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

## Summary

Agrochemicals, especially pesticides, play an important role in the high productivity achieved in agriculture through the control of pests. However, the release of these substances into the environment has serious environmental consequences

The results of this study indicated that OPs (namely chlorpyrifos (CPF) and dichlorvos (DDVP)) may pose risks to Japanese medaka (O. latipes) that are mediated by serious behavioral (swimming, feeding and social) and biochemical alterations (AChE). Accordingly, behavior may serve as a useful biomarker for prediction of the effects of OPs on fish at the population level.

As reported in chapter 2, CPF 96 h LC50 was found to be 0.12 mg L-1 and hypoactivity was observed. In the acute exposure test, fish showed hypoactivity relative to the control, with exposure to 0.018, 0.055, and 0.166 mg L–1 inducing swimming speeds that were 55.6%, 39.0%, and 27.3% of those of the control. Moreover, brain acetylcholinesterase activity and swimming speed were significantly correlated. In addition, in the feeding behavior test, CPF 96 h LC50 (0.12 mg L-1) stopped medaka feeding after 24 and 48 h of exposure, with inhibited swimming speed and brain AChE activity occurring after 48 h of exposure. These results suggest that hypoactivity may be used as a sensitive biomarker to acute exposure of fish to CPF.

As reported in chapter 3.1, increased swimming speed and schooling duration and decreased solitarity were observed in medaka exposed to sublethal levels of chlorpyrifos (Table.5.1). Sublethal exposure to 0.012 mg L–1 chlorpyrifos (10% of LC50) for 8 d resulted in fish hyperactivity, whereas acetylcholinesterase activity was decreased to 68% of that of the control. In addition, fish exhibited significant alterations in social behavior (schooling duration increased and solitary duration decreased). Taken together, these findings indicated that hyperactivity and increased schooling duration may be sensitive biomarkers to sublethal CPF contamination (Table.5.1)

The data presented in Chapter 3.2 confirmed the results reported in chapter 2 and 3.1. Medaka became hypoactive and social behavior (schooling and shoaling) was decreased after 4 d of acute exposure to 0.12 mg L-1. Conversely, fish exhibited hyperactivity and increased schooling duration after 8 d of sublethal exposure to 0.012 mg L-1 followed by normal activity, and decreased schooling duration on day 12. Thus, using a single behavioral response as a biomarker of OPs contamination may be not accurate. Indeed, complex behavioral response is likely the best tool for studying the effect of contaminants as well as their mechanism of action. In addition, behavioral and biochemical alterations in response to CPF exposure were concentration and time dependent.

The condition of recording may influence the behavioral response to OPs. In chapter 2 and 3.1, 0.018 mg L-1 and 0.012 mg L-1 were found to be sublethal concentrations to medaka, respectively; however, they induced different behavioral alterations: namely, hypoactivity (0.018 mg L-1 on day 4) and hyperactivity (0.012 mg L-1 on day 8). These different responses might have been due to different conditions of exposure, recording or measurement of swimming speed in the two experiments. The swimming speed of individual fish was recorded in the acute test, whereas in the subacute test swimming speed was measured in a group setting (six fish). Swimming in a group may enhance swimming activity.

In chapter 4, 24 h DDVP LC50 was recorded to be 26.2 mg L-1. In addition, sublethal exposure to DDVP at 5 mg L-1 decreased schooling and solitary frequency, as well as swimming speed (hypoactivity).

The observed behavioral alterations of medaka in response to OPs exposure suggest that behavior may be used as a sensitive biomarker for prediction of the ecological risk of OPs to the natural ecosystem. Alterations in swimming, feeding and social behaviors, especially schooling, may retard fish growth and reproduction as well as threaten fish survivability at the population level.

AChE inhibition may also be used as an early warning of OPs contamination. AChE assay is quicker and easier than behavioral based measurements. However, correlation between behavioral and biochemical endpoint is essential to understanding and interpretation of behavioral response to OPs. In chapter 3.2, sublethal exposure to CPF (0.012 mg L-1) for 8 days (inhibition of AChE activity to 64.3% of control) was found to cause hyperactivity. However, 0.12 mg L-1 (inhibition of AChE activity to 37.3% of the control) resulted in hypoactivity. These results may be attributed to the action of anticholinesterase agents that can evoke hyperactivity with low levels of AChE impairment and hypoactivity as impairment increases. In conclusion, the reported behavioral alterations in this study reveal that behavior is a useful biomarker for assessment and prediction of the ecological risk OPs pose to the natural ecosystem. Moreover, AChE activity might be an early warning of OPs contamination.