Bull. Environ. Contam. Toxicol. (1990) 45:243–249
© 1990 Springer-Verlag New York Inc.

Bulletin of Environmental Contamination and Toxicology

In Vitro Inhibition of Acetylcholinesterase from Four Marine Species by Organophosphates and Carbamates

François Galgani and Gilles Bocquene

Laboratory of Biochemistry, BP 1049, Institut Français de Recherche pour l'Exploitation, de la Mer/DERO/MR, 44037 Nantes, France

The literature on the biological, physical, and pharmaceutical chemistry of cholinesterase (Ache). Give E.C. number is considerable and includes data on activators and inhibitors. Most of the work on specific anticholinesterasic agents has been concerned with carbamates and organophosphates. Many chemicals of these classes are used as pesticides or other types of biocide and many have thus become environmental contaminants (Coppage 1977 ; Verma et al. 1979). Because of the sensitivity of acetylcholinesterase to carbamates and organophosphates, the enzyme has been used as a biochemical indicator of pollution by these agents (Coppage and Braidech, 1976). However, the chemical reactivity of such chemicals has not been correlated with their effect on Ache and it is impossible to accurately predict biological effects based only on structure.

The objectives of this study were to investigate the sensitivity of various marine animals to both organo-phosphates and carbamates. The study was conducted by assessing the in vitro effect of five organophosphates and three carbamates on acetylcholinesterase activity from the muscle of the shrimp Palaemon serratus, the fishes Scomber scomber and Pleuronectes platessa, and from the whole mussels Mytilus edulis. All these species could be used for the monitoring of effect of pollutants.

MATERIALS AND METHODS

Mussels (Mytilus edulis), plaices (Pleuronectes platessa), mackerels (Scomber scomber) and shrimps (Palaemon serratus) were obtained alive from the local market (Nantes, France) and used immediately for extraction.

Tissues (muscle for all species except Mytilus edulis for which the whole animal was used) were suspended in a Tris 0.1M pH 8 buffer (2/1 V/W) and homogenized for 1 min using an ultraturrax. Then the ultraturrax extract was centrifuged at 25,000 g and the supernatant filtered through a millipore membrane (0.45 µ) and stored for weeks at - 20°C before use as enzyme solution.

Send reprint requests to F. Galgani, IFREMER—rue de l'Ile d'Yeu, 44037 Nantes, France.

The method of Bradford (1976) was used for quantitative determination of protein using bovine serum albumin as the standard. Assays were performed in microplate readers as described previously (Galgani & Bocquene, 1988).

The method used to measure acetylcholinesterase activity in tissues has been described by Ellman (1961) and adapted to microplate readers by Galgani & Bocquene (1988). In a typical assay, 300 µL of 0.1M Tris pH 8 buffer, 20 µL Dithiobis Nitrobenzoic Acid 0.01 M, 10 µL of enzyme solution and 10 µL 0.1M Acetylthiocholine (ACTC) as substrate were successively added.

Send reprint requests to F. GALGANI at the above address.
Inhibition studies were conducted as follows : in the wells containing buffer and DTNB. The enzyme (10 µL) extract were added and preincubated 40 min with 10 µL of 100 % ethanol containing various concentrations of inhibitors. Enzymatic reaction was started with addition of substrate and the activity was measured following the variation of optical density in a Mcc 340 microplate reader (flow laboratory). Four measurements were performed for each assay. One enzymatic unit is the variation of 0.001 unit optical density per min.

The following reagents were used : ACTC, Dithiobis Nitrobenzoic Acid, Dinitrophenyl phosphate (DNP, Paraoxon), eserine (physostigmine) and neostigmine were from Sigma Chem. Co., Carbaryl was from La Littorale (Rhone Poulenc, France) ; and Malathion, Parathion, DDVP and Temephos were from Nanogen (Interchim, France). All other reagents were of analytical grade.

RESULTS AND DISCUSSION

Acetylcholinesterase was found in the four species Palaemon serratus, Mytilus edulis, Pleuronectes platessa, and Scomber scomber. The values of specific activities are given in Table 1.They were found to be high in all species except Mytilus edulis.

Figure 1 shows the effect of different organophosphates on the activity of the enzyme in the four species. Using malathion as inhibitor, no effect was found in invertebrate species. While parathion inhibits statistically significant the enzyme from Palaemon serratus, experimentation conducted with fishes shows that those species are more sensitive to those inhibitors. However, in all cases studied, the level of inhibitor that was necessary to affect the enzyme activity was in the mg/L range. Experiments conducted with paraoxon (DNP) shows that this inhibitor strongly affects the enzyme from all species except for mussel for which no inhibiting effect was observed. The detection level observed for this compound was in the µg/L range. For Pleuronectes platessa, a very effect was observed since about only 10 % of activity was recovered after incubation with 1.3 ppb of Paraoxon (DNP).

