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MECHANISM OF INHIBITION *IN VITRO* OF MAMMALIAN ACETYLCHOLINESTERASE AND CHOLINESTERASE IN SOLUTIONS OF *O,O*-DIMETHYL 2,2,2-TRICHLORO-1-HYDROXYETHYL PHOSPHONATE (TRICHLORPHON)

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Abstract—The rate of decomposition of trichlorphon into DDVP was measured polarographically at pH 7·4. The first order rate constants of decomposition at 25° are $7{\cdot}27 \times 10^{-4}$ and $6{\cdot}05 \times 10^{-4}$ min^{-1} for trichlorphon concentrations of 0·150 and 15·0 mM respectively; at 37° the corresponding rate constants are $53{\cdot}1 \times 10^{-4}$ and $37{\cdot}1 \times 10^{-4}$ min^{-1}. The rate of decomposition of trichlorphon was also calculated from the kinetics of inhibition of acetylcholinesterase (EC 3.1.1.7) and cholinesterase (EC 3.1.1.8) in trichlorphon solutions at 25° and 37° (pH 7·4). The following enzyme sources were used: bovine erythrocytes and rat brain acetylcholinesterase, and human, horse and rat plasma cholinesterase. The rate of decomposition of trichlorphon was calculated by assuming that only DDVP formed from trichlorphon is the enzyme inhibitor, while trichlorphon itself does not act as an inhibitor. The calculated rate constants for the decomposition of trichlorphon are lower or just within the range of the rate constants obtained by the polarographic method. This agreement was taken as kinetic evidence that trichlorphon is not an inhibitor of mammalian cholinesterases. The effect of pH on enzyme inhibition supports this conclusion. The rate of inhibition of bovine erythrocyte acetylcholinesterase by DDVP is the same at pH 7·4 and pH 6·0 (37°). However, the rate of enzyme inhibition in trichlorphon solutions is 30 times faster at pH 7·4 than at pH 6·0, and this agrees with the greater stability of trichlorphon at the lower pH value. The rate of spontaneous reactivation of the enzyme was measured (37°, pH 7·4) after inhibition in trichlorphon solutions of acetylcholinesterase (human and bovine erythrocytes) and cholinesterase (human plasma). For all three enzyme preparations, the rate of spontaneous reactivation was the same as that obtained after inhibition by DDVP. All results point to the conclusion that trichlorphon *in vitro* is not an inhibitor of mammalian cholinesterases.

Trichlorphon (*O,O*-dimethyl 2,2,2-trichloro-1-hydroxyethyl phosphonate) is used as an insecticide and also as an antiparasitic drug, and its toxic effects are attributed to inhibition of cholinesterases. However, there is some controversy on whether trichlorphon itself is the enzyme inhibitor. In aqueous solutions trichlorphon decomposes by two reactions: one reaction is hydrolysis into *O,O*-dimethylphosphate and 2,2,2-trichloroethanol, and the other is rearrangement into *O,O*-dimethyl-2,2-dichlorovinyl phosphate (DDVP) and hydrochloric acid [1–6]. Under acidic conditions both reactions are very slow, and hydrolysis is the predominant reaction. Under neutral and alkaline conditions, decomposition into DDVP occurs almost exclusively, and the rate of the reaction increases with increasing pH. It is known that DDVP is a potent inhibitor of cholinesterases, and it has been postulated that inhibition of the enzyme by trichlorphon, both *in vitro* and *in vivo*, is due to DDVP formed from trichlorphon [7, 8]. However, some authors consider that trichlorphon itself is an inhibitor of cholinesterases [3, 9–12].

The wide use of trichlorphon in chemotherapy of human parasitic diseases (cf. 13) necessitates more knowledge of its mechanism of action.

The purpose of the present work was to establish if trichlorphon itself is an inhibitor *in vitro* of mammalian cholinesterases.

Therefore the rates of decomposition of trichlorphon were determined in two ways. One way was a direct measurement of the trichlorphon decomposition in aqueous solutions by a polarographic method. The other way was indirect, whereby the rate of decomposition was calculated from the kinetics of cholinesterase inhibition, assuming that only DDVP formed from trichlorphon will inhibit the enzyme. If trichlorphon is not an inhibitor of cholinesterases, the rates of decomposition obtained by the two methods should be equal.

