# Mode of Action of Organophosphate Anthelmintics. Cholinesterase Inhibition in *Ascaris lumbricoides*

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Adult female *Ascaris lumbricoides* are poisoned on injection of certain organophosphates into the worms at a dose of 0.2 to 2.0 mg. per kg. The toxic compounds are potent inhibitors, both in vivo and in vitro, of an acetylcholinesterase (AChE) in the anterior end of the worms. Fifty-six compounds are included in this study on the relation among toxicity, potency as in vivo and in vitro AChE inhibitors, and sensitivity of other *Ascaris* esterases to inhibition. AChE activity in poisoned worms recovers only slowly from in vivo inhibition. Detoxication of organophosphates occurs at a very low rate in the worms as compared to insects or mammals; phosphorothionates and phosphorodithioates are not converted in vivo to the more toxic phosphate and phosphorothiolate analogs; other reactions of the organophosphates, including hydrolysis, proceed at a low rate in the worms or homogenates of the worms.

ORGANOPHOSPHORUS compounds are becoming of increasing importance as anthelmintic agents. The following chemicals are of sufficient selectivity in control of helminths without deleterious effect on the host to be of current interest as anthelmintics: *O,O*-dimethyl 2,2,2-trichloro-1-hydroxyethyl phosphonate (trichlorfon) and its dehydrochlorination product, *O,O*-dimethyl 2,2-dichlorovinyl phosphate (dichlorvos); *O,O*-dimethyl *O*-(2,4,5-trichlorophenyl) phosphorothionate (ronnel); *O*-(4-*tert*-butyl-2-chlorophenyl) *O*-methyl methylphosphoramidate (Ruelene); and the *O,O*-di-(2-chloroethyl) phosphate (Haloxon), *O,O*-diethyl phosphate (coroxon), and *O,O*-diethyl phosphorothionate (coumaphos) esters of 3-chloro-4-methyl-2-oxo-2*H*-1-benzopyran-7-ol. Only limited structure-activity data have been published on organophosphate anthelmintics (*22*).

Cholinesterase (ChE) inhibition has been implicated in the mode of action of those organophosphates used in control of other animal pests and would also be presumed to be involved in their anthelmintic activity. Only in the case of *Haemonchus contortus* ChE has the inhibition of helminth esterases by organophosphates been considered in any detail (*21*).

*Ascaris lumbricoides* was selected as the experimental organism for further consideration of helminth esterase inhibition by organophosphates. Studies on esterase localization and substrate specificity served to define the acetylcholinesterase (AChE) system to be used in the toxicological examinations. In order to evaluate the significance of AChE inhibition, the relation of organophosphate structure to both in vivo and in vitro inhibition was considered. Detoxication of organophosphates, in living worms and homogenates of the worms, was also evaluated as a limiting factor in anthelmintic activity.

## Materials and Methods

**Enzyme Source and Homogenization.** Adult female *Ascaris lumbricoides* averaging about 5 grams in weight (4 to 7 grams as a range) were obtained from Oscar Mayer and Co., Inc., Madison, Wis. The helminths, immediately upon removal from swine intestine, were placed in a 0.164*M* sodium chloride solution at 37.5° C. for up to 1 hour, and were then rinsed once with lake water at 37.5° C. For in vivo studies, the worms were then placed in Kronecker's solution (0.164*M* sodium chloride–0.0015*M* sodium hydroxide) at 37.5° C. for up to 3 days. Worms for in vitro studies were frozen after decanting the salt solution and were held at −25° C. for up to 10 days. Homogenates of different *Ascaris* sections were made in bicarbonate buffer (0.357*M* sodium bicarbonate and 0.164*M* sodium chloride) in an all-glass homogenizer at 5° C. Protein was determined by the biuret method of Robinson and Hogden (*27*) using fresh bovine serum albumin (Nutritional Biochemical Corp., Cleveland, Ohio) as the standard.

**Substrates and Inhibitors.** The following esters were utilized as substrates: acetylcholine iodide (ACh), propionylcholine *p*-toluenesulfonate (PrCh), butyrylcholine *p*-toluenesulfonate (BuCh), acetyl-β-methylcholine bromide (MeCh), acetylthiocholine iodide (ATCh), propionylthiocholine chloride (PrTCh), butyrylthiocholine iodide (BuTCh), benzoylcholine chloride (BzCh), phenyl acetate (PhA), phenyl *n*-propionate (PhP), phenyl *n*-butyrate (PhB), glyceryl triacetate (TA), glyceryl tri-*n*-butyrate (TB), ethyl acetate, ethyl *n*-butyrate, methyl *n*-butyrate, β-dimethylaminoethyl acetate, and β-dimethylaminoethyl *n*-butyrate. The aliphatic choline esters and PhP were obtained from California Corp. for Biochemical Research, Los Angeles, Calif.; BzCh was from Nutritional Biochemical Corp.; PhB was obtained from K and K Laboratories, Plainview, N. Y.; all other esters were from Eastman Organic Chemicals, Rochester, N. Y.

Chemical designations and sources for the inhibitors are given in Table IV.

**Estimation of Esterase Activity.** Acid released on ester hydrolysis was determined indirectly at 37.5° C., using a Warburg apparatus, as $CO_2$ evolved from a bicarbonate buffer (0.357*M* bicarbonate and 0.164*M* sodium chloride) in an atmosphere of 95% $N_2$ and 5% $CO_2$. Sodium chloride, at the level used in the assay, had no apparent effect on esterase activity. Single side-arm flasks of 7-ml. total volume were used, usually with the substrate in 0.4 ml. of buffer in the side arm and the enzyme in 1.6 ml. of buffer in the main compartment. Flasks were gassed for 7 minutes and equilibrated for 10 minutes at 37.5° C. prior to initiating the reaction by tipping in the substrate. Manometer readings were taken at 5-minute intervals for 30 minutes. When the rate of $CO_2$ evolution decreased within the 30-minute period, only the initial linear portion was used in deriving the $b_{30}$ value (microliters of $CO_2$ evolved per 30 minutes after correction for nonenzymatic hydrolysis of the substrate and endogenous liberation of $CO_2$ by the enzyme in the absence of substrate).

In experiments where mixtures of substrates were studied, the homogenate was placed in the side arm and the substrates were placed in the main compartment of the flask.

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Esterase activity was studied as a function of substrate concentration with the choline and thiocholine esters. The abbreviation, $pS$, is used to indicate the negative logarithm of the molar substrate concentration. The noncholine esters were used at 10 m$M$. Those which were not water-soluble at this level were emulsified in the bicarbonate buffer with Triton X-100 (Rohm and Haas Co., Philadelphia, Pa.). The emulsified substrates (EM) contained 2.5 mg. of Triton X-100 per ml. as added to the flasks, yielding a final level of 1 mg. of Triton X-100 per flask.

