

Neural Network Patterning and Biochemical Analysis of Fish Behavior as a Biomonitoring System for Detecting Environmental Pollutants

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Copper is a constituent of dopamine- β -hydroxylase, a critical enzyme in the catecholamine biosynthetic pathway. Copper binding proteins play important roles in the establishment and maintenance of metal-ion homeostasis, in deficiency disorders with neurological symptoms (Menkes disease, Wilson disease) and in neurodegenerative diseases (Alzheimer's disease). Copper accumulation is organ- and species-specific in fish, as well as highly dependent on the water quality in which the copper exposure occurs. Accumulation of copper has also been seen in gill and kidney tissue of brown bullhead catfish, bluegill, and coho salmon, as well as in the gonadal tissue of the white sucker. Fluoranthene, a common polycyclic aromatic hydrocarbons (PAHs), exhibits phototoxicity which may affect aquatic organisms. Methodology was developed for quantifying the photocytotoxicity of fluoranthene to a gill cell line from rainbow trout for future use in screening polycyclic aromatic hydrocarbons for their relative photocytotoxicity to fish. However, there have been little reports showing behavioral aspects of fish affected by these common toxic chemicals in the environment. The objective of this study is to develop a biomarker used to monitor abnormal behaviors of Japanese medaka (*Oryzias latipes*) as a model organism caused by hazardous chemicals that are toxic and persistent in the ecosystem.

Each fish was subjected to fluoranthene and copper treatment after starvation for 24 hr. Fish behaviors were observed on a real time basis

using image processing and automatic data acquisition systems. Acetylcholine esterase (AChE) and monoamine oxidase (MAO) activities were assayed for examination of biochemical aspects of the toxicity of the chemicals. For a localization of tyrosine hydroxylase (TH) enzyme expression in tissues, avidin-biotin complex method was used. The untreated individuals showed common behavioral characteristics (i.e., smooth and linear movements with small curvatures). The treatment of fluoranthene (1 ppm), however, caused a significant difference in movement: linear back-and-forth movement in horizontal direction (2-3 cm in distance). By the way, the copper treatment (1 ppm) dramatically increased the vertical movements of the fish. The exposure of medaka fish to copper solution (100-1000 ppb) did not affect acetylcholine esterase activities in both head and body portions. However, cephalic monoamine oxidase, which is involved in catabolism of catecholamine neurotransmitters, was significantly induced by the copper treatment, which might be partially responsible for abnormal behavior of the medaka fish. Treatment of the medaka fish with fluoranthene caused a significant suppression of AChE and MAO activities in the body. The eccentric swimming pattern of medaka fish induced by fluoranthene might be associated with alteration neurotransmitter-metabolizing enzymes. These results may provide biochemical and neurobehavioral bases of a biomonitoring system for such as copper and fluoranthene using a model organism such as fish.