MATERIALS AND METHODS

Inhibitors. Trichlorphon (purity 98·7%; melting point of two batches 79·2 and 78·4°) was obtained from Bayer Ltd., Leverkusen, W. Germany. DDVP (purity 93%) was obtained form the World Health Organization, Geneva, Switzerland. Aqueous solutions of trichlorphon (50 mM) were prepared immediately before use. Stock solutions of DDVP (50 mM) were prepared in dimethylformamid and diluted with water or buffer immediately before use.

Enzyme preparations. The following preparations of acetylcholinesterase (EC 3.1.1.7) and cholinesterase (EC 3.1.1.8) were used: purified bovine erythrocytes (Winthrop Ltd., New York, N.Y., U.S.A.), purified horse serum (Sigma Chemical Co., St. Louis, Mo., U.S.A.), human erythrocytes (erythrocytes from heparinized blood were washed twice with 0·15 M NaCl and made up to the volume of blood, with 0·15 M NaCl), rat brain (rat brain homogenized in 0·15 M NaCl; 160 mg rat brain/ml), human and rat plasma (obtained from heparinized blood). The concentrations of the

enzyme preparation during assay were: bovine erythrocytes 0·005 mg/ml (spectrophotometric method) and 0·25 mg/ml (pH-stat method), horse serum 0·006 mg/ml, human erythrocytes 0·00167 ml/ml, rat brain 1·25 mg/ml, human plasma 0·0067 ml/ml, rat plasma 0·0033 ml/ml.

Buffer. The 1·0 mM buffer was prepared by titrating 1·0 mM NaH_2PO_4 with 1·0 mM Na_2HPO_4 to the required pH. The 0·1 M buffer was prepared by titrating 0·1 M NaH_2PO_4 with 0·1 M Na_2HPO_4 to the required pH.

Enzyme inhibition measured by the spectrophotometric method. All experiments in 0·1 M buffer (pH 7·4) were done by the method of Ellman *et al.* [14]. Enzyme (2·7 ml) and inhibitor (0·3 ml) were incubated for a given time before substrate was added (50 μl acetylthiocholine iodide in water, final concentration 1·0 mM). To control samples, 0·3 ml water was added instead of the inhibitor solution. The reaction mixture contained the thiol reagent 5,5'-dithiobis-2-nitrobenzoate (0·33 mM final concn). The absorbance was read on a Unicam S.P. 500 spectrophotometer at 412 nm in 1·0-cm thermostated cuvettes against a blank. The blank contained buffer and the thiol reagent, except when the activity of human erythrocyte acetylcholinesterase was measured when the blank also contained the enzyme preparation. The absorbance was read at 15-sec intervals for 2·5 min.

The activity was linear with time for both control and inhibited enzyme samples.

Enzyme inhibition measured by the pH-stat method. The pH-stat method of Jensen-Holm *et al.* [15] was used for experiments in 1·0 mM buffer (pH 6·0 and 7·4). Enzyme and inhibitor were incubated in a total volume of 10·0 ml for a given time before substrate was added (0·3 ml acetylcholine in water; final concn 10·0 mM). The liberated acetic acid was titrated with 0·02 M NaOH in a Syringe Burette Unit attached to a titrator and titrigraph (Radiometer Ltd., Copenhagen). The volume of added NaOH did not exceed 0·2 ml, and the time of assay was ≤ 5 min. The reaction mixture was kept under a stream of nitrogen. The enzyme activity was linear with time for both control and inhibited enzyme samples.

Stability of trichlorphon. The stability of trichlorphon was measured polarographically by a modified method of Giang and Caswell [16]. According to this method trichlorphon gives a polarographic wave at a half-wave potential of −0·68 V. Our experimental conditions differed somewhat from those described by the above authors, and our half-wave potential was at −0·8 V. The measurements were made on a Pen-recording polarograph (Cambridge, England) with a dropping mercury cathode and a mercury pool anode (drop time 3 sec). Solutions of trichlorphon (0·150 and 15·0 mM) in phosphate buffer (0·1 M, pH 7·4) were stored at 25°. At suitable time intervals aliquot samples were withdrawn and if necessary suitably diluted with buffer. Gelatine and KCl were added (final concns: 0·002% gelatine and about 0·2–0·4 M KCl), and nitrogen bubbled through the samples for 10 min. After that time, the polarogram was recorded between −0·2 and −1·8 V, the halfwave potential being at −0·8 V. The height of the polarographic wave measured immediately after preparing the trichlorphon solutions was taken as 100% trichlorphon concentration; the heights measured at other time intervals were calculated as per cent of that value.