Table 1. Specific activity of acetylcholinesterase in four marine species.

	Soluble protein (mg/mL extract)	Ache (*) (units/mg protein)
Mytilus edulis (whole animal)	9.9	345 ± 48
Palaemon serratus (muscle)	10.33	8115 ± 285
Pleuronectes platessa (muscle)	10.64	13252 ± 751
Scomber scomber (muscle)	11.05	1422 ± 142

(*) mean value and SD for four assays

Increases of inhibitory effect of Parathion after oxidation that occurs in the environment or after metabolism (Picot Leflond, 1983) could be an interpretation of this result.

For other organophosphates, such as Temephos and DDVP, an inhibition was observed. However, the level of inhibitor that affected enzyme activity was higher than for DNP and corresponded to concentrations never found in water. Species variation in the susceptibility of acute poisoning by organophosphates have been well documented. Murphy et al. (1968) found that the acute toxicity of Paraoxon amoung species followed this order of sensitivity : avian > mammals > piscine. In our study, we found that acetylcholinesterase from whole mussel was less sensitive to organophosphates than the enzyme from Palaemon serratus and fishes. For all organophosphates studied, sensitivity was highest for Pleuronectes platessa. An increase of sensitivity to organophosphates according to the degree of systematic evolution can also explain our results.

Sensitivity to carbamates is somewhat different. For carbaryl, inhibition was maximum in Palaemon serratus and a loss of activity was observed with inhibitor concentrations lower than 0.1 mg/L. Other carbamates such as neostigmine and physostigmine also decreased the acetylcholinesterase activity. In this case, fishes were found to be more sensitive with detection levels under 10 µg/L in both Pleuronectes platessa and Scomber scomber.

In vitro studies have indicated that animals from different classes have widely different species sensitivities to organophosphates and carbamates (Weng & Murphy 1982). Furthermore,the rank order of toxicity differed in different classes of animals. For some insecticides, species differences in the reactivity of

