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Anticholinesterase Properties of Methamidophos and Acephate in Insects and Mammals

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Methamidophos and acephate are active ingredients in two insecticidal formulations bearing the trade names of Monitor and Orthene respectively. Acephate is the N-acetyl derivative of methamidophos (Figure 1). Both chemicals have very good insecticidal properties. However, in mammals, methamidophos is several times more toxic than acephate (Spencer, 1982).

In vitro , methamidophos was found to be a moderate inhibitor of both insect and mammalian cholinesterases compared to paraoxon, a very strong inhibitor (Hussain et al. 1984). This raises the question as to its mode of action in vivo where severe cholinesterase inhibition was observed in insects and mammals. Two mechanisms have been put forward. Kao and Fukuto (1977) have proposed that the mode of action of methamidophos may be due to direct inhibition of the cholinesterase in vivo. Because of the stability of methamidophos, inhibition is prolonged. Eto et al. (1977) have suggested another mechanism, that methamidophos may be activated to its sulfoxide which is a much more potent cholinesterase inhibitor. This activated metabolite is unstable and has not been isolated by the researchers.

In vitro, acephate is a weak inhibitor of insect and mammalian cholinesterases compared to paraoxon. It is a much weaker inhibitor than methamidophos (Hussain et al. 1984). The mode of action of acephate in insects is proposed to be due to metabolic conversion to methamidophos and subsequent inhibition of the cholinesterase (Kao and Fukuto 1977; Eto et al. 1977). However, recent evidence by Chukwudebe et al. (1984) and Hussain et al. (1984) suggests that the amount of methamidophos produced from acephate may not be high enough to cause the severe cholinesterase inhibition observed in vivo. Chukwudebe et al.(1984) therefore proposed that acephate's toxicity in insects might be due to the combined inhibitory effect of acephate and methamidophos. The lower toxicity of acephate to mammals compared to insects may be related to the lower amount of methamidophos produced in mammals.

The objectives of this study were to determine the following:

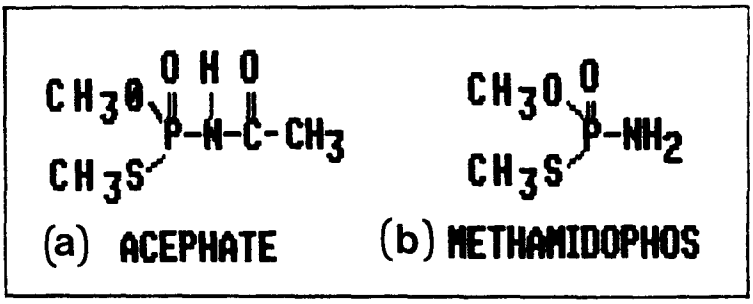

Figure 1. Structure of (a) acephate (O,S dimethyl N-acetylphosphoramidothiolate) and (b) methamidophos (O,S dimethyl phosphoramidothioate).

a) the toxicity of methamidophos to the housefly, the concentration of methamidophos in houseflies exposed to the chemical, and inhibition of the insect's cholinesterase in vivo;

b) the concentration of acephate and methamidophos in houseflies exposed to acephate, and inhibition of the cholinesterase in vivo;

c) the concentration of acephate and methamidophos in tissues of spruce budworm and flour moth exposed to acephate;

d) the combined anticholinesterase effect of acephate and methamidophos on enzymes from spruce budworm, flour moth, rat brain, and human erythrocytes.

Results obtained are discussed in relation to the mode of action of methamidophos and acephate in insects and mammals.

MATERIALS AND METHODS

Three-day old adult houseflies of both sexes were obtained from the insect-rearing facilities at Simon Fraser University. Twenty flies were used for each of the four treatment and one control group, and they were kept in lidded glass jars with sugar and water. The insects were lightly anaesthetised with CO_2 and the appropriate dose of methamidophos was applied to the ventral abdomen. The chemical was prepared in four serial concentrations and the appropriate amount applied to each insect in a 1 ul volume of acetone with a Hamilton microsyringe. Control insects received 1 ul acetone only. Mortality data were collected after 24 h and the LD_{50} value was calculated according to Swaroop (1966). The LD_{50} determination was done twice.

Houseflies were exposed to a concentration of methamidophos equivalent to 30% of the LD_{50}. The chemical was applied in

1 ul acetone to the ventral abdomen after the insects were lightly anaesthetised with CO_2. Twenty insects were used per group. At intervals of 0, 4, 8, 16, 24, 36 and 48 h, two groups of insects were removed and frozen in dry ice for methamidophos analysis. At the same intervals, insects from a third group were frozen for cholinesterase assay.