Spontaneous reactivation of the inhibited enzyme. The enzyme was incubated (10 min) with a suitable inhibitor concentration to obtain 65–85 per cent inhibition. The enzyme–inhibitor solution was then diluted (up to 300-fold) with 0·1 M buffer pH 7·4, and kept at 37°. At suitable time intervals samples were withdrawn and the enzyme activity determined by the spectrophotometric method (cf. above). The detailed procedure is described elsewhere [17].

RESULTS AND DISCUSSION

Kinetics of enzyme inhibition in trichlorphon solutions. The following enzyme sources were used for studying the time course of inhibition at pH 7·4 in 0·1 M buffer: acetylcholinesterase of purified bovine erythrocytes and rat brain homogenate, and cholinesterase of human, rat and horse plasma. For each enzyme preparation 2 to 3 different trichlorphon concentrations were applied, and the rate of inhibition measured at 25° and 37°. The results obtained with horse serum cholinesterase are presented in Fig. 1a. Initially, the rate of inhibition is slow; as the reaction proceeds, the rate of inhibition accelerates. Such characteristics of the inhibition curves were observed

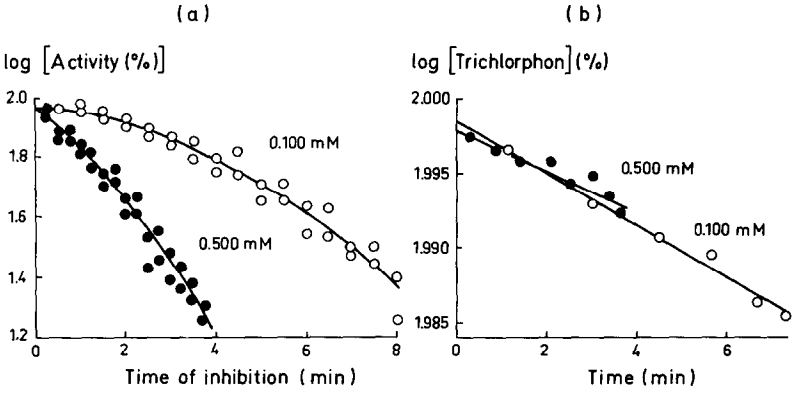

Fig. 1. (a) Inhibition of horse serum cholinesterase in 0·10 and 0·50 mM trichlorphon solutions at 37°. (b) Rate of decomposition of trichlorphon calculated from the kinetics of enzyme inhibition. The lines are calculated regression lines.

with all enzyme preparations, and at both temperatures.

The shape of the inhibition curves indicates that the inhibitor concentration continuously increases during the reaction. Assuming that DDVP was the only inhibitor, the rate of its formation from trichlorphon was calculated in the following way. The inhibition curves were divided into segments which correspond to a decrease of 0·10 log % activity, and through these points lines were calculated. The slopes of these lines (k) are proportional to the inhibitor concentration ($k = k_a[I]$) at that time interval. Knowing k_a for DDVP (cf. following section) the concentrations [I] corresponding to every particular line were calculated. The increase in DDVP concentration must correspond to the decrease in trichlorphon concentration; consequently, the difference [trichlorphon$_{(initial)}$] − [DDVP$_{at\ time\ t}$] should equal [trichlorphon$_{at\ time\ t}$]. By plotting log [trichlorphon] as a function of time, the rate constant of decomposition of trichlorphon can be obtained. This is shown in Fig. 1b, for the results presented in Fig. 1a. The lines in Fig. 1b are calculated regression lines and their slope corresponds to the rate constant of trichlorphon decomposition.

