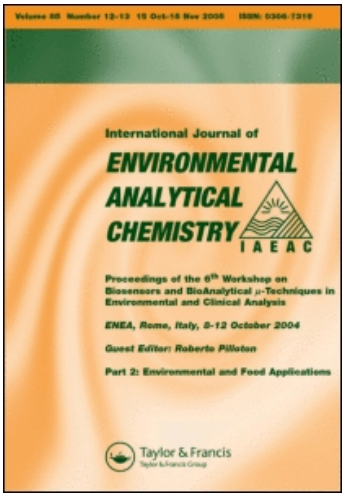

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Measurement of Bimolecular Rate Constants k_i of the Cholinesterase Inactivation Reaction by 55 Insecticides and of the Influence of Various Pyridiniumoximes on k_i

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MEASUREMENT OF BIMOLECULAR RATE CONSTANTS k_i OF THE CHOLINESTERASE INACTIVATION REACTION BY 55 INSECTICIDES AND OF THE INFLUENCE OF VARIOUS PYRIDINIUMOXIMES ON k_i

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The cholinesterase (ChE) inhibition test is a biochemical method for screening insecticides. The possibilities for the detection of different cholinesterase inhibiting substances were investigated.

The inactivation abilities against cholinesterases from 4 different biological origins were measured. General rules for detection and discrimination of insecticides were derived from the bimolecular rate constants k_i of 55 relevant organophosphorus compounds, carbamates and carbamate metabolites. The thionophosphates were converted into the more powerful inhibiting oxones by oxidation with N-bromosuccinimide.

The inactivation values k_i differ in some cases up to 6 orders of magnitude for different insecticides and 3 orders of magnitude for different enzymes.

The inactivation of acetylcholinesterase (AChE) by organophosphorous compounds can be suppressed by reactivation with obidoxime. On the other hand, an acceleration of inactivation of butyrylcholinesterase (BChE) by carbofuran and certain other carbamates was observed under the influence of bispyridiniumoximes.

KEY WORDS: Cholinesterase; insecticides; bispyridiniumoximes, differentiating detection; oxidation; water analysis.

INTRODUCTION

Pesticides are a common danger for the sources of drinking water (surface or groundwater)[1,2]. There is a great need for analytical methods suitable to detect a large number of those water constituents, being limited in the Regulations of the European Community and meanwhile also in the new German Drinking Water Regulation[3]. These methods must be sensitive enough to estimate concentrations of less than 0.1 $\mu g\, l^{-1}$.

The application of bioanalytical screening methods is an interesting possibility for detecting pesticides in water. In laboratories where large numbers of samples must be processed rapidly, fast screening methods with sufficient selectivity and sensitivity are requested.

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A cholinesterase (ChE)* inactivation reaction is often utilized for sensitive detection of organophosphorus and carbamate insecticides[4–12,19,30,31]

The combination of TLC with the ChE inhibition test is one possibility to distinguish between the active substances according to the R_f-values[13–15]. Quantification of inactivation, i.e. determination of enzyme activity as a function of the time of inactivation (=: inhibition time), could not be realized by this chromatographic method. However, a common and exact way of expressing the inhibitory power of an insecticide for a ChE is the bimolecular rate constant, k_i, of the ChE inactivation reaction[16–19]. By measuring the kinetics of the inactivation of reversibly acting[20–26] (not covalently bound) inhibitors, which cause a disturbance of any ChE inhibition test, and irreversibly acting inhibitors both can be distinguished.

The reactivating effect of obidoxime[27–29] is utilized for discrimination of carbamates and organophosphorus compounds[10,31]. A general method for identification of inhibitors like thionophosphates, e.g. parathion, is the conversion into the more powerful inhibiting "oxones" like paraoxon by oxidation[30,31].

Oxidation with bromine and reduction of the oxidant with $Na_2S_2O_3$, as described[30,31], has led sometimes to a disturbance of the enzymatic inhibition test[32]. A slight excess of bromine has caused a significant inhibition of ChE. An excess of $Na_2S_2O_3$, on the other hand, has led to a disturbance of the enzymatic optical test: The variation of extinction per time unit ΔE min^{-1} (proportional to enzymatic activity) is decreased during the 2 minutes of measuring time.

A new method was also developed for oxidation of thionophosphates (yields > 90%) with N-bromosuccinimide (NBS) under mild conditions[32].

Some references report the property of insecticides to differ distinctly in their inhibition ability against ChE preparations from different biological origin[12,17,18,33]. The objective of this paper is to derive general rules for enzymatic detectability and discrimination of ChE-inhibiting compounds used in agriculture by means of their k_i-data.

REAGENTS AND MATERIALS

Chemicals

The chemicals were obtained as follows: AChE from bovine erythrocytes (membrane bound) and 2-PAM, purchased from Sigma Chemical Company, St. Louis, USA.

AChE from bovine erythrocytes (50 mg ≙ 100 U per flask); AChE from electric eel (1000 U per flask); BChE from human serum (100 U per flask); ASCh, BSCh and DTNB, obtained from Boehringer Mannheim, FRG.

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* Abbreviations used: AChE, acetylcholinesterase; BChE, butyrylcholinesterase; ChE, cholinesterase; ASCh, S-Acetylthiocholine iodide, BSCh, S-butyrylthiocholine iodide; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); BSA, albumine from bovine serum; OB, obidoxime chloride, bis(pyridinium-4-aldoxime-1-methyl)-ether dichloride; 2-PAM, 1-methyl-2-(hydroxyiminomethyl)pyridinium iodide; HI-6, 1-[4-(carboxamido)-pyridinio]methyl-2[(hydroxyimino)methyl]pyridinio]methyl ether dichloride; BP-5, 1,1′-pentamethylene-bis(4-acetylpyridinium bromide) dioxime; NBS, N-bromosuccinimide; ASC, ascorbic acid.

BChE from horse serum (500 U per flask); bovine serum albumine (BSA) and N-bromosuccinimide (NBS), obtained from Merck, Darmstadt, FRG.

Eupergit C 30 N oxirane acrylic beads, purchased from Röhm Pharma, Weiterstadt, FRG.

HI-6 (a Hagedorn oxime[41]), received as gift from Prof. Eyer, Institut of Pharmacology and Toxicology of Ludwig Maximilian University, München, FRG.

Aldicarb; azinphosethyl; azinphosmethyl; bromophosethyl; butocarboxim, sulfoxide and sulfone; coumaphos; demeton-S-methylsulfon; dialifos; dioxacarb; omethoate; parathion-ethyl; purchased from Dr. Ehrenstorfer, Augsburg, FRG. Aldicarb sulfone; bendiocarb; bromophosmethyl; carbofuran; carbaryl; chlorfenvinphos; chlorpyriphosethyl; chlorpyriphosmethyl; diazinon; dibrom; dichlorvos; dimetilan; ethiofencarb; etrimphos; fensulfothion; heptenophos; malaoxon; malathion; methidathion; methiocarb; methomyl; mevinphos; oxamyl; paraoxonethyl; paraoxonmethyl; parathionmethyl; phosalone; phosmet; phoxim; pirimicarb; promecarb; propoxur; pyrazophos; tetrachlorvinphos; thionazin; triazophos, obtained from Riedel de Haen, Hannover, FRG.

Aldicarb sulfoxide; azinphosethyloxon; azinphosmethyloxon; 3-hydroxicarbofuran; dimethoate; ethiofencarb sulfoxide and sulfone; fenitroxon; fenoxon; methiocarb sulfoxide and sulfone; 2-hydroxipropoxur, received as gift from Dr. Blass, Bayer Leverkusen, FRG.

Instruments

Photometer Dr. Lange LP6; round cuvettes LCW 906, purchased from Dr. Bruno Lange, Berlin, FRG.

