Anticholinesterase Action of Methyl Parathion, Parathion and Azinphosmethyl in Mice and Fish: Onset and Recovery of Inhibition

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In recent studies we found that the lethal dose ranges for single intraperitoneal injections of parathion, methyl parathion and azinphosmethyl in mice were 13-15, 10-12 and 3-4.5 mg/kg respectively and in sunfish were 10-200, >2500 and 1-10 mg/kg respectively (BENKE, et al., 1973). LC50 determinations in sunfish have also demonstrated their resistance to methyl parathion and susceptibility to azinphosmethyl, relative to parathion (MINCHEW and FERGUSON, 1970, and PICKERING, et al., 1962). Since the acute toxicity of these organophosphorus compounds may be partially a function of the rapidity of acetylcholinesterase (AChE) inhibition (O'BRIEN, 1960) and the degree to which the organism can reverse this inhibition (REINER, 1971, and VANDEKAR and HEATH, 1957), we felt that determinations of the rate of onset and recovery of AChE inhibition in sunfish and mice might partially explain the large differences in acute toxicity of the above organophosphorus insecticides in fish, relative to mice. Furthermore, this information would be of value in other respects. For example, the suggested use of fish brain AChE as a monitor for pollution by organophosphate insecticides (WEISS, 1958, 1959 and 1965, HOLLAND, et al., 1967, and WILLIAMS and SOVA, 1966) requires knowledge of control activities and times required for fish AChE's to return to normal. In addition, speed of onset of poisoning by these compounds in mammals is important from the point of view of prompt efficacious treatment, and furthermore the residual effects in mammals (especially man) following single large exposures is important, for example, in considering when a worker can return to his job.

Materials and Methods

Chemicals: Parathion (0,0-diethyl p-nitrophenyl phosphorothioate), methyl parathion (0,0-dimethyl p-nitrophenyl phosphorothioate) and azinphosmethyl [0,0-dimethyl S(4-oxo-1,2,3-benzotriazin-3(4H)-ylmethyl)phosphorodithioate] were supplied by the manufacturers. All were of 99+% purity. Acetylthiocholine iodide, and 5,5'-dithiobis-2-nitrobenzoic acid were purchased from the Sigma Chemical Company.

Animals and treatments: The compounds were administered ip in corn oil solutions (2 ml/kg for fish and 5 ml/kg for mice).

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Pumpkinseed sunfish (Lepomis gibbosus), 4-15 g were held in aerated 20 gallon tanks at 18-20°C and were fed dried shrimp fish food twice daily except during treatment days. In the time-to-onset studies 2 treated fish were removed at indicated times for each compound and frozen on dry ice. Two or 3 control fish given corn oil only were also removed and processed in the same manner. In the recovery-from-inhibition studies, 4 fish per compound were used for each time period.

Male Charles River mice (25-35 g) were housed in air-conditioned rooms and fed and watered ad libitum. Five mice were used per point for each insecticide.

Enzyme preparations: Brains were dissected from frozen fish and 1% homogenates in 1.0 ml 0.1 M pH 8 phosphate buffer were prepared using a Polytron homogenizer (Brinkman Instruments). Fish muscle samples of about 100 mg were taken from the left anterior-dorsal area, and 2% homogenates were made. Brains and diaphragms were removed from mice when they were sacrificed (by spinal separation) and placed in 1 ml ice-cold 0.1 M phosphate buffer pH 8. Ten % homogenates were prepared and diluted 1:13 in additional buffer. Homogenates of mouse diaphragms (2.5%) were prepared in a similar manner and assayed without further dilution. For both fish and mice, 0.1 ml of the enzyme preparations were used in the AChE assay. Tissue levels were selected so that controls gave absorbance readings in the AChE assay of 0.8-1.0 O.D. units.

Acetylcholinesterase assay: AChE activity was measured colorimetrically using the method of ELLMAN, et al. (1961) essentially as modified by VOSS and SCHULER (1967). Duplicate determinations were performed on all samples.