Houseflies were exposed to a concentration of acephate equivalent to 30% of the LD_{50} as described above. At the same intervals, insects were removed for acephate and methamidophos analyses and cholinesterase assays.

Last instar larvae of spruce budworm and flour moth were exposed to acephate equivalent to 10% of the LD_{50}. The insects were lightly anaesthetised with CO_2 and the chemical was applied in 1 ul acetone to the dorsal thoracic segments. The larvae were then kept in lidded petri dishes with food. At the intervals mentioned above larvae were removed for acephate and methamidophos analyses.

Cholinesterases from spruce budworm, flour moth, rat brain and human erythrocytes were prepared as described by Hussain et al. (1984). Each enzyme was then exposed *in vitro* to acephate and methamidophos individually and in combination. The concentration of each chemical used was equivalent to the I_{25} of the chemical to the enzyme. Each enzyme was exposed to a chemical or combination of chemicals for 10 minutes and then assayed for activity.

Acephate and methamidophos in insect tissues and acetylcholinesterase activity *in vivo* and *in vitro* were analyzed according to Hussain et al. (1984). Briefly, acephate and methamidophos were extracted from the insect tissues with a mixture of acetonitrile and methanol. The liquid extract was evaporated and redissolved in methanol which was passed through a glass column of Nuchar charcoal and Whatman cellulose powder. The residues in the column were eluted out with methanol which was evaporated and redissolved in acetone for gas chromatography. Each chemical was measured in parts per billion and then converted to percent of the amount applied to the insects at time zero.

Cholinesterase enzyme was extracted from insect tissues with phosphate buffer pH 7.6. Protein determination of the enzyme concentration in each extract was done according to Lowry et al. (1951). Enzyme activity in each extract was measured as the amount of acetylthiocholine hydrolyzed per minute per mg protein. Results were obtained in nanomoles of substrate hydrolyzed per minute and then converted to percent of the activity of the uninhibited enzyme at time zero.