**In Vitro Esterase Inhibition Studies.** Solutions of the inhibitors were prepared in water, whenever possible. When the inhibitors were not soluble in water, they were added to the bottom of the flask in acetone solution, and the acetone was evaporated with an air jet prior to adding the enzyme preparation. The enzyme level was 4 sections (14 mg. of protein) per flask with all esters except those of the phenyl series, where one section per flask was used because of the higher activity of the esterase(s) on the aryl substrates. The nature of the sections is considered in the Results (see Selection of Body Region for ChE Studies). The homogenate, with and without inhibitor, was incubated for 20 minutes at 28° C. and 20 minutes at 37.5° C. (except for 62C47, which was incubated for 10 minutes at 28° C. and 20 minutes at 37.5° C.) before tipping to mix it with the substrate. All concentrations stated for inhibitors are those during the inhibition reaction and not during the assay of the esterase. The abbreviations, $pI$ and $pI_{50}$, refer to the negative logarithm of the molar inhibitor concentration and the negative logarithm of the molar inhibitor concentration for 50% inhibition, respectively.

In certain studies, the effectiveness of "protective homogenization" (*3*) with MeCh in reducing the inhibition of ChE in *Ascaris* sections by selected compounds was examined. For "protective" experiments, the MeCh (125m$M$) and inhibitor were added to the sections immediately prior to homogenization at 5° C. The homogenate was then placed in the main compartment of the Warburg flask and the MeCh was diluted to 100 m$M$ by tipping in buffer from the side arm at zero time. For "unprotective" experiments, the MeCh (500m$M$) was placed in the side arm and diluted to 100 m$M$ upon tipping at zero time into the main compartment, which contained the enzyme and inhibitor.

The "protective" method gave a $b_{30}$ of 90 and the "unprotective" method a $b_{30}$ of 80 in the absence of inhibitor. Each value for activity, both with and without inhibitor, is the mean of duplicate determinations in the same run.

**In Vivo Toxicity and ChE Inhibition Studies.** Adult female worms were injected with candidate toxicants at 1, 10, 100, and 1000 μg. per worm, doses approximately equivalent to 0.2, 2, 20, and 200 mg. of toxicant per kg. of worm. Three worms were injected with each dose of each compound using a 50-μl. syringe (Hamilton Co., Inc., Whittier, Calif.). The needle was inserted into the pseudocoel just anterior to the vulva, and the compound (dissolved in distilled water or reagent grade acetone) was injected in a total volume of 10 μl. The three worms were then placed in a culture tube (25 × 300 mm.), nearly filled with sodium chloride–sodium hydroxide solution, which was then stoppered loosely with a cork. After 24 hours at 37.5° C., the worms were individually transferred to a fresh sodium chloride–sodium hydroxide solution at 40° C. and, after simple flexure of the body, the symptoms of poisoning were observed by direct comparison with controls as described later. Acetone (10 μl.) did not produce any apparent ill effects during the 24-hour test period.

For in vivo ChE inhibition studies, the worms were injected with the compound as described above, except that 10 worms were used at each dose, which ranged from 0.03 to 1000 μg. per worm. After injection, the 10 worms were placed in 400-ml. beakers, about one-fourth filled with the sodium chloride–sodium hydroxide solution, which were then placed in a water bath at 37.5° C. After the appropriate time interval, the worms were examined for symptoms of poisoning and dissected into the standard 10-mm. anterior sections as described later. These 10 sections were homogenized, by the "protective" technique, in an all-glass homogenizer containing 120 mg. of MeCh; the homogenate was then made up to a final volume of 4 ml. with the sodium chloride–sodium bicarbonate buffer. A volume of 1.6 ml. of this homogenate was used per flask and 0.4 ml. of buffer was tipped in from the side arm at zero time. Duplicate analyses were made. To evaluate the spontaneous hydrolysis of the substrate and endogenous release of $CO_2$ by the enzyme, 10 sections were placed in a homogenizer containing the MeCh, and enough coroxon ($1 \times 10^{-4}M$) was added to inhibit the ChE activity completely. This residual $CO_2$ evolution was usually around 10% of that represented by the uninhibited ChE activity. The use of acetone as an injection solvent did not alter the ChE activity.

**Inhibitor Destruction Studies.** The hydrolysis of certain organophosphates in vitro by *Ascaris* enzyme(s) was investigated in the presence and absence of $Mn^{+2}$ (1 m$M$). Initial studies were made using a 20% whole worm homogenate as the enzyme source, after adjusting the pH of the homogenate to 7.5 with 0.1$N$ sodium hydroxide, and 10m$M$ DFP and dichlorvos as substrates. Both enzyme and substrate were prepared in bicarbonate buffer (0.357$M$). The enzyme, with or without $Mn^{+2}$, was incubated for 20 minutes at 28° C. and 20 minutes at 37.5° C. prior to tipping in the organophosphate.

Interference in these assays resulted from the high endogenous activity (90 μl. of $CO_2$ per 30 minutes) of the whole homogenate. This endogenous activity was not affected by $Mn^{+2}$ nor by the phosphorothionates studied, but was inhibited about 10% by paraoxon and 110H60 and 25 to 35% by trichlorfon and dichlorvos with the organophosphates at 10m$M$. Centrifugation of the 20% homogenate at 20,600 G for 1 hour at 2° C. yielded a soluble fraction with endogenous activity of 10 μl. of $CO_2$ per 30 minutes and a particulate fraction with no endogenous activity. (It is possible that the particulate fraction contains enzymes active on substrates in the soluble fraction, so that centrifugation gives fractions whose combined endogenous value is less than that of the whole homogenate.) Subsequent experiments were made using aliquots of the soluble and particulate fractions, and of the whole homogenate, as enzyme sources in the presence of $Mn^{+2}$. Water-soluble organophosphates were used as 10m$M$ solutions; others were emulsified with Triton X-100 (2.5 mg. per ml.).

Additional in vitro studies were made with DFP and butonate. The sensitivity of the enzyme(s) hydrolyzing these two organophosphates to inhibition by *p*-chloromercuribenzoate, *o*-iodosobenzoate, or cupric chloride at $1 \times 10^{-4}M$ and the activating effect of the chloride salts of $Mn^{+2}$ and $Co^{+2}$ at 1m$M$ were investigated.

In one study, the in vivo fate of Bidrin was considered. A female worm (4.8 grams) was injected with 25 μg. of Bidrin-$C^{14}$ labeled in the *O*-methyl position. After 6 hours at 37.5° C., the whole worm was homogenized in acetone and the extracted, radioactive metabolites of Bidrin were analyzed by a chromatographic procedure (*24*).