Stock solutions

Methanolic insecticide stock solutions	All insecticides were dissolved in methanol to concentrations from 10 mg ml^{-1} to 10 $\mu g\ ml^{-1}$.
Buffer I:	K_2HPO_4/KH_2PO_4 buffer, 0.05 mol l^{-1}, pH 7.0
Buffer II:	K_2HPO_4/KH_2PO_4 buffer, 0.5 mol l^{-1}, pH 7.0
ASCh solution (0.03 mol l^{-1}):	0.1 g ASCh were dissolved in 10 ml distilled water. Solution for measuring the enzymatic activity of AChE
BSCh solution (0.12 mol l^{-1}):	0.4 g BSCh were dissolved in 10 ml distilled water. Solution for measuring the enzymatic activity of BChE
DTNB solution (0.01 mol l^{-1}):	30 mg $NaHCO_3$ and 60 mg DTNB were dissolved in 20 ml buffer I

Adjusted enzyme suspensions

The enzymes (the contents of one flask) were dissolved either in buffer I: Solutions of AChE from electric eel in 10 ml buffer I, BChE from human serum in 2 ml buffer

I and BChE from horse serum in 5 ml buffer I were prepared. For immobilisation of the enzymes, 2 ml of each enzyme solution were mixed either with 100 mg (AChE from eel) RESP. 1 g (BChE from human RESP. horse serum) Eupergit C 30 N. All mixtures were allowed to stand with gentle shaking, at various time intervals, for 36 hr at room temperature. The beads were thoroughly washed on a porous glass plate with buffer solution and afterwards suspended in 1 ml (AChE from eel) RESP. in 5 ml (BChE) buffer I.

10 μl immobilized preparation were pipetted into 2 ml buffer I in a round cuvette. The enzymatic activity, measured as variation of substrate concentration per time unit, $\Delta c_s \, \text{min}^{-1}$, was determined by the spectrophotometric method of Ellmann *et al.*[35,43]. The variation of extinction per time unit, $\Delta E \, \text{min}^{-1}$, was determined photometrically by adding 0.1 ml DTNB solution and 0.1 ml ASCh solution RESP. 0.1 ml BSCh solution. The round cuvette, containing the enzyme suspension (= photometric determination batch), was agitated permanently (3·6 mm magnetic stick) by a magnetic stirrer, that was installed above the photometer (measuring time: 2 minutes).

The enzymatic activity was calculated as follows:

$$\Delta c_s \cdot \text{min}^{-1} = \frac{\Delta E \cdot \text{min}^{-1}}{\varepsilon \cdot d} = \frac{\Delta E \cdot \mu\text{mol} \cdot \text{mm}}{\text{min} \cdot 1.36 \cdot 10^{-3} \cdot 1 \cdot 10\,\text{mm}} \tag{1}$$

$$\Delta c_s \cdot \text{min}^{-1} = \frac{\Delta E}{13.6} \cdot \frac{\mu\text{mol}}{\text{min} \cdot \text{ml}} = \frac{\Delta E}{13.6} \cdot \frac{\text{U}}{\text{ml}} \tag{2}$$

d: interior diameter of the round cuvette
ε: molar extinction coefficient of 5-thiolo 2-nitrobenzoate[43].

All immobilized preparations were adjusted to an enzymatic activity of 3 U($\pm$1 U) per ml buffer by diluting with an appropriate volume buffer I.

The activity of the immobilized enzymes per mg bead ranged from 0.2 to 1 U mg^{-1} (AChE from eel) and from 0.02 to 0.1 U mg^{-1} (BChE from human serum and BChE horse serum)[33]. The membrane bound enzyme AChE from bovine erythrocytes was directly suspended (50 mg) in 2 ml phosphate buffer I by stirring 5 hr at room temperature.

All adjusted enzyme suspensions were stored in the refrigerator at 4°C.

Adjusted enzyme stock solutions

0.4 g BSA were dissolved in 20 ml buffer I and 20 ml glycerol. Some small crumbs of BChE from horse serum RESP. of BChE from human serum RESP. of AChE from eel RESP. 10 mg AChE from bovine erythrocytes (from Boehringer) were dissolved either in 1 ml glycerol-BSA solution. The enzymatic activities of these stock solutions were adjusted either to 3 $\pm$ 1 U ml^{-1} by diluting with more glycerol-BSA solution as described in the chapter before.

Preparation of 1,1'-pentamethylenebis (4-acetylpyridinium bromide) dioxime (BP-5)

BP-5 was synthesized in a two-step synthesis:

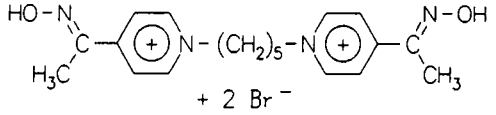
Step 1:

1-(4-pyridyl)ethanone oxime was prepared by refluxing (18 hr) a solution of 30 g 4-acetylpyridine, 35 g hydroxylammonium chloride and 80 g sodium acetate in 500 ml methanol/water (1:1)[34]. After cooling nearly 80% of the solvent was removed in a rotavapor. The remaining solution was poured on breaked ice. The arised precipitate was filtered and desiccated.

Yield (after recrystallisation from ethanol): 23.4 g = 69%.

Step 2:

BP-5 was synthesized by refluxing a solution of 6 g 1-(4-pyridyl)ethanone oxime and 2.7 g 1,5-dibromopentane in 23 ml dimethylformamide for 30 min at 140°C. 50 ml acetone were added after cooling to room temperature and the arised precipitate was filtered. The precipitate was then suspended in 100 ml dichloromethane and refluxed for 2 hr. The purified product was filtered and desiccated. Yield: 4.5 g = 75% ^{1}H-NMR (D_2O/CD_3OD): 1.6 (2 H, q); 2.2 (4 H, q); 2.35 (6 H, s); 4.7 (4 H, t); 8.35 (4 H, d); 9.1 (4 H, d).



BP-5

^{13}C-NMR (D_2O/CD_3OD): 10.3 (q); 23.3 (t); 31.2 (t); 61.4 (t); 124.5 (d); 145.4 (d); 151.1 (s); 153.9 (s)

$C_{19}H_{26}N_4O_2Br_2$	%N	%C	%H
Theor.	11.2	45.4	5.2
Found	10.6	43.3	5.5

Determination of k_i-values[33]

The inhibition batch was prepared from 20 ml inhibitor solution in buffer I (an adequate* volume of a methanolic insecticide stock solution, not more than 200 μl, dissolved in 20 ml buffer I) and from 0.1 ml adjusted enzyme suspension. A repeated observation of the enzymatic activity followed (defined as procedure I): The inhibition batch was permanently stirred in a beaker. 9 round cuvettes were filled either with 2 ml inhibition batch (by pipetting from the beaker) within a few minutes. All cuvettes were stirred (3 · 6 mm magnetic stick) at 25°C.

The variation of extinction per minute, $\Delta E\,min^{-1}$, was measured at least with 6 cuvettes at different inhibition times (0 min; 2.5 min; 5 min; 7.5 min...) by adding DTNB solution and ASCh RESP. BSCh solution as described above.

* If inhibition was either too weak or too strong, the experiment was repeated with an inhibitor concentration suitable to accomplish following condition: 5 min < $t_{0.5}$ < 50 min.

The relative enzymatic activity after the inhibition time t was calculated as follows:

$$Ac_t = \frac{\Delta E_t}{\Delta E_0} \cdot 100\% \tag{3}$$

$\Delta E_0\ \mathrm{min}^{-1}$: The first determined activity from the inhibition batch, obtained by measuring the extinction of the enzyme solution at 405 nm for two minutes, as a control: $0.15\ \mathrm{min}^{-1} < \Delta E_0\ \mathrm{min}^{-1} < 0.25\ \mathrm{min}^{-1}$

$\Delta E_t\ \mathrm{min}^{-1}$: Activity after inhibition time t

Ac_t: Relative activity.

The reaction of insecticides with ChE can be described by the model of a pseudo-first order reaction[30,36], if the concentration of the enzyme is small compared with the concentration of the inhibitor:

$$EH + IB \xrightarrow{k_i} EI + BH$$

$$[EH_t] = [EH_0] \cdot \exp(-k_i \cdot [IB] \cdot t) \tag{4}$$

$[IB]$: Concentration of active substance in the inhibition batch $[\mathrm{mol\,l}^{-1}]$

$[EH]$: concentration of the active enzyme

t: inhibition time

The bimolecular rate constant k_i is given:

$$k_i = \frac{\ln 2}{t_{0.5} \cdot [IB]} \tag{5}[30]$$

The reaction half time $t_{0.5}$ and k_i were calculated by plotting $\log Ac_t$ against t and by performing a linear regression:

$$y = b \cdot x + a$$

$$y_2 - y_1 = [\log(Ac_0/100) - \log(0.5\ Ac_0/100)] = b \cdot t_{0.5}$$

$$\Rightarrow t_{0.5} = \frac{\log 2}{b}$$

$$\Rightarrow k_i = \frac{\ln 2}{\log 2} \cdot \frac{b}{[IB]} \tag{6}$$

This equation is also valid as a good approximation to the reaction of carbamates with cholinesterase. At the beginning of the reaction the velocity of reactivation of the carbamylated enzyme is small compared to the velocity of inactivation of the enzyme.

