A study on behavior of Japanese medaka (Oryzias latipes) as a biomarker to organophosphorus pesticides exposure

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論文題目　：**A study on behavior of Japanese medaka (*Oryzias latipes*) as a biomarker to organophosphorus pesticides exposure**

（魚類の行動に及ぼす有機リン剤の影響に関する研究）

論　文　内　容　の　要　旨

As organophosphorus pesticides (OPs) are widely applied in agricultural and urban systems, a tool is required to monitor and predict their impact on fish and natural ecosystems. Behavioral tests have thus been developed as a new ecological tool for assessing the risk of contaminants on organisms. Using these tests, this study was performed to estimate the risk of the OPs chlorpyrifos (CPF) and dichlorvos (DDVP) on the behavior (swimming, feeding and social) and acetylcholinesterase (AChE) activity of the Japanese medaka (*Oryzias latipes*).

In an acute exposure test, medaka were exposed to 0.018, 0.055, and 0.166 mg L^{-1} of each concentration for 96 h. The LC50 of CPF after 96 h was 0.12 mg L^{-1}, and fish showed hypoactivity (decreased swimming speed) when compared with the control. Swimming speeds reduced to 55.6%, 39.0 %, and 27.3% of the control at exposure concentrations of 0.018, 0.055, and 0.166 mg L^{-1}, respectively. Brain AChE activity and swimming speed were significantly correlated. In a feeding behavior test, the CPF 96 h LC50 (0.12 mg L^{-1}) stopped medaka feeding after 24 h and 48 h exposure, with inhibited swimming speed and brain AChE activity after 48 h exposure. Thus, I suggest that hypoactivity may be a sensitive biomarker of acute exposure of fish to CPF.

In a subacute test, medaka were exposed to 0.012 mg L^{-1} CPF (10% of LC_{50}) for 8 days, and behavior was then recorded for 30 min. During this period, the frequency of social behavior patterns, classified as schooling, shoaling, and solitary, were recorded. Swimming speed was also measured. Fish showed hyperactivity, but AChE activity decreased to 68% of the control. Fish also exhibited significant alterations in their social behavior (increased schooling duration and decreased solitary duration). Thus, hyperactivity and increased schooling behavior may be sensitive biomarkers for identifying sublethal CPF contamination.

To confirm that hypoactivity and hyperactivity occurred in response to different conditions of behavior recording, the medaka were then exposed to CPF at 0.12 and 0.012 mg L^{-1} for 12 days. After 4 days of acute exposure to 0.12 mg L^{-1}, the medaka were hypoactive with a decrease in social behavior (schooling and shoaling). In contrast, fish exhibited hyperactivity and increased schooling duration after 8 days sublethal exposure to 0.012 mg L^{-1}. This was followed by normal activity, and decreased schooling duration on day 12. Thus, using single behavioral responses as biomarkers for OP contamination may give inaccurate results.

In a final test, the LC50 of 24 h exposure to DDVP was found to be 26.2 mg L^{-1}. Medaka were then exposed to 1 and 5 mg L^{-1} DDVP for 96 h. At 5 mg L^{-1}, DDVP significantly decreased schooling and shoaling frequency. Additionally, solitary behavior frequency was significantly lower in the groups exposed to 1 and 5 mg L^{-1} DDVP, compared with the control group. However, shoaling and schooling behavior were not affected by exposure to 1 mg L^{-1} DDVP. The swimming speed of medaka exposed to 5 mg L^{-1} also decreased (hypoactivity). However, 1 mg L^{-1} caused no effect on swimming. Thus, I suggest that sublethal exposure to DDVP may induce hypoactivity and decrease schooling in medaka.

Based on the obtained behavioral responses of medaka to OPs exposure, I suggest that behavior may be used as a sensitive biomarker for predicting the ecological risk of OPs in a natural ecosystem. In addition, inhibition of AChE activity might also be an early warning of OPs exposure.