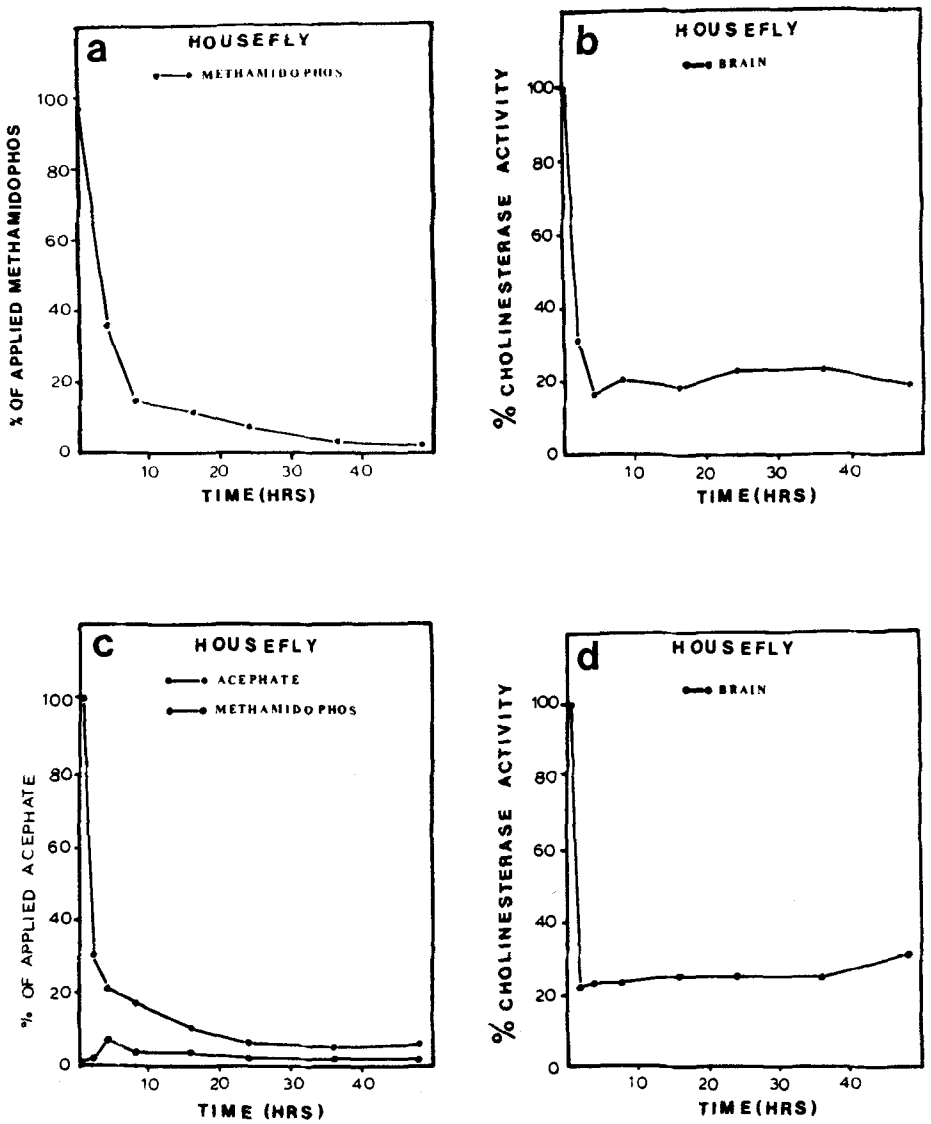

Figure 2. (a) Concentration of methamidophos in housefly tissues following exposure of the insects to 30% of the LD_{50} of the chemical; (b) cholinesterase inhibition from such an exposure; (c) acephate and methamidophos concentration in houseflies after exposure to 30% of the LD_{50} of acephate; (d) cholinesterase inhibition resulting from such an exposure. Each point is the mean of three values.

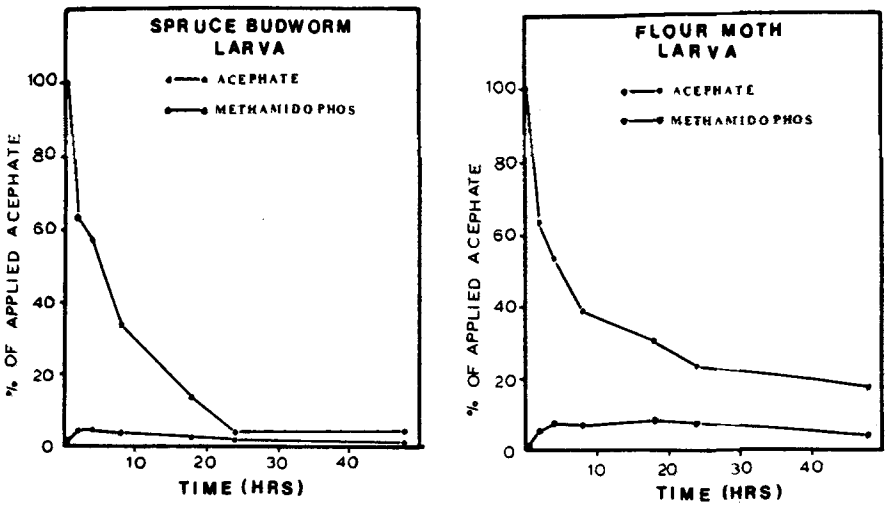

Figure 3. Concentration of acephate and methamidophos in larvae of spruce budworm and flour moth after exposure to 10% of the LD_{50} of acephate. Each point is the mean of 3 values; S.D. not shown.

Each of the experiments described above, except the LD 50, was replicated three times.

Technical acephate (98%) and technical methamidophos (99.6%) used in this study were obtained from Chevron Chemical Co., Richmond, California. Paraoxon (99.5%) was obtained from American Cyanamid, Princeton, New Jersey.

RESULTS AND DISCUSSION

The LD_{50} of methamidophos to the housefly was found to be 0.64 ug/g.

When houseflies are exposed to 30% of the LD_{50} of methamidophos, the chemical rapidly disappears from the insects' tissues. Within 8 h, more than 80% of methamidophos is lost. Thereafter, the chemical gradually dissipates. The cholinesterase also is rapidly and maximally inhibited to almost 80% within 4 h. No further inhibition or recovery of the enzyme was noted over 48 h (Figure 2 a, b).

Acephate also rapidly disappears to more than 80% within 8h from housefly tissues when the insects are exposed to 30% of the LD_{50} of acephate. Dissipation thereafter was gradual. A very small amount (about 7%) of methamidophos was detected. The cholinesterase was maximally depressed after 2 h without further inhibition or recovery over 48 h (Figure 2 c,d).

Table 1. Percent inhibition of cholinesterase from spruce budworm, flour moth, rat brain and human erythrocytes when the enzymes are exposed to acephate (A) and methamidophos (M) individually and in combination (A & M). Each value is the mean ± S.D. of three determinations.