Oxidation of thionophosphates ("thiones")[32]

25 mg NBS were dissolved in 100 ml distilled water. 20 µl methanolic thionophosphate solution ($1\ \mathrm{g\,l}^{-1}$) were dissolved in 10 ml NBS solution. 1 ml solution of 25 mg ascorbic acid in 10 ml water was added after 20 minutes to the oxidised

thionophosphate solution. The solution additionally was diluted with 2 ml buffer II and 7 ml water.

An aliquot of this solution was refilled with phosphate buffer I to a total volume of 20 ml and this inhibition batch was completed by addition of 0.1 ml adjusted enzyme suspension. The repeated observation of the enzymatic activity was performed by procedure I.

Measurement of the extension of the oxidation of thionophosphates

12 insecticide (either 6 thionosphosphates and their corresponding oxones) stock solutions were prepared by dissolving 50 μl (adequate) methanolic insecticide solution in 50 ml water (active substances and their concentrations see Table 6). 0.1 ml NBS solution (0.1 g per 1 water) were added to 1 ml insecticide stock solution. After 15 minutes 0.1 ml buffer II and 0.1 ml of an adjusted enzyme stock solution (AChE from bovine erythrocytes) were added (= inhibition batch). A repeated observation of the enzymatic activity followed (defined as procedure II) by adding either 0.1 ml of the inhibition batch, 0.1 ml ASCh solution and 0.1 ml DTNB solution to 2 ml buffer I in a round cuvette. The cuvette was agitated with a reciprocating-impeller agitator (10 seconds). One minute after adding substrate solution $\Delta E\,\mathrm{min}^{-1}$ was measured photometrically for 2 minutes.

The procedure was performed with each of the 12 insecticides. Ac_i and $t_{0.5}$ were determined as described before. The extension of the oxidation, *Eox*, is defined as follows:

$$\frac{Eox}{100\%} = \frac{k_i(\text{thion})}{k_i(\text{oxon})} = \frac{t_{0.5}(\text{oxon}) \cdot M(\text{thion}) \cdot c(\text{oxon})}{t_{0.5}(\text{thion}) \cdot M(\text{oxon}) \cdot c(\text{thion})} \tag{7}$$

Oxon: Corresponding O=P— analogous compound of a tested thionophosphate
M: molecular weight [μg mol^{-1}]
c: concentration of insecticide stock solution [μg l^{-1}]

Determination of inhibition values k_i under the influence of pyridinium oximes

The oximes are able to catalyse substrate hydrolysis. For example, $8.7 \cdot 10^{-5}$ mol l^{-1} $\approx$ 43.5 mg l^{-1} BP-5 (in the photometric determination batch, consisting of 2 ml buffer, 0.1 ml BSCh solution and 0.1 ml DTNB solution) caused a blank reading, $\Delta E\,\mathrm{min}^{-1}$, of 0.009. Therefore all experiments were performed with oxime concentrations causing a blank reading less than 5% of enzymatic activity.

The oximes were dissolved in distilled water: 15 mg 2-PAM RESP. 10 mg HI-6 RESP. 10 mg obidoxime RESP. 10 mg BP-5 in 1 ml water. 20 μl oxime solution were added to 20 ml of an appropriate insecticide solution in buffer I. The inhibition batch was completed by addition of 0.1 ml adjusted enzyme suspension. The repeated observation of the enzymatic activity followed by procedure I.

Cholinesterase is inhibited by the oximes[37], but this inhibition is not a function of inhibition time (tested by a negative control).

The dependence of k_i (e.g.: carbaryl) on oxime concentration was determined as follows: BP-5 and carbaryl (methanolic stock solution) were dissolved in 10 ml buffer I to concentrations given in Table 9. The inhibition batch was prepared from 1.1 ml carbaryl/BP-5 solution and from 0.1 ml adjusted enzyme stock solution (BChE from horse serum).

The repeated observation of the enzymatic activity followed by procedure II, using BSCh solution as substrate.

Inhibition pattern of spiked water samples

In 50% of experiments an oxidation was performed by addition of 50 µl NBSI solution (0.2 g l^{-1}) and 15 min later 50 µl ascorbic acid (ASC) solution (0.8 g l^{-1}) to 1 ml sample (bidest. water, spiked with 20 µg l^{-1} azinphosmethyl RESP. 10 µg l^{-1} carbofuran). In experiments without oxidation 0.1 ml bidist. water were added to the sample. An addition of 12 µl oxime solution, of 0.1 ml buffer II and of different adjusted enzyme stock solutions (Table 1) followed. The repeated observation of the enzymatic activity was performed by procedure II using ASCh RESP. BSCh solution as substrate. Relative inhibition = [180 min: $t_{0.5}$].

Table 1 Added reagents RESP. enzyme solutions to 1 ml water sample spiked with 20 µg l^{-1} azinphosmethyl RESP. 10 µg l^{-1} carbofuran

Oxidant 0.1 ml	*Oxime 12 µl*	*Enzyme 0.1 ml*
W	—	AChE-E
W	—	AChE-BO
W	—	BChE-HU
W	—	BChE-HO
NBSI/ASC	—	AChE-E
NBSI/ASC	—	AChE-BO
NBSI/ASC	—	BChE-HU
NBSI/ASC	—	BChE-HO
W	OB	AChE-E
W	OB	AChE-BO
W	BP-5	BChE-HU
W	BP-5	BChE-HO
NBSI/ASC	OB	AChE-E
NBSI/ASC	OB	AChE-BO
NBSI/ASC	BP-5	BChE-HU
NBSI/ASC	BP-5	BChE-HO

OB = obidoxime solution (5 g l^{-1}).
BP-5 = BP-5 solution (1 g l^{-1}).
W = bidest. water.
Enzyme solutions: AChE-E (from electric eel); AChE-BO (from bovine erythrocytes); BChE-HU (from human serum); BChE-HO (from horse serum).

RESULTS AND DISCUSSION

Inhibiting effects of organophosphorus compounds and carbamates

The inhibition values k_i of 55 organophosphorus compounds, insecticidal carbamates and carbamate metabolites were determined in combination with AChE from electric eel, AChE from bovine erythrocytes, BChE from human blood and BChE from horse blood (Table 2: diethoxiorganophosphates; Table 3: dimethoxiorganophosphates; Table 4: N-methylcarbamates; Table 5: N-dimethylcarbamates). The thionophosphates were converted into the more powerful inhibiting oxones before measuring k_i.

Altogether, about 400 single experiments were performed either by at least 6 time dependent measurements of enzymatic activity. Nearly 90% of the fitted plots gave a linear regression with correlation coefficient $R > 0.99$ ($10 \geq n \geq 6$). The lowest R