The calculated rate constants are summarized in Table 1. At 25°, the mean value of the rate constants is $3{\cdot}5 \times 10^{-4}$ min^{-1} and at 37° it is 33×10^{-4} min^{-1}. At both temperatures, the range of the rate constants is fairly wide. However, there is no trend in the values of the rate constants, neither with respect to the source of enzyme, nor with respect to the trichlorphon concentration. It is worthwhile noting that during the observed time of enzyme inhibition (wherefrom the above rate constants were calculated), less than 2 per cent of trichlorphon has to decompose into DDVP, in order to quantitatively account for the enzyme inhibition. The wide range of the calculated rate constants is probably due (at least in part) to such a small fraction of the decomposed trichlorphon.

Published experiments on inhibition of cholinesterases in trichlorphon solutions have all been done by applying longer times of inhibition, than used in this paper; furthermore, the concentrations of DDVP formed during that time have not been taken into account for the interpretation of the results. This probably explains why it was suggested by some authors that trichlorphon is an inhibitor of cholinesterases.

Table 1. Decomposition of trichlorphon (in 0·1 M buffer pH 7·4)

Enzyme	Trichlorphon (mM)	$10^4 \times k$ (min^{-1}) 25°	37°
Acetylcholinesterase			
Bovine erythrocytes	0·15	3·69	
	0·50	2·80	
Rat brain	0·15	5·08	28·2
	0·50	3·48	36·4
Cholinesterase			
Human plasma	0·01	7·08	41·6
	0·05	2·39	46·2
	0·10	4·17	
Horse plasma	0·10	3·97	39·4
	0·50	2·68	30·8
	1·00	1·46	
Rat plasma	0·15	3·13	20·0
	0·50	2·47	22·2

Values calculated from the kinetics of inhibition of acetylcholinesterase and cholinesterase. First order rate constants for decomposition (k) are given.

Arthur and Casida [3] and Hassan *et al.* [10] used 30–120 min as the time of inhibition (at 30° and 37°); during that time up to 40 per cent of trichlorphon will decompose into DDVP. Du Bois and Cotter [9] used the manometric technique and measured the enzyme activity for 30 min. Bueding *et al.* [12] calculated the inhibitory power of trichlorphon from the degree of inhibition obtained after 10 min, and not from the time course of the reaction. The same was done by Metcalf *et al.* [7] (time of inhibition 45 min); these authors suggest that DDVP, and not trichlorphon, is the enzyme inhibitor, because the degree of inhibition was more pronounced at high pH (the pH ranged from 5·4 to 9·4).

Miyamoto [8] investigated the mechanism of inhibition of fly head acetylcholinesterase in trichlorphon solutions; the inhibitor was preincubated in buffer (zero, 5 and 20 min preincubation, at pH 6·5 or pH 7·6) before the enzyme was added. Preincubation made trichlorphon a better inhibitor of the enzyme, while preincubation of DDVP in buffer made no difference to the inhibition by DDVP. The amounts of DDVP formed from trichlorphon during the preincubation period were calculated, and the calculated amounts agreed well with the concentrations of DDVP required for inhibition. Miyamoto [8] therefore stated that trichlorphon is not an inhibitor of fly head acetylcholinesterase.

Kinetics of enzyme inhibition by DDVP. The time course of inhibition was measured by varying the concentration of DDVP (2 or 3 different DDVP concentrations for each enzyme source) and the time of inhibition (up to 10 min). The rate of inhibition of acetylcholinesterase and cholinesterase by DDVP follows the kinetics of a bimolecular reaction. A plot of log enzyme activity against time is linear, and first order rate constants of inhibition (k) are linearly related to the DDVP concentration (cf. Fig. 2). The derived second order rate constants for inhibition (k_a) in 0·1 M buffer at pH 7·4 are summarized in Table 2.

The rate of inhibition of bovine erythrocyte acetylcholinesterase was also measured in 1·0 mM buffer at pH 6·0 and 7·4. The k_a constants differ little at these two pH values (Table 3). This finding fits the results on the effect of pH on k_a of other non-charged acylating

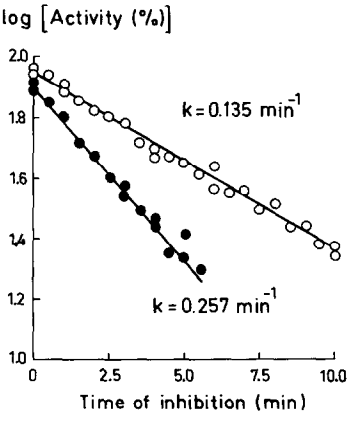

Fig. 2. Inhibition at 37° of horse serum cholinesterase by DDVP. 1·50 μM (○) and 3·00 μM (●). The first order rate constants of inhibition (k) were obtained from the calculated regression lines.