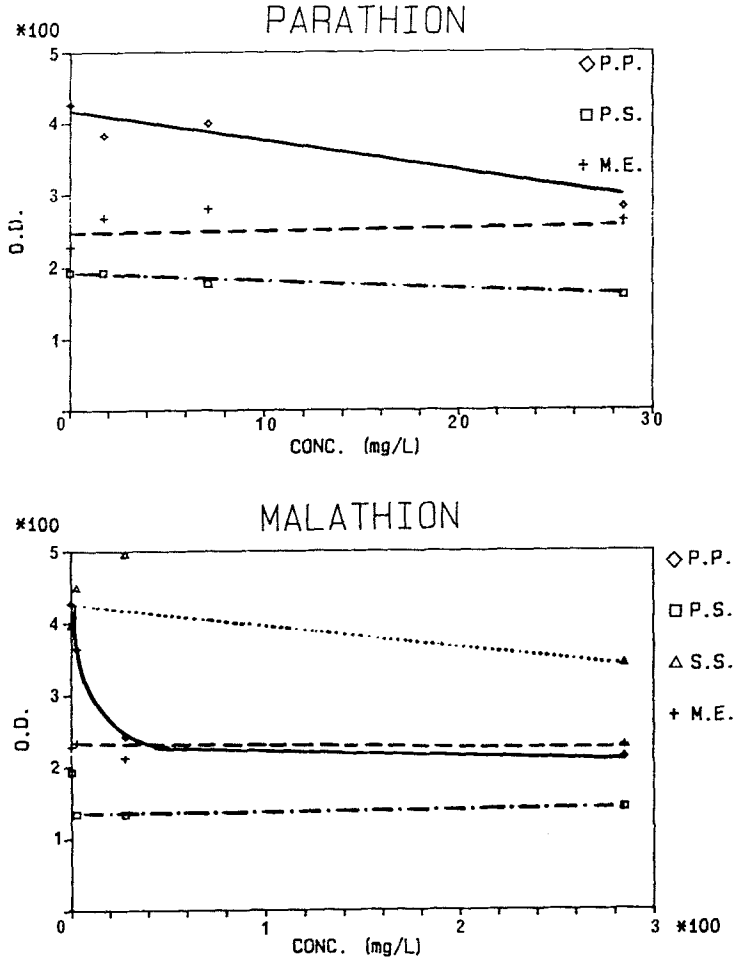
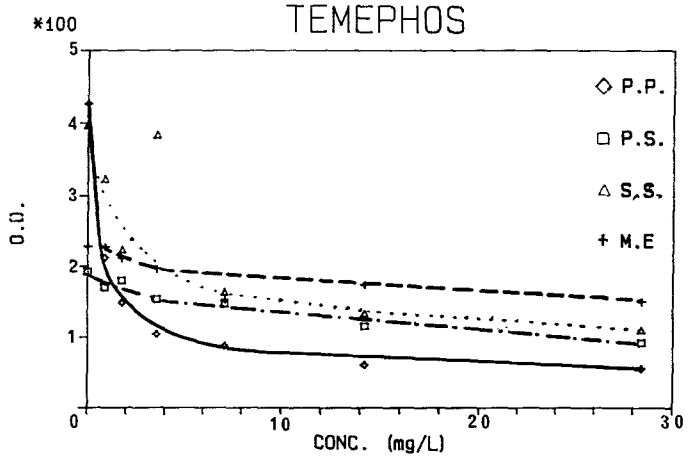
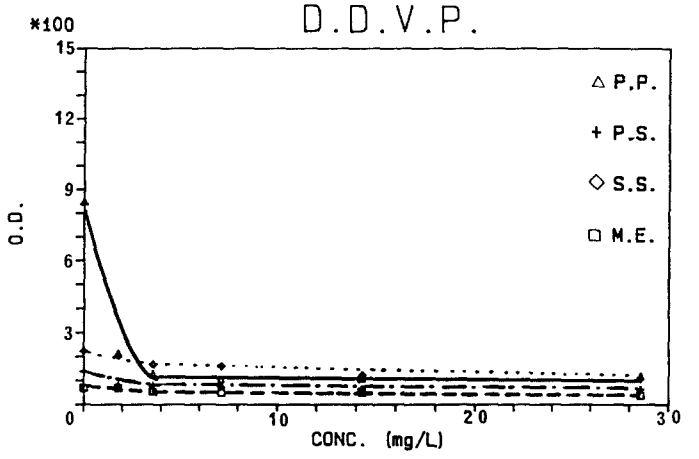


Figure 1 - Effect of organophosphates on the activity of acetylcholinesterase from Palaemon serratus (P.s.), Mytilus edulis (M.e.), Pleuronectes platessa (P.p.) and Scomber scombrus (S.s.) experiments were conducted as described in materials and methods.


TEMEPHOS
*100
O.D.
CONC. (mg/L)
◇ P.P.
□ P.S.
△ S.S.
+ M.E


D.D.V.P.
*100
O.D.
CONC. (mg/L)
△ P.P.
+ P.S.
◇ S.S.
□ M.E.

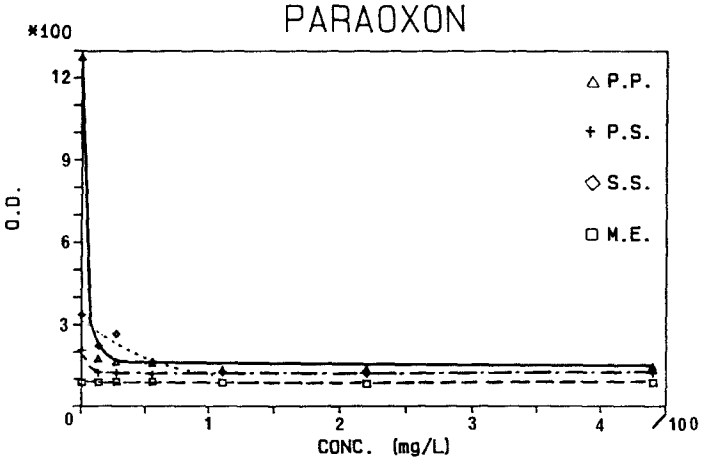

PARAOXON
*100
O.D.
CONC. (mg/L)
△ P.P.
+ P.S.
◇ S.S.
□ M.E.