Table 2 Bimolecular rate constants k_i of diethoxiorganophosphates

Active substance	$k_i[l \cdot mol^{-1} \cdot min^{-1}] \pm (\Delta k_i/k_i) \cdot 100\%\ (n)$			
	AChE eel	*AChE bovine*	*BChE human*	*BChE horse*
Paraoxonethyl	$2.2 \cdot 10^5 \pm 5\%$ (10)	$0.6 \cdot 10^6 \pm 3\%$ (8)	$1.5 \cdot 10^6 \pm 3\%$ (9)	$0.8 \cdot 10^6 \pm 4\%$ (9)
Thionazin	$3.7 \cdot 10^5 \pm 4\%$ (8)	$4.6 \cdot 10^5 \pm 4\%$ (8)	$1.1 \cdot 10^6 \pm 7\%$ (11)	$5.1 \cdot 10^5 \pm 9\%$ (9)
Fensulfothion	$0.8 \cdot 10^4 \pm 7\%$ (10)	$4.4 \cdot 10^3 \pm 4\%$ (9)	$2.1 \cdot 10^5 \pm 15\%$ (9)	$3.9 \cdot 10^4 \pm 8\%$ (10)
Bromophosethyl	$1.1 \cdot 10^5 \pm 11\%$ (8)	$1.8 \cdot 10^5 \pm 3\%$ (8)	$0.6 \cdot 10^8 \pm 10\%$ (8)	$1.3 \cdot 10^7 \pm 3\%$ (8)
Chlorpyriphosethyl	$1.8 \cdot 10^6 \pm 4\%$ (9)	$3.8 \cdot 10^6 \pm 4\%$ (9)	$2.0 \cdot 10^8 \pm 7\%$ (9)	$1.3 \cdot 10^8 \pm 8\%$ (9)
Diazinon	$2.3 \cdot 10^4 \pm 18\%$ (9)	$3.4 \cdot 10^4 \pm 10\%$ (9)	$1.8 \cdot 10^7 \pm 4\%$ (9)	$1.2 \cdot 10^7 \pm 25\%$ (8)
Coumaphos	$1.9 \cdot 10^5 \pm 10\%$ (10)	$0.7 \cdot 10^5 \pm 6\%$ (10)	$0.7 \cdot 10^7 \pm 6\%$ (10)	$3.9 \cdot 10^6 \pm 7\%$ (10)
Triazophos	$3.0 \cdot 10^6 \pm 11\%$ (9)	$0.7 \cdot 10^6 \pm 1\%$ (9)	$0.9 \cdot 10^8 \pm 9\%$ (9)	$0.7 \cdot 10^8 \pm 7\%$ (9)
Pyrazophos	$1.6 \cdot 10^4 \pm 6\%$ (9)	$1.3 \cdot 10^4 \pm 3\%$ (9)	$5.7 \cdot 10^5 \pm 8\%$ (10)	$4.5 \cdot 10^5 \pm 10\%$ (9)
Phoxim	$5.8 \cdot 10^5 \pm 5\%$ (9)	$3.6 \cdot 10^5 \pm 3\%$ (9)	$0.9 \cdot 10^8 \pm 7\%$ (8)	$3.3 \cdot 10^7 \pm 8\%$ (9)
Chlorfenvinphos	$2.9 \cdot 10^4 \pm 7\%$ (9)	$0.9 \cdot 10^4 \pm 4\%$ (10)	$1.0 \cdot 10^6 \pm 4\%$ (10)	$2.1 \cdot 10^6 \pm 5\%$ (9)
Azinphosethyloxon	$1.0 \cdot 10^6 \pm 4\%$ (10)	$1.0 \cdot 10^6 \pm 2\%$ (10)	$3.6 \cdot 10^6 \pm 5\%$ (8)	$1.5 \cdot 10^7 \pm 4\%$ (9)
Dialifos	$5.5 \cdot 10^5 \pm 9\%$ (8)	$0.6 \cdot 10^6 \pm 4\%$ (8)	$3.0 \cdot 10^6 \pm 15\%$ (7)	$1.1 \cdot 10^7 \pm 6\%$ (8)
Phosalone	$2.5 \cdot 10^5 \pm 4\%$ (9)	$0.8 \cdot 10^5 \pm 9\%$ (9)	$1.9 \cdot 10^5 \pm 14\%$ (9)	$3.6 \cdot 10^6 \pm 6\%$ (9)

Δk_i: Confidence interval (0.95, n).
n: Number of determinations of $\Delta E\ min^{-1}$ as a function of inhibition time t.

Table 3 Bimolecular rate constants k_i of dimethoxiorganophosphates

Active substance	$k_i[l \cdot mol^{-1} \cdot min^{-1}] \pm (\Delta k_i/k_i) \cdot 100\%$ (n)			
	AChE eel	*AChE bovine*	*BChE human*	*BChE horse*
Paraoxonmethyl	$0.9 \cdot 10^5 \pm 4\%$ (10)	$2.1 \cdot 10^5 \pm 5\%$ (9)	$1.7 \cdot 10^4 \pm 3\%$ (9)	$2.2 \cdot 10^4 \pm 4\%$ (9)
Fenitroxon	$3.0 \cdot 10^4 \pm 16\%$ (8)	$3.0 \cdot 10^4 \pm 6\%$ (9)	$0.9 \cdot 10^5 \pm 13\%$ (10)	$0.9 \cdot 10^5 \pm 5\%$ (10)
Fenoxon	$2.3 \cdot 10^3 \pm 8\%$ (10)	$3.1 \cdot 10^4 \pm 3\%$ (10)	$2.0 \cdot 10^4 \pm 10\%$ (8)	$0.8 \cdot 10^4 \pm 7\%$ (10)
Bromophosmethyl	$0.8 \cdot 10^4 \pm 16\%$ (9)	$3.4 \cdot 10^4 \pm 13\%$ (8)	$1.4 \cdot 10^6 \pm 9\%$ (9)	$0.9 \cdot 10^6 \pm 7\%$ (9)
Chlorpyriphosmethyl	$0.7 \cdot 10^6 \pm 9\%$ (9)	$1.4 \cdot 10^6 \pm 8\%$ (9)	$2.2 \cdot 10^7 \pm 13\%$ (9)	$1.5 \cdot 10^7 \pm 12\%$ (9)
Etrimphos	$0.9 \cdot 10^6 \pm 10\%$ (8)	$2.6 \cdot 10^5 \pm 3\%$ (9)	$1.1 \cdot 10^6 \pm 13\%$ (9)	$1.8 \cdot 10^6 \pm 5\%$ (8)
Dibrom	$0.7 \cdot 10^6 \pm 9\%$ (8)	$1.3 \cdot 10^6 \pm 3\%$ (9)	$3.4 \cdot 10^6 \pm 9\%$ (10)	$1.6 \cdot 10^6 \pm 7\%$ (8)
Dichlorvos	$4.2 \cdot 10^4 \pm 8\%$ (10)	$2.3 \cdot 10^4 \pm 6\%$ (8)	$2.3 \cdot 10^5 \pm 5\%$ (9)	$0.8 \cdot 10^5 \pm 4\%$ (9)
Heptenophos	$2.4 \cdot 10^4 \pm 6\%$ (9)	$1.6 \cdot 10^4 \pm 6\%$ (9)	$4.8 \cdot 10^5 \pm 7\%$ (9)	$1.2 \cdot 10^5 \pm 7\%$ (9)
Tetrachlorvinphos	$5.0 \cdot 10^3 \pm 4\%$ (9)	$0.8 \cdot 10^3 \pm 5\%$ (9)	$1.7 \cdot 10^5 \pm 7\%$ (9)	$1.2 \cdot 10^6 \pm 12\%$ (9)
Mevinphos	$1.5 \cdot 10^5 \pm 11\%$ (8)	$2.0 \cdot 10^5 \pm 5\%$ (10)	$5.4 \cdot 10^4 \pm 8\%$ (10)	$3.8 \cdot 10^4 \pm 6\%$ (10)
Azinphosmethyloxon	$0.6 \cdot 10^7 \pm 5\%$ (9)	$0.9 \cdot 10^6 \pm 5\%$ (9)	$1.5 \cdot 10^4 \pm 4\%$ (10)	$2.7 \cdot 10^5 \pm 5\%$ (10)
Phosmet	$1.3 \cdot 10^6 \pm 4\%$ (10)	$0.8 \cdot 10^5 \pm 9\%$ (9)	$3.7 \cdot 10^3 \pm 14\%$ (10)	$3.1 \cdot 10^4 \pm 10\%$ (9)
Methidathion	$0.6 \cdot 10^6 \pm 8\%$ (9)	$0.7 \cdot 10^5 \pm 5\%$ (9)	$1.9 \cdot 10^4 \pm 9\%$ (8)	$1.5 \cdot 10^4 \pm 17\%$ (9)
Malaoxon	$1.7 \cdot 10^6 \pm 6\%$ (8)	$2.7 \cdot 10^5 \pm 4\%$ (8)	$2.1 \cdot 10^4 \pm 5\%$ (8)	$1.1 \cdot 10^4 \pm 6\%$ (9)
Omethoate	$2.6 \cdot 10^3 \pm 3\%$ (9)	$0.9 \cdot 10^3 \pm 4\%$ (8)	$0.8 \cdot 10^2 \pm 5\%$ (10)	$1.0 \cdot 10^2 \pm 4\%$ (9)
Demeton-S-methylsulfon	$4.2 \cdot 10^2 \pm 9\%$ (9)	$0.6 \cdot 10^3 \pm 4\%$ (9)	$2.8 \cdot 10^2 \pm 3\%$ (9)	$3.5 \cdot 10^2 \pm 5\%$ (10)

Δk_i: Confidence interval (0.95, n).
n: Number of determinations of ΔE min^{-1} as a function of inhibition time t.

was found >0.92. These results are in good agreement with the model of a pseudo-first order reaction. An example is given in Figure 1.