Table 2. Second order rate constants for inhibition (k_a) of acetylcholinesterase and cholinesterase by DDVP (in 0·1 M buffer pH 7·4)

Enzyme	$10^{-4} \times k_a$ (M^{-1} min^{-1}) 25°	37°
Acetylcholinesterase		
Bovine erythrocytes	2·62	—
Rat brain	6·85	15·4*
Cholinesterase		
Human plasma	50·1	87·0*
Horse plasma	7·77	8·78
Rat plasma	6·90	18·3*

* Škrinjarić-Špoljar *et al.* [17].

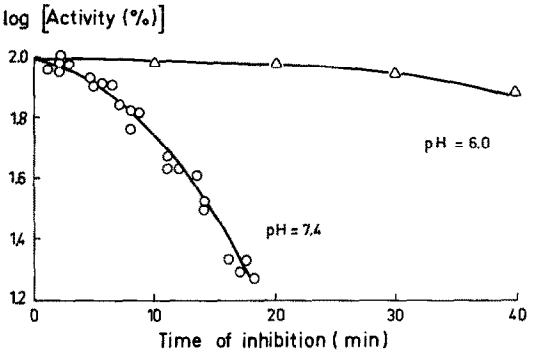

Fig. 3. Effect of pH on inhibition of bovine erythrocyte acetylcholinesterase by a 0·10 mM solution of trichlorphon in 1·0 mM buffer at 37°. The points at pH 6·0 are mean values of four experiments. The points at pH 7·4 represent individual results.

inhibitors, where the k_a was found to be almost independent of pH between 5 and 8 [18].

Effect of pH on inhibition of bovine erythrocyte acetylcholinesterase in trichlorphon solutions. The rate of inhibition of bovine erythrocyte acetylcholinesterase was measured at pH 6·0 and 7·4, in 1·0 mM buffer at 37°. At pH 6·0, the trichlorphon concentrations were 0·100 and 0·500 mM, and at pH 7·4 they were 0·025 and 0·100 mM. The results with 0·100 mM trichlorphon are given in Fig. 3. It is obvious that the rate of inhibition is much slower at pH 6·0 than at pH 7·4. The rate of decomposition of trichlorphon was calculated, and the obtained rate constants are given in Table 3. At pH 6·0 trichlorphon is 30 times more stable than at pH 7·4. This effect of pH agrees with results of Metcalf *et al.* [7] and Miyamoto [8], which were referred to above.

Spontaneous reactivation of the inhibited enzyme. The rate of spontaneous reactivation was measured after inhibition in trichlorphon solutions of acetylcholinesterase (human and bovine erythrocytes) and cholinesterase (human plasma).

For all three enzyme preparations, the rate of spontaneous reactivation was the same as that obtained after inhibition by DDVP (cf. 17). In Fig. 4, this is shown for bovine erythrocyte acetylcholinesterase. For human erythrocyte acetylcholinesterase the determined rate constant for spontaneous reactivation (k_{+3}) was 0·0138 ± 0·0011 min^{-1} (mean of 5 experiments), and this agrees with the k_{+3} obtained after inhibition by DDVP, which is 0·0136 min^{-1}. Human plasma cholinesterase, inhibited in a trichlorphon solution, reactivates 15 per cent within 25 hr; this would correspond to a k_{+3} of >0·00011 min^{-1}, and this too agrees with the results obtained after inhibition by DDVP.

The few published data on the rates of spontaneous reactivation after inhibition by trichlorphon also indicate that the rates are the same as after inhibition by DDVP. The recovery of rat erythrocyte acetylcholinesterase activity after inhibition by trichlorphon was measured by Arthur and Casida [3], and found to be the same as after inhibition by DDVP. The recovery *in vivo* of human plasma cholinesterase activity was measured in children who received therapeutic doses of trichlorphon orally [19]; the half-time, calculated from the published data on enzyme activity, was about 180 hr. This agrees with the slow rate of spontaneous reactivation obtained *in vitro* after inhibition of human plasma cholinesterase by DDVP (half-time <82·5 hr) [17].