## Results

**Selection of Body Region for ChE Studies.** In order to select a suitable body region as the ChE source for manometric studies, the anterior 15 mm. of *Ascaris* worms was severed, the intestinal tract was removed, and the body wall with its stroma was then cut into three 5-mm. sections. The activity for hydrolysis of 3m$M$ ACh was approximately 42 for the anterior 5-mm. section, 22 for the 5- to 10-mm. region, and 22 for the 10- to 15-mm. region, when expressed as micromoles of ACh hydrolyzed per 100 mg. of protein per hour. The anterior 10-mm. region minus the intestinal tract was accordingly used for all subsequent experiments and contained approximately 3.5 mg. of protein per section. The $b_{30}$ values were directly proportional to the homogenate concentration with ACh, ATCh, and MeCh as substrates.

**Substrate Specificity of Ascaris Esterases.** The hydrolysis of choline and thiocholine esters was inhibited by excess substrate, the levels for optimum activity being 3m$M$ for ACh, PrCh, and BuCh; 30m$M$ for ATCh, PrTCh, and BuTCh; and 100m$M$ for MeCh (Figure 1).

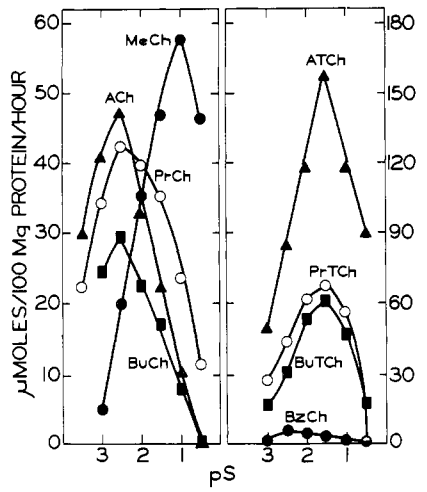


Figure 1. Activity-substrate concentration curves for hydrolysis of choline and thiocholine esters by *Ascaris* sections

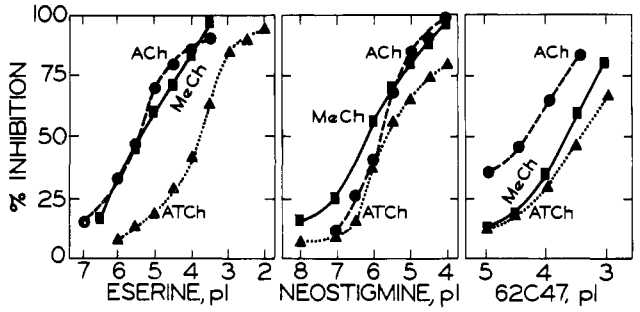


Figure 2. Inhibition of ChE in *Ascaris* sections by eserine, neostigmine, and 62C47 as assayed with ACh (3mM), ATCh (30mM), and MeCh (100mM)

Relative rates of hydrolysis at optimum substrate levels were acetyl > propionyl > butyryl for both the choline and thiocholine esters. Thiocholine esters were hydrolyzed at a faster rate than the corresponding choline esters. BzCh was not appreciably hydrolyzed. MeCh was hydrolyzed more rapidly than the choline esters when assayed at the optimum substrate concentration, and the rate of $CO_2$ evolution under this condition was undiminished for at least a 3-hour assay period. Only one of the *d*- or *l*-MeCh isomers was appreciably hydrolyzed, because the hydrolysis rate decreased markedly when half the theoretical $CO_2$ was evolved.

Hydrolysis of the phenol esters proceeded at the greatest rate of the substrates examined (Table I), even though the limited water solubility of these compounds required the use of emulsions (EM) in their assay. Glycerol triesters were also hydrolyzed rapidly, while most other esters examined were hydrolyzed slowly if at all. Hydrolysis rates for other esters at 10m*M* concentration, expressed as micromoles per 100 mg. of protein per hour, were as follows: β-dimethylaminoethyl acetate, 0; β-dimethylaminoethyl *n*-butyrate, 11; ethyl acetate, 0; ethyl *n*-butyrate, 0; methyl *n*-butyrate, 5. In contrast to the choline and thiocholine esters, the rate of hydrolysis of esters of glycerol, phenol, and β-dimethylaminoethanol increased with longer acyl groups.

The rate of $CO_2$ evolution ($b_{30}$) was compared for substrates individually and in paired mixtures. Substrates and levels examined were as follows: ACh, ATCh, and MeCh at their optimal substrate level and one half that value; TB at 1.5 and 10m*M*; PhA at 10m*M*. The $b_{30}$ values obtained with mixtures of any of the choline esters with TB closely approximated the sum of the $b_{30}$ values obtained with the choline ester and TB alone, while PhA mixed with the choline esters yielded $b_{30}$ values only slightly lower than that expected from summation of the $b_{30}$ values when the substrates were tested alone. In the case of mixtures of the choline esters or the PhA-TB mixture, the $b_{30}$ value was larger than with either substrate alone, but not as great as the sum of the individual $b_{30}$ values resulting with each substrate alone.

Esterases in the *Ascaris* homogenate appeared in several fractions obtained by differential centrifugation at 2° C. in the sodium bicarbonate–sodium chloride buffer. The distribution of esterase activity among the centrifugal fractions varied with the efficiency of homogenization; the most consistent results were obtained by using a small homogenizer (140 × 15 mm.). The enzyme hydrolyzing choline esters sedimented more readily than those hydrolyzing phenol or glycerol esters. For example, centrifugation at 20,600 G for 30 minutes resulted in sedimentation of 60 to 75% of the activity for hydrolysis of ACh, ATCh, BuCh, and MeCh but less than 40% of the activity for hydrolysis of TB, PhA, or PhB. The particulate fraction from 600 G centrifugation was always at least four times more active in hydrolyzing ACh, ATCh, MeCh, and PhB than the particulate fraction subsequently obtained when the supernatant, after removal of the 600 G precipitate, was centrifuged at 20,600 G.

**Selective Inhibition of Hydrolysis of Choline and Noncholine Esters.** The efficiency of 62C47 and the carbamate ChE inhibitors varied somewhat with the substrate, possibly owing in part to differences in the concentrations of the various choline or thiocholine esters used in the assays (Figure 2, Table I). Enzymatic hydrolysis of choline and thiocholine esters by *Ascaris* esterases was relatively resistant to inhibition by eserine, neostigmine, and 62C47. Eserine was less effective in inhibition of thiocholine than of choline or β-methylcholine ester hydrolysis. Neostigmine was not as selective as eserine in differentiating ACh and ATCh hydrolysis, whereas the hydrolysis of MeCh was more susceptible to inhibition. With 62C47, the choline ester hydrolysis was more easily inhibited than the thiocholine ester or MeCh hydrolysis. Inhibition of *Ascaris* ChE by organophosphates was influenced to a lesser degree by the nature of the substrate than with 62C47 and the carbamate inhibitors (Tables I and II). The degree of inhibition resulting from coroxon, mevinphos, and Ruelene did not vary with the substrates, ACh, ATCh, or MeCh, used in the assays. The enzymatic hydrolysis of MeCh was more severely inhibited by dichlorvos than that of ACh or ATCh, and ATCh hydrolysis was less sensitive to DFP and paraoxon than ACh or MeCh hydrolysis. The nature of the acyl group, whether acetyl or butyryl, did not greatly alter the susceptibility of choline or thiocholine ester hydrolysis to inhibition by eserine, 62C47, DFP, or paraoxon (Table I).

