strongly suggest that precocene II metabolism, like that of carbaryl and methomyl, is catalyzed by a mixed-function oxidase system located in the fat body tissue. The reason why precocene II degradation was only mildly affected during incubation in an atmosphere of nitrogen (-oxygen) is not known, but it is possible that more endogenous oxygen was present in the reaction medium for the precocene studies. The response following various additions to the reaction medium was remarkably similar for each substrate.

The largest quantities and greatest diversity of ether-soluble metabolites resulted from incubation of [^{14}C]-precocene II with cabbage looper fat body homogenates. Hence, this system was used as a source for biosynthesis of sufficient amounts of metabolities for potential positive identification. The major metabolite comprised 60.3% of the total organosoluble radioactive products and had an R_f value of 0.12 in 4:1 ether–hexane and 0.31 in ether. The unknown was assigned the structure 6,7-dimethoxy-2,2-dimethylchroman-3,4-diol because it cochromatographed with the chemically synthesized diol in both TLC systems, had the same retention times on GPC and GC, and had an identical mass spectrum as that of synthetic precocene II diol.

The second largest metabolite (17.1% of total) moved on TLC with an R_f of 0.44 in 4:1 ether–hexane and 0.75 in ether. Its retention time on GC and its mass spectrum were the same as that of chemically synthesized 6,7-dimethoxy-2,2-dimethylchroman-3-ol. Interestingly, none of the unknown bands or peaks on TLC or GC corresponded to the dimethylchroman-4-ol.

Finally, one of the minor products (3.9%) was identified as epoxy precocene II based on its R_f values in 4:1 ether–hexane (0.85) and 93:7 benzene–ethyl acetate (0.41), its GC retention time, and its mass spectrum which was identical with 3,4-epoxy-6,7-dimethoxy-2,2-dimethylchroman. At this time, it is not known whether epoxy precocene II serves as an intermediate in the formation of precocene II diol, but it is certainly a possible metabolic pathway.

All of the synthetic metabolites were bioassayed for antijuvenile hormone activity using the milkweed bug as the test organism (Bowers et al., 1976). However, none of the compounds were active.

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LITERATURE CITED

Bowers, W. S., Ohta, T., Cleere, J. S., Marsella, P. A., *Science* **193**, 542 (1976).
Crosby, D. G., Berthold, R. V., *J. Org., Chem.* **27**, 3083 (1962).
Davis, A. C., Kuhr, R. J., *J. Econ. Entomol.* **67**, 681 (1974).
Hepworth, J. D., Livingstone, R., *J. Chem. Soc. C*, 2012 (1966).
Kuhr, R. J., *J. Agric. Food Chem.* **18**, 1023 (1970).
Kuhr, R. J., *J. Econ. Entomol.* **64**, 1373 (1971).
Kuhr, R. J., *Pestic. Biochem. Physiol.* **3**, 113 (1973).
Kuhr, R. J., Davis, A. C., *Pestic. Biochem. Physiol.*, **5**, 330 (1975).
Kuhr, R. J., Hessney, C. W., *Pestic. Biochem. Physiol.*, in press (1977).

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Mechanisms by Which Methamidophos and Acephate Circumvent Resistance to Organophosphate Insecticides in the Housefly

Pin Suksayretrup and Frederick W. Plapp, Jr.*

Methamidophos and acephate are nearly as toxic to resistant strains of the housefly as to susceptible strains. Biochemical data indicate these insecticides are poor substrates for microsomal oxidases and glutathione-dependent transferases, enzymes important in detoxifying and conferring resistance to insecticides. The lack of resistance to methamidophos and acephate may be due, therefore, to their lack of reactivity with these enzymes. Both insecticides inhibit housefly cholinesterase and aliesterase in vivo but are poor inhibitors in vitro. Bioassay data show slow knockdown rates for flies exposed to lethal doses of the insecticides. Therefore, the chemicals may have to be metabolically activated in vivo to become toxic.

Methamidophos and its *N*-acetylated derivative, acephate, are relatively new phosphoramidothioate insecticides. Methamidophos shows a broad spectrum of activity against houseflies resistant to various organophosphate (OP), chlorinated hydrocarbon, and carbamate insecticides as well as susceptible housefly strains (Khasawinah, 1970; Quistad et al., 1970). Both insecticides are also toxic to susceptible and OP-resistant *Heliothis* sp. that attack cotton (Plapp, 1972). Acephate is as toxic as methamidophos to the housefly and is much less toxic to mammals (Chevron Chemical Company, 1973a,b).