The inactivation values vary over a wide range. BChE from human blood is inhibited by chlorpyriphosethyl (-oxon) 10^6 times more than by omethoate. Also the relative response of the various ChE on the same inhibitor is considerably different:

AChE from bovine erythrocytes is inhibited by tetrachlorvinphos by a factor of 1000 less than it is BChE from horse blood. In case of carbofuran, the response of AChE from electric eel is more than 200 times greater than it is observed with BChE from human blood.

An interesting synopsis results when one compares the k_i-values of insecticides of similar chemical structure. An example is given in Figure 2.

Table 4 Bimolecular rate constants of N-methylcarbamates

Active substance	$k_i[l \cdot mol^{-1} \cdot min^{-1}] \pm (\Delta k_i/k_i) \cdot 100\%$ (*n*)			
	AChE eel	*AChE bovine*	*BChE human*	*BChE horse*
Carbaryl	$3.3 \cdot 10^4 \pm 9\%$ (7)	$1.8 \cdot 10^4 \pm 9\%$ (6)	$1.9 \cdot 10^3 \pm 5\%$ (9)	$0.7 \cdot 10^4 \pm 8\%$ (8)
Dioxacarb	$1.4 \cdot 10^4 \pm 14\%$ (7)	$0.8 \cdot 10^4 \pm 10\%$ (7)	$1.8 \cdot 10^3 \pm 3\%$ (8)	$4.3 \cdot 10^3 \pm 5\%$ (8)
Bendiocarb	$5.6 \cdot 10^5 \pm 16\%$ (7)	$2.7 \cdot 10^5 \pm 10\%$ (7)	$4.8 \cdot 10^3 \pm 8\%$ (10)	$2.6 \cdot 10^4 \pm 13\%$ (9)
Carbofuran	$1.7 \cdot 10^6 \pm 12\%$ (6)	$0.8 \cdot 10^6 \pm 8\%$ (7)	$0.6 \cdot 10^4 \pm 6\%$ (9)	$3.1 \cdot 10^4 \pm 6\%$ (8)
3-Hydroxicarbofuran	$3.5 \cdot 10^5 \pm 12\%$ (8)	$1.8 \cdot 10^5 \pm 8\%$ (9)	$1.1 \cdot 10^4 \pm 6\%$ (9)	$3.7 \cdot 10^4 \pm 12\%$ (9)
Propoxur	$1.3 \cdot 10^5 \pm 20\%$ (5)	$3.4 \cdot 10^4 \pm 12\%$ (8)	$0.8 \cdot 10^3 \pm 9\%$ (9)	$3\text{-}.8 \cdot 10^3 \pm 13\%$ (7)
2-hydroxipropoxur	$0.8 \cdot 10^2 \pm 18\%$ (8)	$1.6 \cdot 10^2 \pm 40\%$ (8)	$0.9 \cdot 10^2 \pm 48\%$ (7)	$0.5 \cdot 10^2 \pm 23\%$ (9)
Ethiofencarb	$3.0 \cdot 10^4 \pm 18\%$ (6)	$3.5 \cdot 10^3 \pm 14\%$ (7)	$1.5 \cdot 10^3 \pm 4\%$ (9)	$0.9 \cdot 10^3 \pm 2\%$ (9)
Ethiofencarb sulfoxide	$1.4 \cdot 10^3 \pm 27\%$ (6)	$1.1 \cdot 10^3 \pm 5\%$ (9)	$3.5 \cdot 10^2 \pm 7\%$ (9)	$1.9 \cdot 10^2 \pm 22\%$ (9)
Ethiofencarb sulfone	$5.8 \cdot 10^2 \pm 16\%$ (9)	$5.6 \cdot 10^2 \pm 10\%$ (9)	$5.3 \cdot 10^2 \pm 8\%$ (9)	$0.6 \cdot 10^3 \pm 10\%$ (9)
Promecarb	$2.9 \cdot 10^5 \pm 13\%$ (6)	$2.1 \cdot 10^5 \pm 12\%$ (7)	$1.5 \cdot 10^5 \pm 5\%$ (8)	$1.2 \cdot 10^5 \pm 5\%$ (9)
Methiocarb	$1.0 \cdot 10^5 \pm 10\%$ (7)	$5.1 \cdot 10^4 \pm 7\%$ (7)	$1.3 \cdot 10^4 \pm 7\%$ (9)	$1.0 \cdot 10^4 \pm 5\%$ (8)
Methiocarb sulfoxide	$0.7 \cdot 10^5 \pm 22\%$ (7)	$4.0 \cdot 10^4 \pm 10\%$ (8)	$4.3 \cdot 10^4 \pm 9\%$ (8)	$2.8 \cdot 10^4 \pm 4\%$ (7)
Methiocarb sulfone	$0.7 \cdot 10^4 \pm 12\%$ (9)	$0.7 \cdot 10^4 \pm 13\%$ (7)	$3.2 \cdot 10^3 \pm 13\%$ (8)	$1.7 \cdot 10^3 \pm 6\%$ (8)
Aldicarb	$5.0 \cdot 10^4 \pm 13\%$ (7)	$1.3 \cdot 10^4 \pm 11\%$ (6)	$2.4 \cdot 10^4 \pm 8\%$ (8)	$1.0 \cdot 10^4 \pm 9\%$ (7)
Aldicarb sulfoxide	$1.3 \cdot 10^6 \pm 11\%$ (7)	$5.6 \cdot 10^5 \pm 10\%$ (7)	$0.6 \cdot 10^6 \pm 10\%$ (9)	$2.5 \cdot 10^5 \pm 8\%$ (8)
Aldicarb sulfone	$1.5 \cdot 10^4 \pm 8\%$ (7)	$0.6 \cdot 10^4 \pm 8\%$ (8)	$2.5 \cdot 10^4 \pm 9\%$ (10)	$2.6 \cdot 10^4 \pm 11\%$ (9)
Butocarboxim	$0.7 \cdot 10^4 \pm 14\%$ (7)	$0.9 \cdot 10^3 \pm 12\%$ (9)	$0.6 \cdot 10^3 \pm 12\%$ (8)	$5.2 \cdot 10^2 \pm 17\%$ (9)
Butocarboxim sulfoxide	$4.7 \cdot 10^5 \pm 8\%$ (9)	$2.2 \cdot 10^3 \pm 10\%$ (9)	$0.8 \cdot 10^3 \pm 13\%$ (10)	$3.1 \cdot 10^2 \pm 9\%$ (10)
Butocarboxim	$0.8 \cdot 10^4 \pm 15\%$ (7)	$1.1 \cdot 10^3 \pm 13\%$ (9)	$0.6 \cdot 10^3 \pm 5\%$ (7)	$4.5 \cdot 10^2 \pm 6\%$ (8)
Methomyl	$1.5 \cdot 10^5 \pm 12\%$ (7)	$4.6 \cdot 10^4 \pm 8\%$ (7)	$3.9 \cdot 10^3 \pm 5\%$ (9)	$1.5 \cdot 10^3 \pm 5\%$ (9)
Oxamyl	$3.5 \cdot 10^5 \pm 16\%$ (9)	$0.8 \cdot 10^5 \pm 10\%$ (7)	$3.0 \cdot 10^4 \pm 9\%$ (7)	$3.5 \cdot 10^4 \pm 18\%$ (10)

Δk_i: Confidence interval (0.95, *n*).
n: Number of determinations of ΔE min^{-1} as a function of inhibition time *t*.