Figure 1

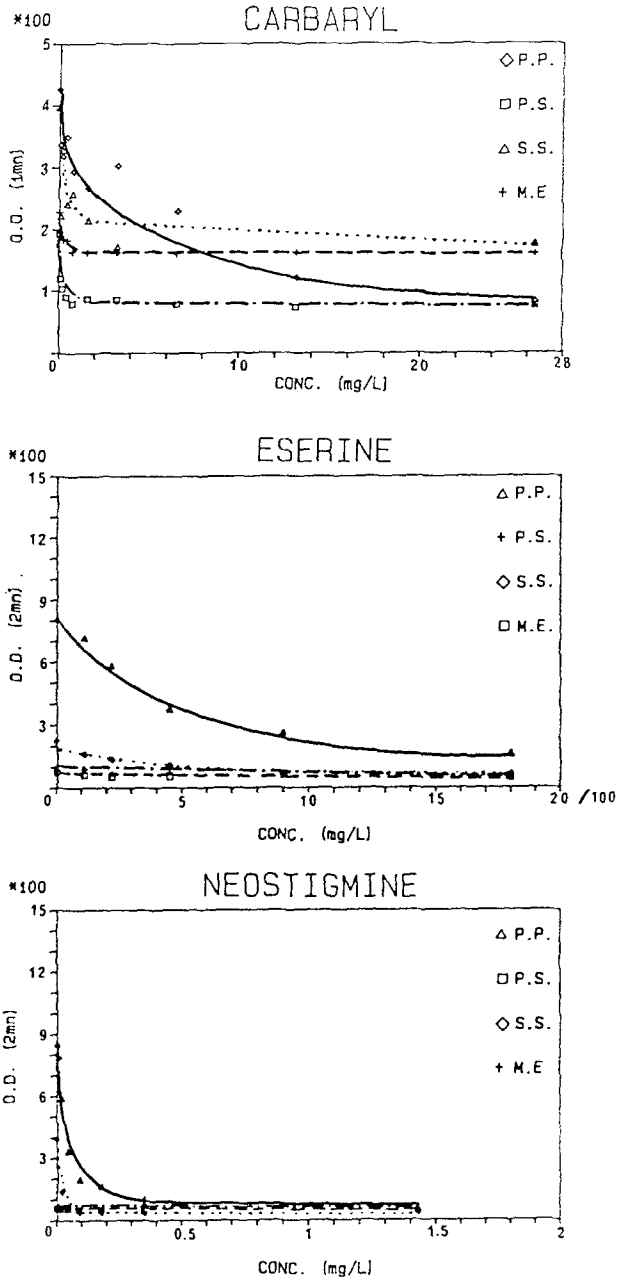


Figure 2 - Effect of carbamates on the activity of acetylcholinesterase from Palaemon serratus (P.s.), Mytilus edulis (M.e.), Pleuronectes platessa (P.p.) and Scomber scombrus (S.s.) experiments were conducted ad described in materials and methods.

organophosphates. For other species, basic work is needed concerning the interspecificity of effect of organophosphates and carbamates and also the comparative sensitivity of acetylcholinesterase to understand the mode of action of pesticide in marine animals.

Acknowledgments : The authors are grateful to P. Truquet and L. David for figures and typescript. We also thank D. Cossa for reviewing the article.

REFERENCES

Bradford M (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dyebinding. Anal Biochem 72 : 248-254.

Coppage D.L (1972) Organophosphates pesticides specific brain AChEinhibition related to death in sheepshead minnows.Trans Am FishSoc 101 : 534-536.

Coppage D.L, Bradeich (1976) River pollution by anticholinesterase agents. Water Res. 10 : 19-24.

Ellman G.L, Countney K.D, Andreas V.J, Featherstore R.M (1961) A new rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 7, 88-95.

Galgani F, Bocquene G, (1990) Semi automated colorimetric and enzymatic assays for oceanographic samples using microplates readers. Water Res., In Press.

Murphy S, Lawwerys R, Cheever K, (1968) Comparative anticholinesterase action of organophosphates insecticides in vertebrates. Toxicol Appl Pharmacol 12,22-35.

Picotleflond C, (1985) Acetylcholinesterase : inhibiteurs irréversibles et réactivateurs. PhD Thesis University of Lille, France.

Verma S, Tyagi A, Bhatnagar M, Dalela R, (1979) Organophosphate poisoning to some fresh water teleosts. Bull Environ Contam Toxicol 21, 502-506 (1979).

Wang C, Murphy S, (1982) Kinetic analysis of species differences in acetylcholinesterase sensitivity to organophosphate insecticides. Toxicol Appl Pharmacol 66, 409-419.

Winners W, Neff J, Wachtendonk von (1978) Distribution of cholinesters and cholinesterases in hémolymphs and smooth muscles of molluscs. Comp Biochem Physiol 61 c, 121-131.
Receved February 15, 1989 ; accepted September 15, 1989.