Flies treated with lethal doses of methamidophos respond slowly (Khasawinah, 1970). Symptoms of poisoning correspond to maximum in vivo thoracic and head cholinesterase (ChE) inhibition. Aliesterase (ali-E) is also inhibited in vivo. No activation or degradation products were found in extracts of houseflies treated with [^{32}P]-methamidophos, but a number of hydrolytic products were detected by in vitro incubation of methamidophos with housefly preparations. It was concluded by Khasawinah (1970) that the relative stability of methamidophos to degradation in vivo is of critical importance in maintaining

Department of Entomology, Texas A&M University, College Station, Texas 77843.

Table I. Data on Test Housefly Strains

Strain	Resistance spectrum	Resistance mechanisms	References
Orlando Regular	Susceptible		
R-Parathion, *clw*[a]	Resistant to parathion and related OP's	Altered aliesterase	Bigley and Plapp, 1960 Hoyer et al., 1965
R-Baygon, *bwb*, *ocra*[b]	Resistant to carbamates and many OP's	High level of microsomal oxidases	Plapp and Casida, 1969
R-Diazinon	Resistant to diazinon, many other OP's, and carbamates	High levels of microsomal oxidase and GSH transferases	Motoyama and Dauterman, 1972

[a] *clw* = mutant symbol for chromosome II mutant classic wing. [b] *bwb* and *ocra* = mutant symbols for brown body and ocra eye, chromosome III and V mutants, respectively.

the compound unchanged for a sufficient period of time to accumulate in the necessary concentration at the site of action, thus compensating for its moderate in vitro anti-ChE action. Acephate is also an exceptionally poor in vitro inhibitor of ChE with an I_{50} much greater than 10^{-2} M. It also possesses lethal inhibitory properties in vivo, suggesting that it is biologically converted to a more potent inhibitor than its in vitro activity demonstrates.

The high toxicity of methamidophos and acephate to OP-resistant insects observed in *Heliothis* and in preliminary experiments with houseflies prompted us to try to evaluate the relationship of these insecticides to enzymes known to be important in OP metabolism and resistance. The enzymes include NADPH-dependent microsomal oxidases, glutathione (GSH) dependent alkyl- and aryltransferases, aliesterase, and cholinesterase. The purpose was to elucidate the mechanism by which these insecticides circumvented the resistance problem occurring with many other OP insecticides.

MATERIALS AND METHODS

Strains of Houseflies. Four strains of the housefly were used. These strains differed in their resistance to insecticides and in the biochemical mechanisms responsible for the resistance. Strain names and properties are listed in Table I.

Chemicals. Methamidophos (*O,S*-dimethyl phosphoramidothioate), purity 75+%, and acephate (*O,S*-dimethyl *N*-acetylphosphoramidothioate), purity 98+%, were gifts from Chevron Chemical Company. Methyl parathion, purity 80+%, and ethyl parathion, purity 99.5+%, were gifts from American Cyanamid Company. [*ethyl*-^{14}C]Parathion, specific activity 5.29 μCi/mg, was purchased from Mallinckrodt Company. [*methyl*-^{14}C]-Azinphosmethyl, specific activity 1.04 μCi/mg, was a gift from Drs. N. Motoyama and W. C. Dauterman, North Carolina State University. A mixture of [*S-methyl*-^{14}C]methamidophos and [*S-methyl*-^{14}C]acephate, specific activity approximately 16 μCi/mg, was a gift from Chevron Chemical Company.

The purification of [^{14}C]methamidophos and acephate was done on 20 cm × 20 cm × 0.5 mm cellulose TLC plates prewashed with 15% NH_4OH in 1:1 (v/v) benzene–2-propanol. The radioactive sample was applied to TLC plates in acetone, and the plates were then developed with the same solvent system. The insecticides were located by exposure of developed plates to x-ray film. Identification of insecticide bands was done by developing identical TLC plates spotted with pure methamidophos and acephate and detecting the bands in an iodide fume chamber. By this system, the R_f's of methamidophos and acephate were 0.35 and 0.8, respectively. Radioactive bands were carefully scraped from TLC plates and extracted with methanol.

Bioassay Study. Flies were bioassayed by exposure to insecticide residues on the inner surface of 450-mL glass jars, following the procedure of Plapp and Casida (1969). Food and water were put in the jars along with ten female and ten male flies, 3–5-days old. Observations were made on % mortality 24 h later and on % knockdown at frequent intervals for the first several hours after the start of treatment. LC_{50}, LC_{90}, and slope values were obtained by computer analysis of the data.