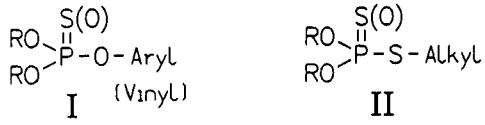
Table 5 Bimolecular rate constants k_i of N-dimethylcarbamates

Active substance	$k_i[l \cdot mol^{-1} \cdot min^{-1}] \pm (\Delta k_i/k_i) \cdot 100\%$ (n)			
	AChE eel	AChE bovine	BChE human	BChE horse
Dimetilan	$1.1 \cdot 10^4 \pm 13\%$ (8)	$0.7 \cdot 10^4 \pm 12\%$ (9)	$1.1 \cdot 10^4 \pm 11\%$ (9)	$4.1 \cdot 10^4 \pm 8\%$ (8)
Pirimicarb	$1.0 \cdot 10^3 \pm 21\%$ (7)	$5.1 \cdot 10^3 \pm 5\%$ (7)	$2.4 \cdot 10^4 \pm 6\%$ (9)	$3.0 \cdot 10^4 \pm 6\%$ (8)

Δk_i: Confidence interval (0.95, n).
n: Number of determinations of ΔE min^{-1} as a function of inhibition time t.

The response pattern of chlopyriphosethyl and -methyl are of a significant resemblance in opposition to those of parathionethyl and -methyl.

General rules can be derived from all obtained k_i-data: Diethoxyorganophosphates inhibit BChE more than AChE. The response of AChE to N-methylcarbamates is greater than it is with BChE. Most of organophosphorus insecticides can be classified into the following two groups:



$R = -CH_3 ; -C_2H_5$

Large substituents linked with the aromatic ring system (I) cause a decreasing sensitivity to AChE and an increasing sensitivity to BChE:

k_i bromophosethyl > k_i parathion (BChE)
k_i bromophosethyl < k_i parathion (AChE)
k_i diazinon > k_i thionazin (BChE)
k_i diazinon < k_i thionazin (AChE)
k_i fenitrothion, bromophosmethyl > k_i parathionmethyl (BChE)
k_i fenitrothion, bromophosmethyl < k_i parathionmethyl (AChE)

On the other hand it is remarkable that all dimethoxiorganophosphorus compounds of group II inhibit AChE more than BChE (azinphosmethyl, phosmet, malathion, methidathion, omethoate, demeton-S-methylsulfon).

Inactivation effects of carbamate metabolites

Ethiofencarb sulfoxide and sulfone, methiocarb sulfone and 2-hydroxipropoxur are less active than the corresponding insecticides (sulfides, propoxur RESP.). Aldicarb sulfone, methiocarb sulfoxide, and 3-hydroxicarbofuran show a decreasing effective-

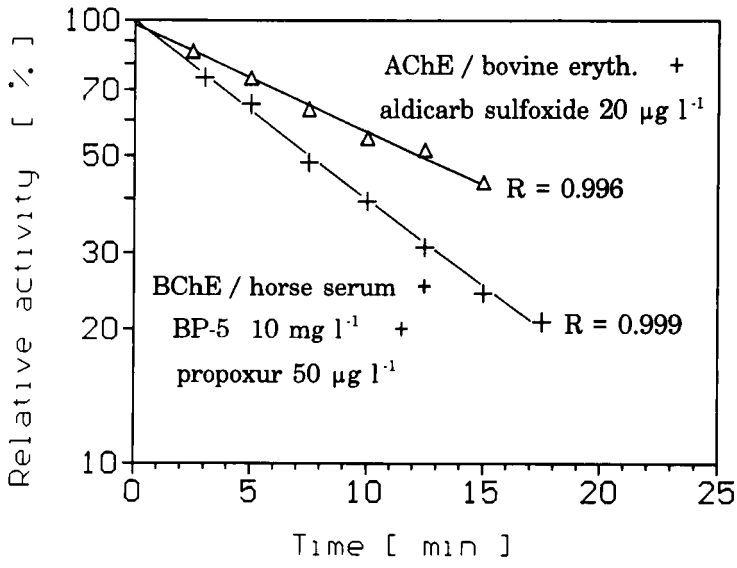


Figure 1 Inactivation kinetics for the reactions of aldicarb sulfoxide with AChE from bovine erythrocytes and (influenced by BP-5) of propoxur with BChE from horse serum.

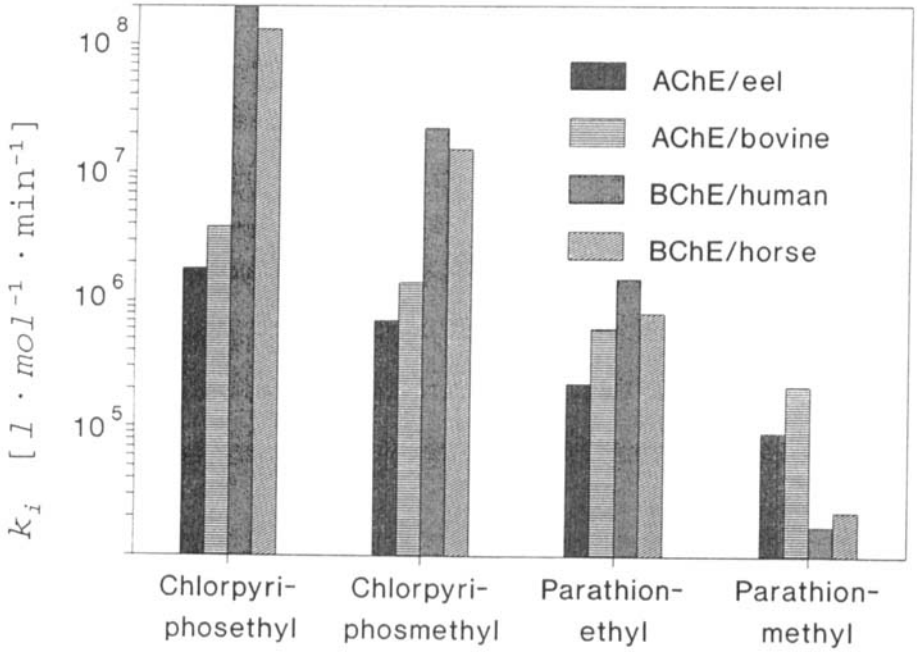
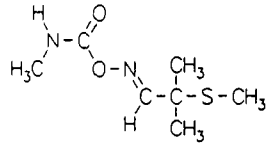


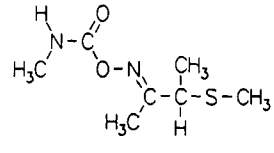
Figure 2 Response pattern of ChE inhibiting insecticides of similar chemical structure.

ness to AChE and an increasing effectiveness to BChE. No significant variations of response occur in case of butocarboxim metabolites.

Aldicarb sulfoxide[38] is a very remarkable derivative: It is 10 times more efficient inhibitor than it is aldicarb or aldicarb sulfone (AChE and BChE). There is a considerable difference in the response of aldicarb RESP., aldicarb sulfoxide RESP. and aldicarb sulfone compared to the response of butocarboxim RESP., butocarboxim sulfoxide RESP. and butocarboxim sulfone.



Aldicarb



Butocarboxim

Extension of oxidation of thionophosphates

The results are given in Table 6. Azinphosethyl, azinphosmethyl, malathion, parathionethyl and parathionmethyl obtained after oxidation with NBS exhibit nearly the same inhibitory power as the corresponding oxones.

Omethoate is one of the weakest ChE inhibiting oxones. Therefore some difficulties exist to test the oxidation of dimethoate under the selected conditions. Indeed,

Table 6 Measured extension of oxidation of thionophosphates by NBS

Active substance	*M* [$\mu g\ mol^{-1}$]	*c* [$\mu g\ l^{-1}$]	$t_{0.5}$ [*min*]	(*n*)	Eox $\pm$ ΔEox
Paraoxonethyl	275	22	18.3	(9)	
Parathionethyl	291	22	20.9	(9)	92 $\pm$ 10%
Paraoxonethyl	275	25	16.4	(9)	
Parathionethyl	291	25	17.8	(9)	98 $\pm$ 14%
Paraoxonmethyl	247	50	19.1	(8)	
Parathionethyl	263	50	21.5	(8)	94 $\pm$ 17%
Azinphosethyloxon	329	10	23.1	(9)	
Azinphosethyl	345	10	21.8	(9)	111 $\pm$ 13%
Azinphosmethyloxon	301	10	29.7	(9)	
Azinophosmethyl	317	10	29.8	(9)	105 $\pm$ 17%
Malaoxon	314	10	26.7	(9)	
Malathion	330	10	27.7	(9)	101 $\pm$ 11%
Omethoat	213	7500	36.7	(9)	
Dimethoat	229	7500	181	(9)	22 $\pm$ 7%
Negative control (without active substance)	—	—	275	(9)	—

Eox: Extension of oxidation.