**Table I. Substrate and Inhibitor Specificity for Esterases in *Ascaris* Sections**

| Substrate | | Activity, μMoles/ 100 Mg. | Inhibition, % | | | |
|---|---|---|---|---|---|---|
| Ester | Concn., mM | Protein/Hr. | Eserine, $10^{-4}$M | 62C47, $10^{-4}$M | DFP, $10^{-6}$M | Paraoxon, $10^{-7}$M |
| MeCh | 100 | 57 | 84 | 34 | 68 | 77 |
| ACh | 3 | 49 | 85 | 64 | 63 | 85 |
| BuCh | 3 | 30 | 81 | 64 | 68 | 81 |
| ATCh | 30 | 157 | 43 | 30 | 50 | 68 |
| BuTCh | 30 | 63 | 50 | 20 | 59 | 75 |
| TA | 10 | 11 | 97 | 45 | 72 | 97 |
| TB | 10(EM) | 54 | 69 | 10 | 89 | 86 |
| PhA | 10(EM) | 357 | 13 | 20 | 22 | 75 |
| PhP | 10(EM) | 748 | 3 | 10 | 14 | 82 |
| PhB | 10(EM) | 1080 | 6 | 3 | 12 | 88 |

**Table II. Sensitivity of Hydrolysis of ACh, ATCh, and MeCh by *Ascaris* Sections to in Vitro Inhibition by Selected Organophosphates**

| Organophosphate | $pI_{50}$ ACh, 3mM | ATCh, 30mM | MeCh, 100mM |
|---|---|---|---|
| Coroxon | 8.1 | 8.1 | 8.2 |
| DFP | 6.3 | 6.0 | 6.4 |
| Dichlorvos | 6.6 | 6.6 | 7.2 |
| Mevinphos | 6.7 | 6.6 | 6.7 |
| Ruelene | 5.3 | 5.3 | 5.3 |

For differentiating *Ascaris* esterases hydrolyzing choline and noncholine esters, eserine and 62C47 were found to be the most selective, DFP intermediate, and paraoxon the least selective (Table I). Paraoxon was very potent for inhibiting the hydrolysis of all the esters examined, and DFP for all except the phenol esters. Hydrolysis of the glycerol esters was more sensitive to inhibition than that of the choline esters with these organophosphates. Eserine and 62C47 were relatively poor inhibitors for the hydrolysis of the phenol esters. Eserine was more potent in inhibiting glycerol ester than thiocholine ester hydrolysis, while 62C47 was the most selective of the inhibitors studied for the ACh- and BuCh-hydrolyzing enzyme relative to other esterases.

**Toxicity Studies.** Adult female worms were poisoned by many known ChE inhibitors following injection of doses of 2 or even as low as 0.2 mg. of toxicant per kg. of body weight (Table IV). The symptoms of organophosphate poisoning in *Ascaris* appeared to differ from those observed in insects and mammals in lacking a definite hyperactivity period. The worms became progressively less active with time after treatment with the toxicants and only by critical comparison with control or untreated worms was it possible to rate effectiveness.

The compounds most toxic to the worms were: the vinyl phosphates, dichlorvos, SD 9129, and OS-1808; the saligenin cyclic phosphate, salioxon; the coumarinyl phosphate, coroxon; and the carbamate, Isolan. The di-(2-chloroethyl)phosphates were less toxic than their diethyl analogs (Haloxon *vs.* coroxon and 110H60 *vs.* paraoxon). The phosphonates and phosphorothiolates were also toxic, while the phosphorotrithioate, phosphorothionates, and phosphorodithioates were nontoxic. The phosphorothionates and phosphorodithioates were much less toxic than their phosphate and phosphorothiolate analogs (parathion *vs.* paraoxon, famphur *vs.* famoxon, coumaphos *vs.* coroxon, ronnel *vs.* ronnoxon, Bayer 22408 *vs.* 9002, salithion *vs.* salioxon, malathion *vs.* malaoxon, and dimethoate *vs.* dimethoxon). Phosphoramidates were nontoxic except for Ruelene. Piperazine adipate and santonin were as toxic as or more toxic than the carbamates that were examined, other than Isolan.

**Cholinesterase Inhibition and Recovery Studies.** Acetyl-β-methylcholine (MeCh) at 100m*M* was selected as the standard substrate for comparing the potency of a variety of organophosphates and carbamates as in vitro and in vivo inhibitors of *Ascaris* ChE because hydrolysis of this substrate is as sensitive or more sensitive to the organophosphates than ACh or ATCh hydrolysis. The most potent in vitro inhibitors were coroxon, tetram, OS 1808, paraoxon, Haloxon, and Bayer 9002 (Tables I, II, and IV).

For assay of ChE inhibition in worms injected with toxicants, the "protective homogenization" technique was utilized and served to minimize the further reaction of free inhibitor and enzyme on homogenization (Table III). Progressive inhibition of *Ascaris* ChE occurred in the first 4 hours following injection of coroxon, dichlorvos, or Haloxon, with the relative potency of the organophosphates decreasing in that order. Recovery of ChE activity was nearly complete by 32 hours following dichlorvos or Haloxon treatment but not with the more active coroxon (Figure 3). For comparison of ChE inhibition in vivo from other compounds, the time after treatment was standardized at 2 hours and the dose was varied (Table IV). As would be expected, in vivo inhibition was dose-dependent with all compounds studied.

The most potent compounds for in vivo ChE inhibition in *Ascaris* were tetram, coroxon, dichlorvos, SD 9129, and DFP, all of which poisoned the worms at very low dosages. The other toxic compounds also resulted in marked in vivo ChE depression, while the nontoxic organophosphates caused little if any ChE inhibition. Only in the case of butonate was the in vivo ChE inhibition greater than that anticipated from the in vitro study, based on the assumption that the organophosphates were uniformly distributed within 2 hours in the worms (Table IV).

