ICES Statutory Meeting 1993

ICES Marine Environmental Quality Committee

C. M. 1993/E:13

SCREENING OF ORGANOPHOSPHATE AND CARBAMATE PESTICIDES BY CHOLINESTERASE INHIBITION

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Abstract

The acetylcholinesterase inhibition test from Boehringer (Mannheim) was used for the determination of organophosphate and carbamate pesticides in sea water and sediment samples from the Belgian coast, the open sea and the Scheldt. The applicability of the test for use with sea water samples was tested. The lower detection limit was judged almost similar to the value (approx. 0.05 µg paraoxone l^{-1}) reported by Boehringer. No acetylcholinesterase inhibition could be detected with the method used.

Introduction

Acetylcholinesterase (acetylcholine acetylhydrolase ; EC 3.1.1.7. ; AChE) terminates the action of acetylcholine, after it had served as a neurohumoral transmitter in nervous function. The activity of the enzyme is sensitive to a number of molecules of economical, pharmacological or military interest

(Heath, 1961 ; Hart and O'Brien, 1974). These inhibitors include organophosphorus compounds, carbamates and methane sulfonates. The usefulness of these compounds as pesticides (organophosphorus compounds and carbamates (Aldridge and Reiner, 1972 ; Wang and Murphy, 1982 ; McHenery et al., 1991)), as medical drugs (carbamates (Green, 1983 ; Giacobini et al., 1987 ; Taylor, 1980)) and as chemical weapons (organophosphates (Robinson, 1971)) arizes from their ability to irreversibly inhibit AChE (Liu and Tsou, 1986). A large number of reviews have dealt with AChE : Rosenberry (1975), Brimijoin (1983), Rosenberry (1985), Silman and Futerman (1987) and Chatonnet and Lockridge (1989).

The use of AChE as an analytical tool in environmental quality assessment prompted Boehringer (Mannheim, Germany) to develop a colorimetric screening test for insecticide determination in water. This test was used in our study to monitor AChE inhibitors in water and sediment from the Belgian Continental Shelf and the Scheldt.

Determination of Acetylcholinesterase was made mandatory by the Flemish governmental regulations on environmental quality control (Anon., 1992). The US Environmental Protection Agency (1973) recommended a safety margin of 1/20 of LC_{50} values.

Materials and methods

Samples

Water and sediment were sampled along the Belgian coast, in the Scheldt and in open sea (fig. 1). 4 sites in open sea are dredge dumping sites (stations 140, 700, 710, 780). The samples were stored frozen prior to extraction and analysis. The sampling period was May and June 1992.

Determination of cholinesterase inhibition

The cholinesterase inhibition test for screening of AChE inhibitors in water samples (Boehringer Mannhein, Germany) was used without modification. Sediment samples were treated as follows : 100 g sediment was extracted with 120 ml sea water or 100 ml dichloromethane. Extractions were performed by shaking (125 rpm) during 24 hrs. The sea water extract was then filtered through a paper filter and treated further like the water samples. The extracts with dichloromethane were filtered (Whatmann n° 2) and gently evaporated to near dryness in a rotary evaporator (vacuum, < 40 °C). The remaining solvent was evaporated to dryness under a stream of nitrogen. The residue was then treated as described by the cholinesterase inhibition test. Ethyl-paraoxone (Promochem, Wesel, Germany) was used as a test control. This pesticide was dissolved in sea water prior to use to avoid precipitation.

Results and discussion

The applicability of the test for use with sea water samples was tested by comparing the AChE activity values yielded by pure water, seawater and preparations with paraoxone. The lower detection limit was judged almost similar to the value (approx. 0.05 µg paraoxone l^{-1}) reported by Boehringer. Galgani and Bocquene (1989) reported respective detection levels of 0.1 µg l^{-1} and 0.01 µg l^{-1} for carbaryl and diethyl p-nitrophenyl phosphate by cholinesterase analysis. Gas chromatographic analysis revealed a lower detection limit of 0.02 µg l^{-1} for dichlorvos (Tully and Morrissey, 1989). No AChE inhibition could be detected with the method used. This was expected for the sea water samples and the sediment extracts with sea water since precipitation of the pesticides is obvious because of the low salt solubility of these compounds, depending on the nature of the side chains. As

a consequence one might expect the organophosphate and carbamate distribution, if present by runoff, to be restricted to sediment of coastal areas and estuaries of rivers. This study indicates that these pesticides are not likely to persist in the Southern North Sea and presumably not in the whole area, except locally in areas with intensive fish farming activity where organophosphates (e.g. dichlorvos) are used as parasitic control agents. Nevertheless Tully and Morrissey (1989) reported only trace levels of dichlorvos (0.001 to 0.02 $\mu g\ l^{-1}$) in water and sediment, sampled near salmon farms in Beirtreach Bui bay in the west of Ireland. The authors did not observe a cumulative increase in dichlorvos levels and concluded that dichlorvos dissipates quickly after release. Major dissipation processes are presumably volatillization (Maguire, 1991) and microbial degradation. Colwell et al. (1989) observed enhanced bacterial growth in media containing phosphorothiolate pesticides as a sole source of carbon and phosphorus. Although AChE is specifically and almost uniquely inhibited by organophosphate or carbamate pesticides the previous reported observations raise questions on the interpretation of AChE inhibition in marine biota whether the inhibition is caused by organophosphate or carbamate pesticides or by other substances. In this context the role of the lipid membrane environment in mediating membrane-bound AChE activity should be investigated because pollutants interrupting the membrane structure indirectly might inhibit the enzyme. No evidence pointing in that direction was obtained by now. Recent investigations by Spinedi et al. (1989 ; 1991) suggested that human erythrocyte AChE activity is not sensitive to changes of membrane physical state.

Despite the high sensitivity of the method used, sublethal to lethal concentrations of organophosphate pesticides of only a little higher than the

lower detection limit have been reported for some aquatic species. 0.1 µg l^{-1} dichlorvos is in the lethal range for the freshwater crustaceans *Daphnia pulex* and *Simnocephalus serratus* (Sanders and Cope, 1966) and exposure of maturing juveniles of the estuarine mysid, *Mysidopsis bahia*, to 166ng l^{-1} fenthion postponed the onset of reproduction by 4 d (McKenney, 1986). Upgrading the extraction procedure and replacing bovine erythrocyte AChE by a more sensitive one (e.g. from bees head) are two possibilities to increase the sensitivity.

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Fig. 1. Sampling locations of water and sediment.