Stability of trichlorphon. The stability of trichlorphon in 0·1 M buffer pH 7·4 was measured polarographically at 25° and 37°. The purpose of these studies was to establish the stability under the same experimental conditions as used for enzyme inhibition, and also to find if the rate of decomposition will be independent of the initial trichlorphon concentration. Two concentrations were tested, 0·150 and 15·0 mM. The latter concentration is the lowest which could be measured by this method. For both concentrations, the rate of decomposition was followed until about 50 per cent of the initial trichlorphon decomposed. At both

Table 3. Effect of pH on (a) inhibition of bovine erythrocyte acetylcholinesterase by DDVP and (b) on the rates of decomposition of trichlorphon

	pH 6·0	pH 7·4
(a) k_a (second order rate constant of inhibition by DDVP (M^{-1} min^{-1})	$4·2 \times 10^4$	$4·7 \times 10^4$
(b) k (first order rate constant of decomposition of trichlorphon) (min^{-1})	$(0·73 \pm 0·12) \times 10^{-4}$	$(22·2 \pm 1·7) \times 10^{-4}$

Values calculated from the kinetics of inhibition of bovine erythrocyte acetylcholinesterase in trichlorphon solutions. Both effects were measured in 1·0 mM buffer at 37°.

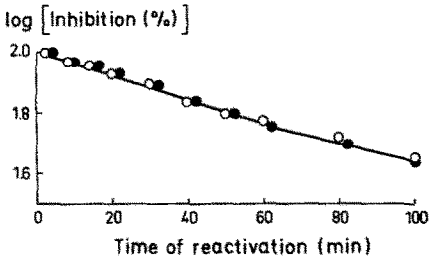

Fig. 4. Spontaneous reactivation of bovine erythrocyte acetylcholinesterase (pH 7·4 and 37°) after inhibition by trichlorphon (○) and DDVP (●).

temperatures and for both concentrations, the rate of decomposition followed the kinetics of a first order reaction. This is shown in Fig. 5, for the decomposition of 0·150 mM trichlorphon at 37°. All calculated first order rate constants for decomposition are given in Table 4.

Trichlorphon appears to be more stable at 15·0 mM than at 0·150 mM. However, the difference is small, particularly at 25°. The half-time of decomposition at 37° is 2·64 hr (mean for both concentrations) and at 25° it is 17·5 hr (mean for both concentrations).

DDVP, which is formed from trichlorphon, has a very similar stability. The half-time of DDVP (0·010 and 0·500 mM solution in 0·1 M buffer pH 7·4) is 2·9 hr at 37° (unpublished data) and for the same experimental conditions at 25° it is 15·6 hr [20].

There are no published data on the stability of trichlorphon measured under the same experimental conditions as in this paper. However, our results compare with published data on the stability of trichlorphon determined under comparable conditions. Metcalf *et al.* [7] and Dedek and Schwarz [21] found that at 37·5° the half-time of trichlorphon is 6·4 hr at pH 7·0 and about 1 hr at pH 8·0; Miyamoto [8] found that at pH 7·6 and 37° the half-time is 1·7 hr; our value of 2·64 hr at pH 7·4 and 37° fits into that range (cf. Table 4). Metcalf *et al.* [7] reported also that at pH 6·0 trichlorphon is 14 times more stable than at pH 7·0 and Miyamoto [8] found that at pH 6·5 trichlorphon is 7 times more stable than at pH 7·6; this too fits with our data that trichlorphon is 30 times more stable at pH 6·0 than at pH 7·4 (cf. Table 3).

Little is known about the kinetics of decomposition with respect to the initial trichlorphon concentration. Miyamoto [8] reported the same rate of decomposition for initial trichlorphon concentrations ranging from 20 to 300 mM. We observed a trend in stability

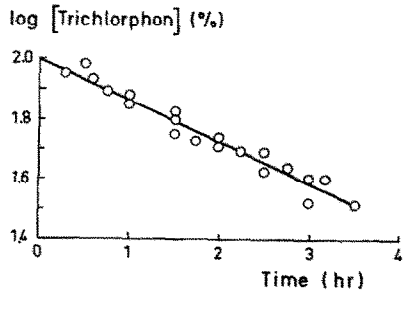

Fig. 5. Decomposition of trichlorphon (0·15 mM) measured polarographically in 0·1 M buffer pH 7·4 at 37°. The line is the calculated regression line.