**Inhibitor Destruction Studies.** DFP hydrolysis was catalyzed by soluble (S) and particulate (P) fractions obtained on centrifugation of whole worm homogenates at 20,600 G for 1 hour. This activity was stimulated by $Mn^{+2}$ (1m*M*) and inhibited by $Co^{+2}$ (1m*M*) as follows (activity expressed as micromoles per 100 mg. of protein per hour): with no divalent cation, S = 19 and P = 3; with $M^{+2}$, S = 48 and P = 5; with $Co^{+2}$, S = 10 and P = 2. With the $Mn^{+2}$-activated hydrolysis of DFP by *Ascaris* S and P fractions, *p*-chloromercuribenzoate and *o*-iodosobenzoate at $1 \times 10^{-4}M$ were not inhibitory, while $Cu^{+2}$ at $1 \times 10^{-4}M$ resulted in 67% inhibition of the P and complete inhibition of the S activity. Paraoxon was also hydrolyzed by the $Mn^{+2}$-activated S fraction at a rate of 6 μmoles per 100 mg. of protein per hour. There was no hydrolytic activity of the P fraction on paraoxon or of either fraction with parathion, 110H60, dichlorvos, malathion, trichlorfon, or tetra-*n*-propyldithionopyrophosphate (Stauffer B-8999-S). Butonate hydrolysis differed in many respects from that of DFP because the following values, as micromoles per 100 mg. of protein per hour, were obtained with this compound: with no divalent cation, S = 38 and P = 5; with $Mn^{+2}$, S = 39 and P = 12; with $Co^{+2}$, S = 14 and P = 5. The activity in the presence of $Mn^{+2}$ was not inhibited by $1 \times 10^{-4}M$ *p*-chloromercuribenzoate; however, *o*-iodosobenzoate at $1 \times 10^{-4}M$ inhibited the S and P fractions 34 and 9%, respectively. Cupric ion at $1 \times 10^{-4}M$ caused 42% inhibition of the soluble and 98% inhibition of the particulate fraction activities.

Oxidative *N*-demethylation of Bidrin occurred very slowly if at all in living *Ascaris* worms. Seventy per cent of the administered radioactivity was recovered by extraction with acetone 6 hours after injection of Bidrin-*O*-methyl-$C^{14}$; of this amount, 91% chromatographed as

**Table III. Effectiveness of Protective Homogenization with MeCh in Reducing in Vitro Inhibition of ChE in *Ascaris* Sections by Selected Compounds**

| Inhibitor | Concn., M | % Inhibition: Protective | Unprotective |
|---|---|---|---|
| Coroxon | $1 \times 10^{-7}$[a] | 56 | 90 |
| DFP | $3 \times 10^{-6}$ | 68 | 93 |
| Dichlorvos | $3 \times 10^{-7}$ | 56 | 72 |
| Ruelene | $1 \times 10^{-5}$ | 68 | 99 |
| Neostigmine | $3 \times 10^{-5}$ | 76 | 80 |

[a] Complete inhibition obtained with coroxon at molar levels of $3 \times 10^{-7}$ or higher, even with protective technique.

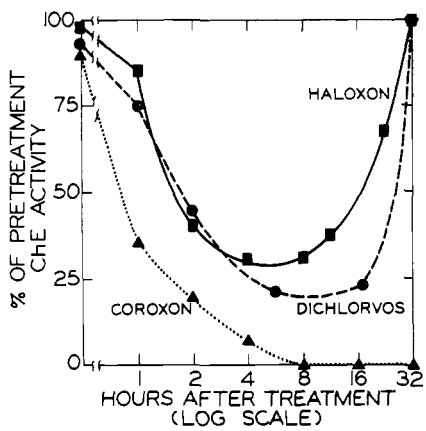


**Figure 3. Inhibition of ChE in *Ascaris* sections following injection of coroxon (0.3 μg./worm), dichlorvos (0.3 μg./worm), and haloxon (1.0 μg./worm)**
Assayed by protective MeCh technique

**Table IV. Relative Potency of Selected Toxicants in Poisoning *Ascaris***

| Toxicants[a] | Symptoms, 24 Hr.,[b] μG./Worm 1 | 10 | 100 | 1000 | In Vivo ChE Inhib., %, 2 Hr., μG./Worm 0.1 | 1.0 | 10 | 100 | 1000 | In Vitro ChE Inhib., $pI_{50}$ or % at $1 \times 10^{-4}M$[c] |
|---|---|---|---|---|---|---|---|---|---|---|
| Phosphates, (alkyl-O)$_2$P(O)O-aryl | | | | | | | | | | |
| 1. Ronnoxon, (MeO)$_2$P(O)OPhCl$_3$-2,4,5 | 0 | 1 | 2 | 3 | 0 | 4 | 27 | ... | .. | 6.3 |
| 2. Famoxon, (MeO)$_2$P(O)OPh-(SO$_2$NMe$_2$)-4 | 0 | 1 | 2 | 3 | 34 | 56 | 85 | ... | .. | 6.5 |
| 3. Paraoxon, (EtO)$_2$P(O)OPhNO$_2$-4 | 0 | 0 | 3 | 3 | 8 | 66 | 93 | ... | .. | 7.5 |
| 4. Coroxon, (EtO)$_2$P(O)O-(3-Cl-4-Me-2-oxo-2*H*-1-benzopyran-7-yl) | 1 | 2 | 3 | 3 | 54 | 96 | 100[d] | ... | .. | 8.2 |
| Phosphates, (ClCH$_2$CH$_2$O)$_2$P(O)O-aryl | | | | | | | | | | |
| 5. Haloxon, (ClCH$_2$CH$_2$O)$_2$P(O)O-(3-Cl-4-Me-2-oxo-2*H*-1-benzopyran-7-yl) | 0 | 0 | 2 | 3 | 5 | 60 | 100[d] | ... | .. | 7.5 |
| 6. 86H60, (ClCH$_2$CH$_2$O)$_2$P(O)O-(4-Me-2-oxo-2*H*-1-benzopyran-7-yl) | 0 | 1 | 2 | 3 | 0 | 57 | 81 | ... | .. | 7.4 |
| 7. 110H60, (ClCH$_2$CH$_2$O)$_2$P(O)OPhNO$_2$-4 | 0 | 0 | 1 | 3 | 1 | 76 | 97 | ... | .. | 7.2 |
| 8. 112H60, (ClCH$_2$CH$_2$O)$_2$P(O)OPhCl$_3$-2,4,5 | 0 | 0 | 1 | 2 | 1 | 1 | 65 | ... | .. | 6.3 |
| Phosphates, vinyl | | | | | | | | | | |
| 9. Dichlorvos, (MeO)$_2$P(O)OCH=CCl$_2$ | 0 | 3 | 3 | 3 | 30 | 83 | 100[d] | ... | .. | 7.2 |
| 10. Mevinphos, (MeO)$_2$P(O)OC(Me)=CHC(O)OMe | 0 | 1 | 3 | 3 | .. | ... | ... | ... | .. | 6.7 |
| 11. Ciodrin, (MeO)$_2$P(O)OC(Me)=CHC(O)OCH(Me)-Ph | 0 | 0 | 2 | 3 | .. | ... | ... | ... | .. | 7.4 |
| 12. SD 11319, (MeO)$_2$P(O)OC(Me)=CHC(O)NH$_2$ | 0 | 1 | 2 | 3 | 6 | 71 | 88 | ... | .. | 5.1 |
| 13. SD 9129, (MeO)$_2$P(O)OC(Me)=CHC(O)NHMe | 0 | 2 | 3 | 3 | 41 | 82 | 91 | ... | .. | 5.9 |
| 14. Bidrin, (MeO)$_2$P(O)OC(Me)=CHC(O)NMe$_2$ | 0 | 0 | 2 | 3 | 0 | 70 | 88 | ... | .. | 5.7 |
| 15. OS 1836, (EtO)$_2$P(O)OCH=CHCl | 0 | 1 | 2 | 3 | 0 | 12 | 87 | ... | .. | 6.3 |
| 16. GC 4072, (EtO)$_2$P(O)OC(PhCl$_2$-2,4)=CHCl | 0 | 0 | 0 | 2 | .. | ... | ... | ... | .. | 6.1 |
| 17. OS 1808, (EtO)$_2$P(O)OC(Me)=CHC(O)OEt | 1 | 1 | 3 | 3 | 4 | 53 | 98 | ... | .. | 7.7 |
| Phosphates, others | | | | | | | | | | |
| 18. Bayer 9002, (EtO)$_2$P(O)O-(naphthaloximido) | 0 | 0 | 1 | I[e] | 7 | 77 | 94 | ... | .. | 7.5 |
| 19. TOCP, (2-Me-PhO)$_3$P(O) | 0 | 0 | 0 | 0 | .. | ... | 0 | 0(0)[f] | 0 | (0) |
| 20. Methyldi-(*o*-cresyl)phosphate, (2-Me-PhO)$_2$P(O)OMe | 0 | 0 | 0 | 0 | .. | ... | 0 | 0(0)[f] | 9 | (0) |
| 21. Salioxon, MeOP(O)OPh-2-CH$_2$ | 0 | 2 | 3 | 3 | 6 | 49 | 89 | ... | .. | 6.1 |
| Phosphonates | | | | | | | | | | |
| 22. Butonate, (MeO)$_2$P(O)CH[OC(O)C$_3$H$_7$-*n*]CCl$_3$ | 0 | 0 | 3 | 3 | .. | ... | 36 | 96(63)[f] | 97 | 5.3 |
| 23. Trichlorfon, (MeO)$_2$P(O)CH(OH)CCl$_3$ | 0 | 0 | 3 | 3 | 0 | 24 | 94 | ... | .. | 6.2 |
| 24. DFP, (iso-PrO)$_2$P(O)F | 0 | 1 | 3 | 3 | 30 | 89 | 100 | ... | .. | 6.3 |
| Phosphoramidates | | | | | | | | | | |
| 25. Ruelene, MeO(MeNH)P(O)OPhCl-2-CMe$_3$-4 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | ... | .. | 5.3 |
| 26. Narlene, MeO(MeNH)P(S)OPhCl-2-CMe$_3$-4 | 0 | 0 | 0 | 0 | .. | ... | 0 | 0 | 0 | (5) |
| 27. Nellite, (MeNH)$_2$P(O)OPh | 0 | 0 | 0 | 0 | .. | ... | ... | ... | .. | (37) |
| 28. Hexamethylphosphoramide, (Me$_2$N)$_3$P(O) | 0 | 0 | 0 | 0 | .. | ... | 0 | 0(9)[f] | 0 | (15) |