ΔEox: Confidence interval (absolute).

c: Concentration of active substance (insecticide stock solution).

M: molecular weight.

n: Number of determinations of $\Delta E\ min^{-1}$ as a function of inhibition time.

$t_{0.5}$: Reaction half time.

7.5 mg l^{-1} dimethoate were not oxidised completely. Probably, there was not enough excess of NBS.

The mechanism of oxidation was not investigated.

Influence of bispyridiniumoximes on the inactivation reaction

The inactivation of AChE by organophosphorus compounds can be suppressed by obidoxime (Figure 3) through reactivation of the enzyme. On the other hand, a 20-fold acceleration of inhibition by carbaryl occurs. Under the influence of an oxime neither a significant increase ($\leq$ 4-fold) nor a significant decrease ($\leq$ 2-fold) of inhibitory power of any other tested carbamate to AChE was observed. Contrary to this the inhibiting effect of *several* carbamates on BChE increased under the influence of bispyridiniumoximes. Table 7 demonstrates the promising effect of BP-5 in relation to some known antidotes.

The k_i-values of carbofuran, bendiocarb, propoxur, carbaryl, promecarb, dioxacarb, methomyl and ethiofencarb were increased by a factor k_i(oxime)/k_i (see Table 8). No significant variability of rate constants k_i was observed in case of methiobarb, aldicarb, aldicarb sulfone, dimetilan and pirimicarb.

The dependency of k_i on the concentration of BP-5 was investigated (Table 9). At concentrations of BP-5 $\geq$ 10 mg l^{-1}, k_i reaches a saturation value.

Only few organophosphorus compounds were investigated on their influence towards inhibition of BChE. First results show a slight (k_i oxime/$k_i \approx 5$) increase of inhibition (paraoxon, dichlorvos, omethoate, methamidophos). The response of BChE to azinphosmethyloxon and chlorfenvinphos decreases under the influence of obidox-

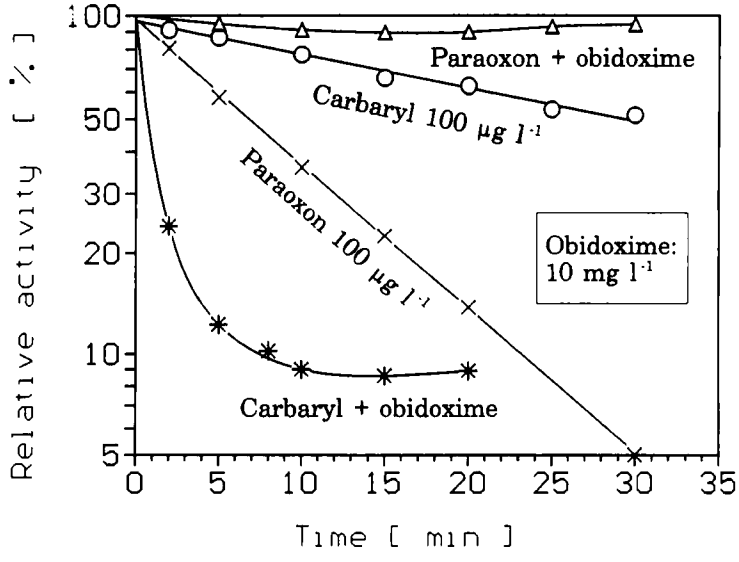


Figure 3 Influence of obidoxime towards inhibition of AChE from electric eel by paraoxon and carbaryl.

Table 7 Influence of different pyridiniumoximes on the inhibition of immobilized BChE from human serum by carbofuran

Pyridiniumoxime	$k_i[l \cdot mol^{-1} \cdot min^{-1}]$
without oxime	$0.7 \cdot 10^4$
2-PAM	$4.8 \cdot 10^4$
HI-6	$4.5 \cdot 10^4$
OB	$2.0 \cdot 10^5$
BP-5	$5.1 \cdot 10^6$

Table 8 Bimolecular rate constants under the influence of BP-5

Active substance	*AChE (bovine eryth.)*		*BChE (human serum)*		*BChE (horse serum)*	
	k_i^* $\Delta cf;(n)$	$\frac{k_i^*}{k_i^0}$	k_i^* $\Delta cf;(n)$	$\frac{k_i^*}{k_i^0}$	k_i^* $\Delta cf;(n)$	$\frac{k_i^*}{k_i^0}$
Carbaryl	$3.1 \cdot 10^5 \pm 6\%$ (7)	17	$2.5 \cdot 10^5 \pm 6\%$ (9)	130	$3.7 \cdot 10^5 \pm 9\%$ (8)	53
Dioxacarb	$3.5 \cdot 10^4 \pm 16\%$ (7)	4.4	$5.6 \cdot 10^4 \pm 17\%$ (8)	31	$0.8 \cdot 10^5 \pm 11\%$ (7)	19
Bendiocarb	$1.8 \cdot 10^5 \pm 12\%$ (9)	0.7	$0.8 \cdot 10^6 \pm 9\%$ (9)	170	$5.5 \cdot 10^5 \pm 17\%$ (7)	21
Carbofuran	$4.9 \cdot 10^5 \pm 18\%$ (8)	0.6	$5.1 \cdot 10^6 \pm 12\%$ (7)	850	$2.4 \cdot 10^6 \pm 10\%$ (8)	77
Propoxur	$0.9 \cdot 10^5 \pm 12\%$ (8)	2.6	$3.3 \cdot 10^5 \pm 10\%$ (9)	410	$3.9 \cdot 10^5 \pm 5\%$ (8)	100
Promecarb	$1.4 \cdot 10^5 \pm 6\%$ (9)	0.7	$2.0 \cdot 10^6 \pm 12\%$ (8)	13	$1.9 \cdot 10^6 \pm 9\%$ (8)	16
Methiocarb	$3.8 \cdot 10^4 \pm 11\%$ (9)	0.7	$2.1 \cdot 10^4 \pm 8\%$ (8)	1.6	$1.4 \cdot 10^4 \pm 6\%$ (9)	1.4
Ethiofencarb	$3.2 \cdot 10^3 \pm 8\%$ (9)	0.9	$0.8 \cdot 10^4 \pm 6\%$ (9)	5.3	$1.9 \cdot 10^4 \pm 13\%$ (7)	21
Aldicarb	$1.4 \cdot 10^4 \pm 13\%$ (9)	1.1	$4.2 \cdot 10^4 \pm 15\%$ (8)	1.8	$2.5 \cdot 10^4 \pm 9\%$ (9)	2.5
Aldicarb sulfone	$4.9 \cdot 10^3 \pm 20\%$ (9)	0.8	$1.1 \cdot 10^4 \pm 10\%$ (9)	0.4	$0.9 \cdot 10^4 \pm 5\%$ (8)	0.35
Methomyl	$3.0 \cdot 10^4 \pm 9\%$ (9)	0.7	$0.8 \cdot 10^5 \pm 8\%$ (8)	20	$3.0 \cdot 10^4 \pm 10\%$ (9)	20
Dimetilan	$3.7 \cdot 10^3 \pm 7\%$ (9)	0.5	$0.6 \cdot 10^4 \pm 9\%$ (9)	0.5	$1.8 \cdot 10^4 \pm 10\%$ (9)	0.4
Pirimcarb	$5.5 \cdot 10^3 \pm 11\%$ (9)	1.1	$5.9 \cdot 10^4 \pm 13\%$ (8)	2.5	$2.6 \cdot 10^4 \pm 8\%$ (9)	0.9

k_i^*: Bimolecular rate constant under the influence of BP-5 (10 mg l^{-1} related to the inhibition batch) [$l \cdot mol^{-1} \cdot min^{-1}$].
k_i^0 Bimolecular rate constant, non-influenced (see Tables 4 and 5).
Δcf: Confidence interval (0.95; n) = $(\Delta k_i^*/k_i^*) \cdot 100\%$.
n: Number of determinations of ΔE min^{-1} as a function of inhibition time t.

