibioprobe, being hydroxylated in a stereo- and regio-selective way by different CYPs, decreases for almost all the considered monooxygenases were seen after 3 day exposure at R1 point. At point network R2, up-regulations for all monooxygenases (up to 90% for 6β-OHT, $p <0.01$), were observed. Finally, after 6 days of exposure, a general drop for all monooxygenases was seen at F point; on the contrary, significant ($p <0.01$) increases for 16α- and 2β-OHT at the network point A (up to 200 and 215 %, respectively) and point R2 (380 and 116%, respectively) were registered. These data indicate that complex chemical mixtures may simultaneously determine inductive and/or inactivating effects to different CYPs. If reproduced in humans-after lower dose administration and longer exposure period-such effects can alter endogenous metabolism (e.g., biosynthesis of bile acids, corticosteroids, fatty acids) and cellular function (e.g., cellular homeostasis, differentiation, apoptosis, neuroendocrine functions) where these catalysts are physiologically involved. Furthermore, the epigenetic implications could explain the increased tumor stated by observational studies. Our results can contribute to the definition of experimental approaches to supplement the control measures already provided for water intended for human consumption, and as a possible criterion for assessing the quality of water.