[a] Sources. Compounds 2, 3, 29, 30, 34, 37, 42, 43, and 46 from American Cyanamid Co., Princeton, N. J.; 4, 18, 23, 32, 35,38–40, 44, 45, and 47 from Chemagro Corp., Kansas City, Mo.; 9 to 17 and 36 from Shell Development Co., Modesto, Calif.; 5 to 8 from Cooper Technical Bureau, Berkhamsted, Herts, England; 25 to 27 and 33 from Dow Chemical Co., Midland, Mich.; 49 from J. R. Geigy S. A., Basle, Switzerland; 31 from Imperial Chemical Industries, Ltd., Jealott's Hill Research Station, Bracknell, Berks, England; 20, 21, and 41 from M. Eto, Department of Agricultural Chemistry, Kyushu University, Fukuoka, Japan; 50, 51, 54, and 56 from California Corp. for Biochemical Research, Los Angeles, Calif.; 24, 28, and 53 from Aldrich Chemical Co., Inc., Milwaukee, Wis.; 19 and 52 from Eastman Organic Chemicals, Rochester, N. Y.; 48 from Burroughs Wellcome and Co. (U.S.A.), Inc., Tuckahoe, N. Y.; 55 from K and K Laboratories, Plainview, N. Y.; 1 from nitric acid oxidation (*28*) of 33; and 22 from 23 according to a described procedure (*2*). Abbreviations for designating structures: Me = methyl; Et = ethyl; Pr = propyl; Cl = chloro; and Ph = phenyl.

Bidrin, 2% as SD 9129, and 7% as further decomposition products, none of which were SD 11319 or *N*-hydroxymethyl intermediates in the demethylation reaction.

### Discussion

An acetylcholinesterase (AChE) is present in the anterior regions of the *Ascaris lumbricoides* body wall, as shown in this investigation and previous studies with *Ascaris* from swine. Bueding (*7*), also using a manometric technique, reported that homogenates of *Ascaris* muscle hydrolyze ACh (40m*M*) faster than BuCh (40m*M*), and Lee (*20*), using a histochemical method, has shown that ATCh is hydrolyzed to a greater degree by somatic muscles of the anterior end than by the other, more posterior, muscles of the body. In the present study, it was found that the rate of hydrolysis of choline esters by *Ascaris* anterior section homogenates is highest with ACh and decreases with increasing acyl chain length. Inhibition by excess choline ester substrate, which occurs with the *Ascaris* enzyme, is characteristic of AChE (*4*, *18*). The AChE from honey bee heads is similar to that from *Ascaris*, in that MeCh is hydrolyzed much faster than ACh at optimum substrate levels, and the optimum level for MeCh is much higher than that for ACh (*19*).

Inhibition by eserine of choline ester hydrolysis by *Ascaris* homogenates occurs at somewhat higher levels than is necessary for inhibition of AChE from mammalian and insect species. However, invertebrate AChE from a planarian (*13*) and from the parasitic nematode, *Haemonchus contortus* (*21*), are also relatively resistant to inhibition by this carbamate. Compound 62C47, a "selective" AChE inhibitor (*5*, *11*, *30*), and DFP, a "selective" pseudocholinesterase inhibitor (*1*, *25*), are not selective in their inhibition of ACh and BuCh hydrolysis, indicating that no pseudocholinesterase activity is assayed with the *Ascaris* homogenates. Lui *et al.* (*23*), using a histochemical technique similar to that employed by Lee (*20*), except that no selective inhibitors were used, reported the presence of an AChE and a pseudocholinesterase in *Ascaris* and identical distribution of the two enzymes. It would appear from the present study and that of Lee (*20*) that the two enzymes reported by Lui *et al.* (*23*) are one and the