Cholinesterase	Inhibitor+	% Inhibition ±S.D.
Spruce budworm	A	26 ± 2.5
	M	22 ± 2.5
	A & M*	37 ± 3.8
Flour moth	A	22 ± 1.5
	M	24 ± 2.7
	A & M*	40 ± 2.1
Rat Brain	A	27 ± 1.4
	M	19 ± 1.7
	A & M*	31 ± 6.6
Human erythrocytes	A	25 ± 3.3
	M	26 ± 2.9
	A & M*	42 ± 1.4

+Concentration of each inhibitor used is equivalent to the I_{25} of the chemical to the enzyme.
*Concentration of acephate and methamidophos used here is the same as that used when each chemical was tested individually.

Figure 3 shows the concentration of acephate and methamidophos when spruce budworm and flour moth larvae are exposed to 10% LD_{50} of acephate. In both species, acephate concentration rapidly drops by about 65% within 8 h. Thereafter, the concentration in spruce budworm continues to dissipate rapidly to about 3% after 24 h. In the flour moth, acephate dissipates gradually over 48 h. A small amount (about 5-8%) of methamidophos was observed in both insects.

Table 1 shows the percentage inhibition of cholinesterase from spruce budworm, flour moth, rat brain and human erythrocytes when the enzymes are exposed for 10 min to acephate and methamidophos individually and in combination.

The concentration of inhibitor used was equivalent to the I_{25} of each chemical to each enzyme. Acephate and methamidophos individually inhibit all four enzymes to about the same extent (19-27%). In combination, the inhibition was 31-42% indicating an additive effect of the two inhibitors to enzymes from insects and mammals in vitro.

The results in this study indicate that methamidophos is very toxic to the housefly (LD_{50} = 0.64 ug/g). The study

further suggests that this toxicity may be related to the rapid inhibition of the cholinesterase. It appears that methamidophos is removed from the tissues by becoming bound to the enzyme. The housefly cholinesterase therefore seems to be very sensitive to inhibition by methamidophos *in vivo*.

Hussain et al. (1984) have shown previously that acephate's toxicity to the housefly is nearly the same as that of methamidophos found in this study. The LD_{50} value reported for acephate was 0.88 ug/g. Results in this study and in that by Hussain et al. (1984) now show that acephate, like methamidophos, quickly disappears from the housefly tissues by becoming bound to the cholinesterase. The housefly cholinesterase therefore appears to be as sensitive to inhibition by acephate as it is by methamidophos *in vivo* and the nearly equal toxicity of the two chemicals may be related to this inhibition.

Compared to houseflies, larvae of spruce budworm and flour moth are several times less sensitive to the toxic effects of acephate. LD_{50} values reported were 23 ug/g for spruce budworm and 47 ug/g for flour moth (Hussain et al. 1984). This difference in toxicity may be due to a combination of several species-specific factors including access of the inhibitor to the enzyme as may have been indicated by the results in this study. However, the results in this study and in that by Hussain et al. (1984) indicate that acephate rapidly disappears from these insects' tissues, although not as rapidly as in the case of houseflies, by becoming bound to the larval cholinesterases *in vivo*. The enzymes therefore appear to be very sensitive to inhibition by acephate *in vivo*.

Thus, from the above discussion of results on the housefly, spruce budworm and flour moth, both acephate and methamidophos appear to be equally strong inhibitors of the insect cholinesterase *in vivo*. Differences in toxicity of acephate to different insect species may be related to accessibility of the inhibitor to the enzyme and not to differences in affinity of the inhibitor to the enzyme *in vivo*.

In vitro, acephate is a weak inhibitor of the insect and mammalian cholinesterases compared to methamidophos (Hussain et al. 1984). This suggests that the high cholinesterase inhibition observed for acephate *in vivo* may not be due to acephate per se but perhaps to an activated form which is equally as toxic as methamidophos. However, Hussain et al. (1984) revealed that a more active metabolite of acephate was not produced when insect and mammalian mixed function oxidases (MFO) were incubated with acephate *in vitro*. Methamidophos, a known metabolite of acephate, could not be found either. This suggests that the small amount of

methamidophos found *in vivo* when insects and mammals are exposed to acephate is produced mainly by hydrolysis.

The methamidophos produced may play a role in the inhibition of the cholinesterase, considering that it is a strong inhibitor of the enzyme *in vivo*. However, it cannot account for all the inhibition observed when insect or mammal are exposed to acephate only. A similar conclusion had been reached by Chukwudebe et al. (1984).

Even the combined effect of acephate and methamidophos on cholinesterases from insects and mammals *in vitro* cannot account for the total cholinesterase inhibition observed *in vivo*. Acephate and methamidophos together have an additive rather than a synergistic effect. But it does appear that acephate would have to be converted into a highly active state to produce the strong inhibition of the enzyme observed *in vivo*. What this active state is and how it is produced is yet to be determined.

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