Table 4. Decomposition of trichlorphon in 0·1 M buffer pH 7·4

Trichlorphon (mM)	$10^4 \times k$ (min^{-1})	
	25°	37°
0·150	7·27 ± 0·39 (33)	53·1 ± 1·2 (20)
15·0	6·05 ± 0·52 (18)	37·1 ± 1·2 (40)

The rate of decomposition was measured polarographically. First order rate constants (k) ± standard errors are given. The numbers in parentheses are the number of individual results from which k was calculated.

with respect to the trichlorphon concentration, but our initial concentrations were smaller and the concentration range larger (cf. Table 4), which makes a strict comparison difficult.

The effect of temperature on the stability of trichlorphon at pH 1–5 was extensively investigated by Mühlmann and Schrader [4]; the activation energy calculated from their data is 97 kJ mole^{-1} (temperature range 0°–70°). Rybakov and Ermishkin [22] report an activation energy of 104 kJ mole^{-1} at neutral and alkaline pH and for a temperature range from 20° to 70°. The activation energy calculated from our results at 25° and 37° is 122 kJ mole^{-1}, which is close to the published data.

CONCLUSION

The stability of trichlorphon in aqueous solutions was determined in two ways i.e. from the kinetics of enzyme inhibition (which is an indirect method) and from polarographic data on the concentrations of trichlorphon (which is the direct method). The rate constants for decomposition of trichlorphon calculated from the kinetics of enzyme inhibition are lower or just within the range of the constants obtained by the polarographic method (cf. Tables 1 and 4). In the experiments of enzyme inhibition less than 2 per cent of the total trichlorphon decomposes into DDVP, whereas in the polarographic studies at least 50 per cent of trichlorphon was allowed to decompose. Taking that into account, the agreement between the two methods is good, and we consider it as a kinetic proof that DDVP, and not trichlorphon, is the enzyme inhibitor. If trichlorphon had inhibitory properties, the rate of decomposition calculated from the enzyme kinetics would be larger than that obtained polarographically. However, in our results the rate constants are not larger, but the same or lower than those obtained polarographically.

The mechanism of inhibition of cholinesterases by an organophosphorus compound is considered analogous to the mechanism of the nonenzymic, OH^--catalysed, hydrolysis of that compound [23]. The hydrolysis of trichlorphon is H^+-catalysed (cf. 4) and at neutral and alkaline conditions the rate of hydrolysis is almost negligible [3, 4]; it therefore seems plausible that this compound would not inhibit cholinesterases.

The rate constants of trichlorphon decomposition calculated from the enzyme kinetics are the same irrespective of which source of mammalian cholinesterase was used (cf. Table 1). This suggests that, under our experimental conditions, other enzymes do not take part in the conversion of trichlorphon into DDVP.

The rate of inhibition of bovine erythrocyte acetylcholinesterase in trichlorphon solutions is much faster at pH 7·4 than at pH 6·0 (cf. Table 3). However, the rate of inhibition of bovine erythrocyte acetylcholinesterase by DDVP is almost the same at these two pH values. Consequently, the difference can be attributed to a faster conversion of trichlorphon into DDVP at pH 7·4 than at pH 6·0; and this in turn agrees with published data on the stability of trichlorphon as a function of pH (cf. section on Stability of trichlorphon).

Finally, the rate of spontaneous reactivation of cholinesterases after inhibition by trichlorphon solutions, was the same as after inhibition by DDVP. This is no proof that DDVP is the enzyme inhibitor in trichlorphon solutions, but it is a necessary condition which must be fulfilled, if DDVP is the inhibitor.

Consequently, all the results presented in this paper point to the conclusion that trichlorphon is not an inhibitor of mammalian cholinesterases. Inhibition of the enzymes by trichlorphon solutions can be quantitatively explained as due to DDVP formed nonenzymatically from trichlorphon.

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