**and as in Vivo and in Vitro Inhibitors of ChE in Anterior Sections**

| Toxicants[a] | Symptoms, 24 Hr.,[b] μg./Worm 1 | 10 | 100 | 1000 | In Vivo ChE Inhib., % 2 Hr., μg./Worm 0.1 | 1.0 | 10 | 100 | 1000 | In Vitro ChE Inhib. $pI_{50}$ or % at $1 \times 10^{-4}M$[c] |
|---|---|---|---|---|---|---|---|---|---|---|
| Phosphorothiolates | | | | | | | | | | |
| 29. Dimethoxon, $(MeO)_2P(O)SCH_2C(O)NHMe$ | 0 | 0 | 2 | 3 | .. | ... | 3 | 22 | 74 | 4.5 |
| 30. Malaoxon, $(MeO)_2P(O)SCH[C(O)OEt]CH_2C(O)OEt$ | 0 | 0 | 0 | 2 | .. | ... | ... | ... | .. | 6.5 |
| 31. Tetram, $(EtO)_2P(O)SCH_2CH_2NEt_2$, hydrogen oxalate | 0 | 0 | 3 | 3 | 88 | 100 | 100 | ... | .. | 7.8 |
| Phosphorotrithioate | | | | | | | | | | |
| 32. DEF, $(n\text{-}C_4H_9S)_3P(O)$ | 0 | 0 | 0 | 0 | .. | ... | ... | ... | .. | (3) |
| Phosphorothionates | | | | | | | | | | |
| 33. Ronnel, $(MeO)_2P(S)OPhCl_3$-2,4,5 | 0 | 0 | 0 | 0 | .. | ... | 0 | 0 | 9 | (1) |
| 34. Famphur, $(MeO)_2P(S)OPh$-$(SO_2NMe_2)$-4 | 0 | 0 | 0 | 1 | .. | ... | 0 | 30 | 59 | (10) |
| 35. Fenthion, $(MeO)_2P(S)OPhMe$-3-SMe-4 | 0 | 0 | 0 | 0 | .. | ... | 5 | 5(5)[f] | 37 | (2) |
| 36. Methyl parathion, $(MeO)_2P(S)OPhNO_2$-4 | 0 | 0 | 0 | 0 | .. | ... | ... | ... | .. | (5) |
| 37. Parathion, $(EtO)_2P(S)OPhNO_2$-4 | 0 | 0 | 0 | 0 | .. | ... | 0 | 3 | 24 | (24) |
| 38. Bayer 22408, $(EtO)_2P(S)O$-(naphthaloximido) | 0 | 0 | 0 | 0 | .. | ... | 0 | 0 | 16 | (5) |
| 39. Coumaphos, $(EtO)_2P(S)O$-(3-Cl-4-Me-2-oxo-2*H*-1-benzopyran-7-yl) | 0 | 0 | 0 | 0 | .. | ... | 0 | 0(0)[f] | 8 | (8) |
| 40. Potosan, $(EtO)_2P(S)O$-(4-Me-2-oxo-2*H*-1-benzopyran-7-yl) | 0 | 0 | 0 | 0 | .. | ... | 0 | 0(12)[f] | 12 | (2) |
| 41. Salithion, $MeOP(S)OPh$-2-$CH_2$ (O bridge) | 0 | 0 | 0 | 0 | .. | ... | 0 | 0 | 0 | (17) |
| Phosphorodithioates | | | | | | | | | | |
| 42. Dimethoate, $(MeO)_2P(S)SCH_2C(O)NHMe$ | 0 | 0 | 0 | 1 | .. | ... | 0 | 0 | 5 | (2) |
| 43. Malathion, $(MeO)_2P(S)SCH[C(O)OEt]CH_2C(O)OEt$ | 0 | 0 | 0 | 0 | .. | ... | 6 | 11(7)[f] | 40 | (3) |
| 44. Guthion, $(MeO)_2P(S)S$-(4-oxo-1,2,3-benzotriazin-3(4*H*)-yl methyl | 0 | 0 | 0 | 0 | .. | ... | 0 | 0(6)[f] | 5 | (3) |
| 45. Ethyl Guthion, $(EtO)_2P(S)S$-[4-oxo-1,2,3-benzotriazin-3(4*H*)-yl methyl] | 0 | 0 | 0 | 0 | .. | ... | 4 | 19(30)[f] | 54 | (10) |
| 46. Phorate, $(EtO)_2P(S)SCH_2SEt$ | 0 | 0 | 0 | 0 | .. | ... | 5 | 7(30)[f] | 75 | (5) |
| 47. Di-syston, $(EtO)_2P(S)SCH_2CH_2SEt$ | 0 | 0 | 0 | 0 | .. | ... | 0 | 4(0)[f] | 5 | (0) |
| Nonphosphorus-Containing | | | | | | | | | | |
| 48. 62C47, $[(4\text{-}Me_3\overset{+}{N})\text{-}PhCH_2CH_2]_2CO$, diiodide | 0 | 0 | 2 | I[e] | .. | ... | ... | ... | .. | 3.7 |
| 49. Isolan, $Me_2NC(O)O$-(1-*i*-Pr-3-Me-5-pyrazolyl) | 0 | 2 | 3 | 3 | 17 | 67 | 80 | ... | .. | 6.2 |
| 50. Eserine sulfate, physostigmine | 0 | 0 | 0 | 2 | .. | ... | 11 | 42 | 60 | 5.4 |
| 51. Neostigmine methylsulfate, prostigmine | 0 | 0 | 1 | 3 | .. | ... | 7 | 50 | 80 | 6.2 |
| 52. Diphenylcarbamyl chloride, $Ph_2NC(O)Cl$ | 0 | 0 | 0 | 2 | .. | ... | ... | ... | .. | (37) |
| 53. Phenothiazine | 0 | 0 | 0 | 2 | .. | ... | ... | ... | .. | (45) |
| 54. Santonin | 0 | 1 | 2 | 3 | .. | ... | ... | ... | .. | (19) |
| 55. Piperazine adipate | 0 | 0 | 2 | I[e] | .. | ... | ... | ... | .. | (52) |
| 56. Acetylcholine iodide | 0 | 0 | 0 | 0 | .. | ... | ... | ... | .. | ... |

[b] Symptoms. 0 = treated worms as active as controls; 1 = treated worms less active than controls; 2 = no spontaneous movement in treated worms, but movement after touched with probe; 3 = no movement in treated worms even after touched with probe.

[c] Inhibition time 20 minutes at 28° C. and 20 minutes at 37.5° C. except with Compound 48, in which case it was 10 minutes at 28° C. and 20 minutes at 37.5° C.

[d] Additional data indicating that in vivo ChE inhibition at 2 hours increases progressively with dosage: for coroxon, 13% at 0.03 μg., 74% at 0.3 μg., and 100% at 3.0 μg.; for Haloxon, 40% at 0.3 μg. and 85% at 3.0 μg.; for dichlorvos, 56% at 0.3 μg. and 100% at 3.0 μg.

[e] Insolubility in both acetone and water at necessary concentration for injection prevented assaying at indicated dosage.