Results and Discussion

The acetylcholinesterase (AChE) activities at various times after the ip administration of methyl parathion, parathion and azinphosmethyl are shown in Figure 1. Doses were selected which caused mild to moderate signs of poisoning but not death. In fish these signs included loss of equilibrium, lethargy and infrequent bursts of hyperactivity. As shown, mouse AChE's were inhibited much more rapidly than fish AChE's. This may be a function of 1) a faster rate of production of the respective oxygen analogues and/or 2) greater sensitivity of the AChE of mouse tissues relative to fish. Of the 3 compounds, methyl parathion produced the most rapid AChE inhibition in fish, with maximal levels of inhibition within 2-4 hr. Azinphosmethyl-treated fish had slight inhibition at 2 hr and maximal inhibition at 4 hr. AChE in fish dosed with parathion did not reach maximum inhibition until 20 hr and was only slightly inhibited at 10 hr. Clearly, the greater toxicity of parathion in fish relative to methyl parathion could not be related to the speed of onset of AChE inhibition. Similarly, azinphosmethyl, which is considerably more toxic to sunfish than methyl parathion, had a slower onset of AChE inhibition.

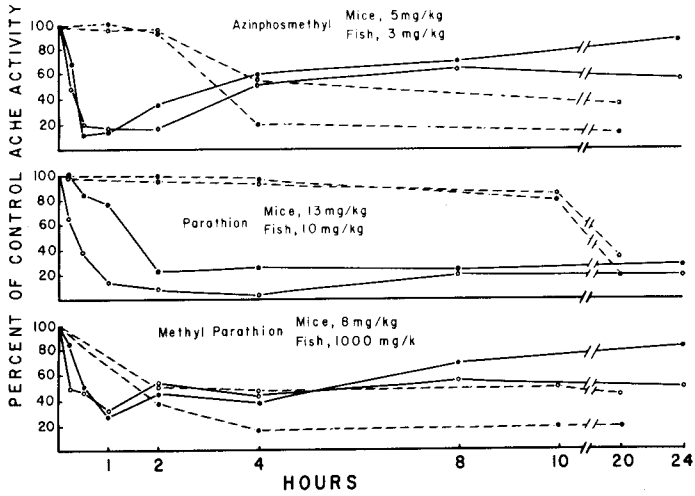

Figure 1. Onset of inhibition of AChE in tissues of mice and fish treated with azinphosmethyl, parathion and methyl parathion. Legend: solid lines - mice, broken lines - fish, solid circles - brain, open circles - muscle.

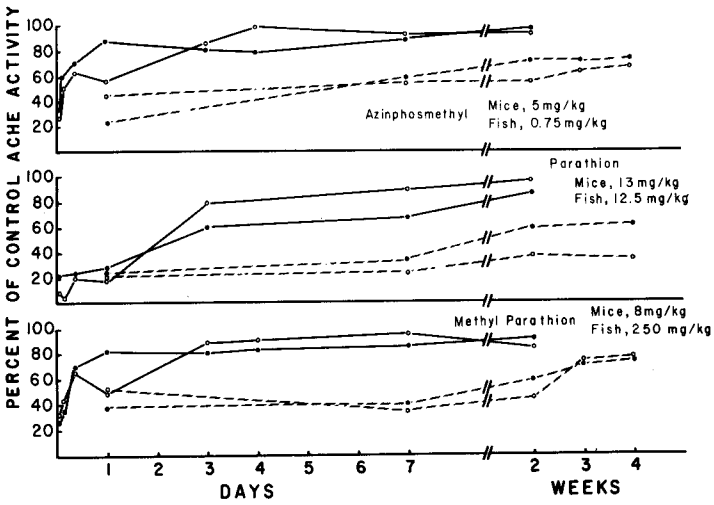

Figure 2. Recovery of AChE activity following inhibition by azinphosmethyl, parathion and methyl parathion. Legend as above.

As mentioned, mouse AChE's were inhibited much more rapidly than fish AChE's, but , as in fish, AChE activities of parathion-treated mice were depressed less rapidly than following either methyl parathion or azinphosmethyl. The onset of mouse AChE inhibition was slightly more rapid following azinphosmethyl than after methyl parathion. If these results are applicable to humans, more time would be available, in the case of an acute dose of parathion, to begin antidotal therapy than with methyl parathion or azinphosmethyl. On the other hand, the more rapid recovery of AChE activity after inhibition by azinphosmethyl and methyl parathion indicates that the duration of acute effects after sublethal doses would be shorter than for parathion. The much lower dermal toxicity (to rats) of methyl parathion, relative to parathion (GAINES, 1969) may indicate that methyl parathion reactivation of AChE more nearly keeps pace with a slower rate of absorption from the skin. VANDEKAR, et al. (1971) have suggested that such an explanation may account for low dermal toxicity of some carbamate insecticides, and they have also noted in rats given slow iv infusions of methyl paraoxon and paraoxon, that the dimethyl analogue was much less acutely toxic at the lower dose rates than was paraoxon.