Table 9 Dependency of k_i on the concentration of BP-5 (BP-5 (BChE from horse serum inhibited by carbaryl)

c(Oxime) $[mg \cdot l^{-1}]$	*c(Carbaryl)* $[mg \cdot l^{-1}]$	$t_{0.5}$ *[min]*	k_i $[l \cdot mol^{-1} \cdot min^{-1}]$
0	0.15	15.0	$0.6 \cdot 10^4$
0.39	0.015	14.5	$0.6 \cdot 10^5$
0.77	0.015	8.8	$1.0 \cdot 10^5$
3.85	0.031	16.5	$2.7 \cdot 10^5$
7.7	0.031	16.0	$2.8 \cdot 10^5$
77	0.031	11.5	$3.9 \cdot 10^5$

c: Concentration related to the inhibition batch.
$t_{0.5}$: Reaction half time.

ime. Plots of log Ac_t against t demonstrated slight reactivation effects (deflexion from the straight line of a pseudo first order reaction) (Figure 4).

The influence of pyridiniumoximes on ChE inhibition by organophosphorus compounds was discussed[39]:

An acceleration of inhibition may be caused by a phosphorylated oxime, a powerful inhibitor, that is formed by reaction of the organophosphorus compound with the oxime anion. De Jong and Ceulen[40], who found a great inhibitory power of diethoxiphosphorylobidoxime ($k_i = 3.8 \cdot 10^8\, l \cdot mol^{-1} \cdot min^{-1}$; AChE from bovine erythrocytes) compared to paraoxon ($k_i = 6.3 \cdot 10^5\, l \cdot mol^{-1} \cdot min^{-1}$), agreed with that hypothesis.

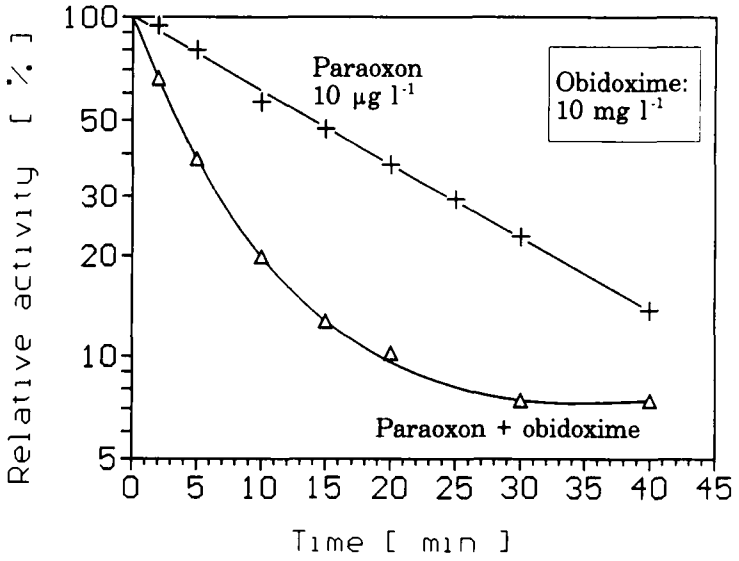


Figure 4 Influence of obidoxime towards inhibition of BChE from human serum by paraoxon.

16 parameter inhibition pattern, small scale test

The kinetics of ChE inactivation of two spiked water samples were determined under various conditions (Figure 5).

$$(t_{0.5} \gg 180 \text{ min} \Rightarrow \text{relative inhibition} \approx 0)$$

The inhibition pattern of azinphosmethyl (AZM) and carbofuran (CBF) demonstrate the predicted differences: The thionophosphate AZM is not detectable without oxidation. Adding OB, inhibition of AChE, caused by the oxidised product, dis-

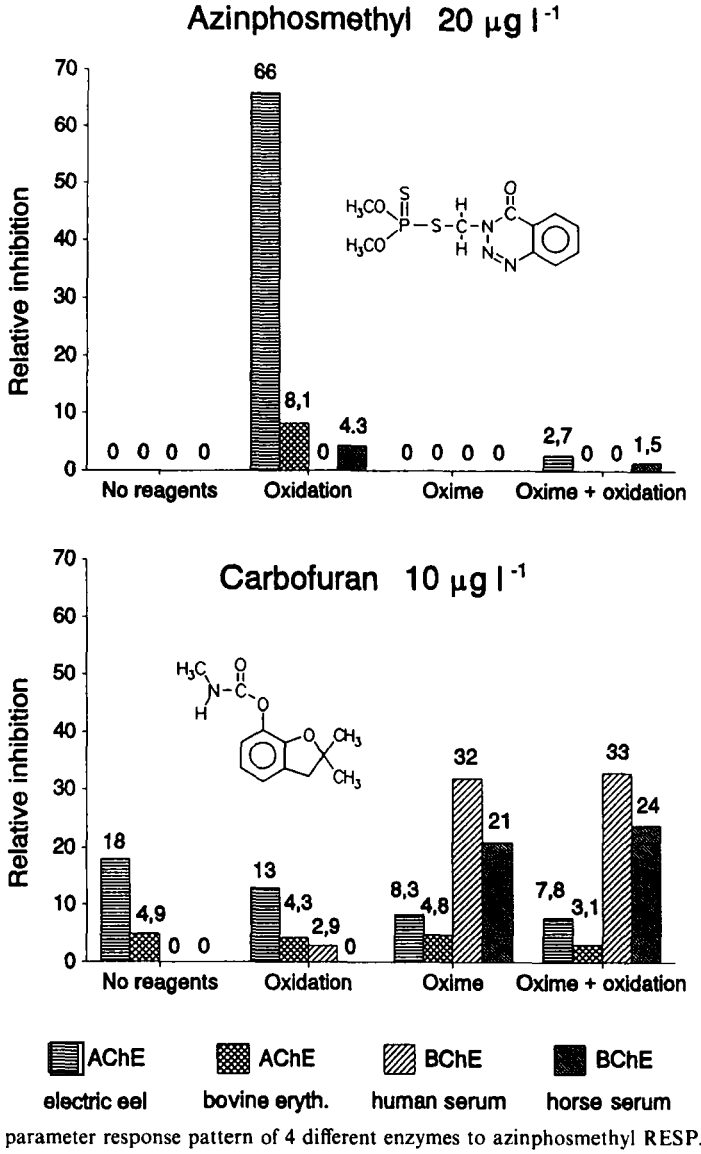


Figure 5 16 parameter response pattern of 4 different enzymes to azinphosmethyl RESP. carbofuran.

appears. The carbamate CBF is detectable without preceding oxidation. BChE is inhibited by CBF after adding BP-5.

The kinetic of inhibition seems to be influenced by either NBSI, succinimide (reduction product of NBSI), ASC or dehydroascorbic acid (oxidation product of ascorbic acid). The inhibition of BChE from human serum by CBF after adding NBS and ASC was not expected. These effects are of a distinctly less amount than the effects described above.

This small scale test demonstrates one possibility for qualitative discrimination of active substances in a water sample. The next step will be the investigation of the applicability of this method for samples spiked with mixtures of insecticides.

CONCLUSIONS

Discrimination of insecticides in a water sample by a biochemical method that is normally used as a screening test seems possible.

Phosphorous containing insecticides can be identified by reactivation of AChE. An inhibition, which occurs only after oxidation, is characteristic for thionophosphates like parathion or azinphos.

Bispyridiniumoximes are normally used as antidots against organophosphorous poisoning[41,42]. By addition of BP-5 a group of certain carbamates can be identified by an acceleration of inhibition of BChE. For example, the important water contaminants aldicarb (aldicarb is oxidised in natural waters[38] mostly to the sulfoxide) and carbofuran can be distinguished. There is a large inhibiting effect of aldicarb sulfoxide on AChE and BChE. The response of AChE-preparations to carbofuran are of a greater extent than those of BChE. Carbofuran will be identified by the inactivation of BChE, that occurs when a bispyridiniummoxime is added.

The discrimination of some insecticides, for example parathionethyl, azinphosmethyl and chlorpyriphosethyl, by their differences in their inactivation abilities against ChE from different sources is also possible.

The development of a cholinesterase array consisting of various enzymes applied toward the same sample is very promising and the inhibition test may become an alternative to the classical chromatographic methods like GC or HPLC for differentiated detection of insecticide residues in water.

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