[f] Figures in parentheses are per cent inhibition values in vitro with 2.5 μg. of compound/4 sections. This amount of compound is that which would have appeared in the 4 sections from in vivo studies at 2 hours, assuming uniform distribution throughout organism. Conversion in vivo to a more potent ChE inhibitor would be indicated by greater inhibition from in vivo than in vitro assay.

same. The greater activity using ATCh and BuTCh than with ACh and BuCh may be related in part to the fact that the data were not corrected for the ionization of thiocholine ($pK_a$ 7.7) (*14*), which would also cause some $CO_2$ evolution from the buffer at the pH of the assay (7.6). Also, hydrolysis of thiocholine esters by enzymes other than ChE has been reported with other organisms (*6*, *17*) and may also contribute to differences in the inhibition pattern for hydrolysis of these substrates by *Ascaris*.

It is difficult to conclude the number of esterases acting on each of the substrates from the experimental results reported. Substrate-summation and inhibition studies failed to indicate more than a single AChE acting on esters of choline, β-methylcholine, and probably thiocholine. The *Ascaris* AChE activity is present to the greatest degree in the particulate fraction obtained at low-speed centrifugation. With housefly and honey bee heads, the same AChE appears in the soluble or particulate fractions, depending on the conditions of preparation (*29*, *31*). The lack of any appreciable hydrolysis of ethyl acetate, ethyl *n*-butyrate, or methyl *n*-butyrate suggests that this type of aliesterase(s) is not present in the *Ascaris* homogenates.

*Ascaris lumbricoides* is susceptible to poisoning by very low dosages of organophosphates and carbamates that are also very potent in vitro and in vivo inhibitors of the AChE in this species. None of the organophosphates which did not inhibit the AChE were toxic. This suggests that the mode of anthelmintic action of these toxicants is related to inhibition of AChE. The structure-activity relationship for organophosphates as in vitro inhibitors of *Ascaris* AChE is not greatly different from that already established with the same or related compounds and other esterase sources (*16*, *26*). Attempts have frequently been made with various organisms to correlate the in vitro and in vivo potency of ChE inhibitors with their toxicity. Such correlations can be of no more than a superficial nature except within restricted series of closely related analogs. However, the toxicity of the organophosphates to *Ascaris lumbricoides* appears to be generally related to their activity both in vivo and in vitro as inhibitors of the AChE in the anterior sections as assayed with MeCh. Only the excep-

tions to this relationship will be discussed further.

Butonate, dimethoxon, salioxon, SD 9129, and SD 11319 are more toxic and/or more potent AChE inhibitors in vivo than would be predicted from their inhibitor potency in vitro. Toxicity of butonate may be related to its ability to form the more potent dichlorvos by enzymatic butyryl ester hydrolysis and subsequent spontaneous dehydrochlorination and rearrangement. No direct evidence is available on this point, however, other than the demonstration of esterases in *Ascaris* which release an acid moiety from the butonate molecule. There is at present no explanation for these apparent anomalies with the other compounds. Several of the organophosphates are more potent AChE inhibitors in vitro than their toxicity and/or in vivo AChE inhibitor potency would indicate. The chloroethyl phosphates fall in this category. Haloxon-inhibited AChE rapidly recovers activity in vivo, presumably by cleavage of the resulting *O,O*-di-(2-chloroethyl) phosphorylated-AChE; the related compounds (110H60 and 86H60) may also yield the same easily reactivatable form of cholinesterase inhibition in vivo. Other compounds with higher relative in vitro than in vivo potency were Bayer 9002, Ciodrin, GC 4072, malaoxon, paraoxon, and tetram, where the apparent deviation is as yet unexplained.

The in vitro reaction of Haloxon with *Haemonchus contortus* AChE is bimolecular and irreversible, whereas the reaction with sheep erythrocyte AChE, although probably bimolecular, is reversible (*21*). With *Ascaris* in the in vivo AChE inhibition studies, the enzymatic activity recovers to near pretreatment levels within 32 hours after treatment with Haloxon or dichlorvos but not with coroxon. This indicates that the order of in vivo stability for these compounds in *Ascaris* is coroxon > dichlorvos > Haloxon and/or that the rate of dephosphorylation of the inhibited esterase is di-(2-chloroethyl) > dimethyl > diethyl.

Metabolism of the ChE inhibitors by *Ascaris* proceeds much more slowly than has been previously noted for the same compounds with most mammals and insects. This conclusion is based on in vitro studies in which the hydrolysis of organophosphates was considered, toxicity and in vivo AChE inhibition studies, and limited radiotracer investigations.

Hydrolysis of organophosphates occurs at a relatively low rate in the intact worms and homogenates of the whole worms. Dichlorvos and Haloxon are probably largely detoxified in vivo within 18 hours. Homogenates failed to hydrolyze most compounds studied, were low in activity on paraoxon, and hydrolyzed only DFP and butonate at an appreciable rate. Differences in the inhibition and $Mn^{+2}$-activation patterns for the hydrolysis of DFP and butonate suggest that more than one esterase is involved. The *n*-butyryl group may be the primary site of initial enzymatic attack with butonate, particularly since the worms are active in hydrolysis of other butyryl esters but not in hydrolysis of dichlorvos or trichlorfon. The phosphorofluoridate group is probably the cleavage site with DFP, although in no case were the hydrolysis products characterized.

*Ascaris* apparently lacks the ability to convert many "precursor" organophosphates to potent anti-ChE metabolites. These reactions are usually catalyzed by a microsome-reduced nicotinamide adenine dinucleotide phosphate system; certain essential components of this system may be absent from the worms. Salioxon, or an AChE-inhibitor or toxicant of similar potency, is not formed in *Ascaris* from salithion or methyl di-*o*-cresyl phosphate; salioxon is formed from both of these precursors in houseflies (*9, 10*). Oxidative *N*-demethylation of Bidrin to yield toxic metabolites occurs very slowly if at all in *Ascaris* relative to the rate in houseflies and mammals, where extensive conversion of the dimethylamide (Bidrin) to the monomethylamide (SD 9129), unsubstituted amide (SD 11319), and *N*-hydroxymethyl intermediates has been noted (*24*). Phosphorothionates and phosphorodithioates are nontoxic to *Ascaris* relative to the toxicity of their "activation products," the phosphate and phosphorothiolate analogs. A similar observation has been made with horse strongyle larvae and *O,O*-diethyl-*O*-(2,4,5-trichlorophenyl) phosphorothionate and its phosphate analog (*22*). In the present studies, it was found that *Ascaris* converts less than 0.1% (or less than 1 μg. out of 1000 μg.) of the phosphorothionates to effective anti-ChE metabolites. However, certain phosphorothionates, such as ronnel, coumaphos, and famphur, are effective in controlling some helminths in host mammals (*8, 12, 15*). It is not clear whether some helminths other than *Ascaris* can readily carry out such conversions, or whether the active anthelmintic agent is formed in the host tissues in these cases.


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