It has been suggested by WEISS (1958, 1959 and 1965) that sunfish brain AChE is useful to determine the presence of aquatic contamination by organophosphorus insecticides. WILLIAMS and SOVA (1966) stated that the use of fish brain AChE has "considerable potential" in monitoring studies when used in conjunction with chemical water analysis. Our results in Figure 2 indicate that not only fish brain but also fish muscle may be useful in detecting single acute exposures of fish occurring up to 1 month (or more) previously. The use of fish muscle for this purpose has not been considered previously; however, it could facilitate sampling for assays, especially with small fish where brain dissections may be difficult.

In a study of rates of AChE recovery in rats following injection with methyl paraoxon and paraoxon, DAVISON (1955) observed that a change in slope occurred at about 55% recovery of control values and suggested that the remainder of the AChE was more permanently inhibited. REINER (1971) has indicated that the more easily reversed AChE is accomplished by a hydrolytic mechanism. O'BRIEN (1960) and VANDEKAR and HEATH (1957) discuss the possibility of 2 fractions of AChE, one being less reversible than the other. The less easily reversed fraction is probably "aged" inhibited enzyme (several mechanisms are discussed by O'BRIEN, 1960) which is resistant to hydrolysis. Our results in mice also suggest 2 slopes for rates of recovery from inhibition by the 3 compounds. In fish very little, if any, early AChE recovery occurs (Fig. 2) and the return to control levels is so slow as to suggest that it may be due to synthesis of new enzyme. Fish AChE's in general apparently lack the rapid protective reactivation mechanism.

The slow recovery of AChE activity that occurs in fish may be of practical significance in the case of multiple exposures to orga-

nophosphate pesticides, since it might provide more opportunity for additive effects to occur. To test this possibility, groups of fish were treated with either azinphosmethyl or corn oil (ip). Two weeks later the fish in each of these groups were randomly divided into 2 additional groups and were given either azinphosmethyl or corn oil. One day later AChE activities were assayed. Fish that died before 24 hr were frozen at death. The results are shown in Table 1.

TABLE 1

AChE activity and mortality in sunfish following pretreatment-challenge with azinphosmethyl

Pretreatment Dose[a]	Challenge Dose[a,b]	% AChE Inhibition[c] Brain	Muscle	No. treated	No. dead
Corn oil	Corn oil	0	0	4	0
Azinphosmethyl	Corn oil	11.8±1.9	39.3±1.7	4	0
Corn oil	Azinphosmethyl	72.7±1.5	56.8±3.4	6	0
Azinphosmethyl	Azinphosmethyl	88.2±0.7	74.5±2.9	8	2

a. Azinphosmethyl dose, 0.75 mg/kg, ip
b. Interval between doses, 14 days
c. Twenty-four hours after challenge dose

It can be seen that retreatment 2 weeks after an initial dose of azinphosmethyl produced an approximately additive inhibition of brain and muscle AChE. All fish that had been pretreated with azinphosmethyl had significantly greater AChE inhibition after challenge than those pretreated with corn oil ($p < 0.01$ students "t" test). It was also observed that none of a total of 18 fish died within 24 hr after a single treatment of 0.75 mg/kg azinphosmethyl while 2 of 8 fish (25%) died when retreated with an additional 0.75 mg/kg. This difference in mortality is not significant at the $P < 0.05$ level (Chi-Square test). However, if one includes the 20 fish injected with this dose in the recovery experiments (Fig. 2), the mortality difference is significant ($P < .03$, X^2 test).

In summary, rates of onset of AChE inhibition do not explain the greater toxicity of parathion and azinphosmethyl relative to methyl parathion in sunfish. The rate of onset and recovery from AChE inhibition by all 3 pesticides in fish brain and muscle is much slower than in mice and the slow recovery of AChE's in fish may make them more susceptible to cumulative injury by these compounds.

Acknowledgments

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