Inhibitory Effects on Esterase Enzymes Buche and Ache in Rainbow Trout (Oncorhyncus mykiss) Produced by the Slow Release Insecticide Chitosan Diethyl Phosphate

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The study on the toxicity of chitosan diethyl phosphate (ChDP), a controlled release insecticide, on the activities of butyrylcholinesterase (BuChE) and acetylcholinesterase (AChE) in rainbow trout exposed to this pesticide was carried out. It was found that ChDP reduced BuChE activity in O. mykiss by a factor of eight at 6 days, with high fluctuation to the end of the exposition time at 12 days. The in vitro analysis of brain AChE treated with ChDP and Phenamiphos showed that it was competitively inhibited by both organophosphates. The values obtained for $K_m$ and $V_{max}$ for the AChE-ChDP ($K_m$: 21.23 µM; $V_{max}$: 43.10 µmol/min/g) and AChE-Phenamiphos ($K_m$: 38.62 µM; $V_{max}$: 38.91 µmol/min/g) systems were relatively low compared to values of the AChE (control) system ($K_m$: 62.99 µM; $V_{max}$: 63.29 µmol/min/g). Results reported in this study confirmed that chitosan diethyl phosphate performs similarly to organophosphate pesticides, producing inhibition in cholinesterases in rainbow trout.

Key Words: Esterase enzymes; Rainbow trout; Chitosan; Organophosphorous; Toxicity.

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INTRODUCTION

Organophosphorous pesticides began to replace the organochloride insecticides for pest control in the 1950s. Conventional formulations of these compounds produced undesirable side effects such as loss to the atmosphere through evaporation, photochemical degradation, and most seriously, migration of the active agent into nontarget areas. These factors resulted in applications of ever larger doses of the pesticides to achieve desired pest control, but produced problems of environmental pollution.

The characteristic effect of the organophosphorous pesticides is inhibition of esterase activity, for example, that of butyrylcholinesterase (BuChE: E.C. 3.1.1.8) and acetylcholinesterase (AChE: E.C. 3.1.1.7). The inhibition of these enzymes is due to phosphorylation of the serine hydroxyl group at the active site. BuChE commonly occurs in the blood plasma, pancreas, heart, and liver of vertebrates; it does not have a neurotransmitter function. One of the functions of this enzyme is detoxification of organophosphate compounds, for which it has been considered as a protective buffer for AChE.

AChE is abundant in vertebrates and invertebrates and is of major importance as a neurotransmitter by means of hydrolysis of acetylcholine (ACh). The inhibition of this esterase is indicated by the accumulation of ACh in nervous systems of organisms, e.g., brain in mammals. Studies of this enzyme in brain and nervous tissues have produced reliable methodologies, which may be applied for the detection of water pollution by organo( phosphates/thiophosphates). Measurements of BuChE concentrations in the blood plasma of fish and birds have been used to indicate sublethal levels of exposure of these organisms to the pesticides.

Numerous acute toxicity bioassays of organophosphorous pesticides have been reported. In order to mitigate environmental problems caused by applications of high concentrations of conventionally used organophosphorous pesticides, formulations have been introduced for slow release of these substances. These pesticide formulations are inert in bulk form and allow release over time of biologically active pesticidal moieties. These pesticide formulations typically include the pesticide moiety bonded to a synthetic or natural polymer. Two formulations of this type include chitosan alkyl carbamates and chitosan diethyl phosphate (ChDP), synthesized by our group. Chitosan (poly-β-D-(1-4)-glucosamine) is a naturally occurring polysaccharide mainly found in the fungal cell wall.

In a previous work it was described from acute toxicity assays, i.e., *Daphnia pulex* and Microtox assays, that ChDP was less toxic than conventional pesticides. It was also observed from rainbow trout sublethal assays that ChDP, at concentrations as high as 20 mg/L, produced alterations to biochemical and cytological levels, but caused no mortality during the assays.