Measurement of in Vitro NADPH-Dependent Parathion Metabolism. The procedure of Rhee and Plapp (1974) for estimating microsomal oxidase activity was used with 1 nmol of [^{14}C]ethyl parathion (= parathion) in 20 μL of 2-propanol as substrate. Competitive inhibition of parathion metabolism was measured by adding 1, 10, or 100 nmol of [^{12}C]parathion, methyl parathion, methamidophos, or acephate to reaction flasks along with [^{14}C]parathion before adding fly homogenate.

In vitro microsomal oxidase activity in the R-Diazinon strain was also measured with 1, 2, 4, or 8 nmol of [^{14}C]methamidophos as substrate. Competitive inhibition was measured by adding 20 nmol of either [^{12}C]methamidophos or [^{12}C]methyl parathion. The preparation of incubation mixtures, the steps of reaction, and detection of radioactive samples were as with [^{14}C]parathion as substrate.

Measurement of in Vitro GSH-Dependent Azinphosmethyl Metabolism. The procedure of Motoyama and Dauterman (1972) was utilized to measure in vitro GSH-dependent metabolism of azinophosmethyl. Homogenates containing 10 mg of 4–5-day old female fly per mL of 0.1 M phosphate buffer (pH 7.4) were centrifuged at 12000 rpm for 15 min. The supernatant was used as the enzyme source.

The incubation mixture contained 1 mL of enzyme, 3 mg of GSH in 0.3 mL of water, 0.7 mL of 0.1 M phosphate buffer, 1 nmol of [^{14}C]azinphosmethyl in 35 μL of 2-propanol as substrate, and when needed, 500 nmol of methamidophos, acephate, or methyl parathion as an inhibitor. Controls contained all ingredients except GSH. The incubation was carried out for 15 min and terminated by the addition of 1 mL chloroform. The detection of radioactive water-soluble metabolites followed the procedure of Rhee and Plapp (1974).

In vitro GSH-dependent metabolism of 1, 2, 4, or 8 nmol of [^{14}C]methamidophos was measured in the R-Diazinon strain. Competitive inhibition was measured by adding 200 nmol of either [^{12}C]methamidophos or [^{12}C]methyl parathion. The incubation mixture contained the same chemicals as described above except one whole fly per milliliter of buffer was used as the enzyme source. The incubation period was 30 min.

Measurement of ali-E Activity. The procedure of Gomori (1953) was adapted and utilized in this experiment. α–Naphthol acetate hydrolysis was measured in flies exposed to insecticides in vivo. Four-to-five-day old female flies were exposed to doses of insecticides at ten times the LC_{90} until knockdown occurred. Flies were homogenized

Table II. Bioassay of Houseflies

Insecticides	Fly strain	LC_{50}, μg/jar	LC_{90}, μg/jar	Slope	Resistance factor[a] (R/S at LC_{50})	LT_{50} of 100 μg/jar, h
Methamidophos	Orlando Regular	6.8	22.7	0.25	1.0	1.2
	R-Baygon	8.7	17.0	0.45	1.3	2.9
	R-Parathion	5.1	11.3	0.37	0.8	3.1
	R-Diazinon	17.6	33.1	0.47	2.6	5.5
Acephate	Orlando Regular	6.4	19.7	0.26	1.0	2.3
	R-Baygon	12.7	30.5	0.34	2.0	5.9
	R-Parathion	4.9	17.0	0.24	0.8	5.9
	R-Diazinon	27.0	73.4	0.30	4.2	12.1
Methyl parathion	Orlando Regular	4.7	9.6	0.41	1.0	0.8
	R-Baygon	12.5	46.4	0.23	2.7	2.1
	R-Parathion	20.9	35.5	0.58	4.4	4.0
	R-Diazinon	163.1	291.6	0.51	34.8	>24.0

[a] Assume R/S at LC_{50} of Orlando Regular (susceptible) strain = 1.0.

Table III. Inhibition of [^{14}C]Parathion Microsomal Metabolism in Houseflies

Inhibitor	Amount, nmol	% inhibition of [^{14}C]parathion metabolism[a]		
		Orlando Regular	R-Baygon	R-Diazinon
Methamidophos	1	19.1	27.1	25.5
	10	15.4	20.0	9.8
	100	6.1	0	21.5
Acephate	1	21.2	25.0	24.0
	10	17.4	17.1	13.3
	100	0	14.5	16.1
Methyl parathion	1	37.6	33.6	30.0
	10	73.0	77.6	58.8
	100	93.6	100.0	96.5
Parathion	1	48.6	47.2	41.2
	10	83.8	79.3	77.1
	100	87.8	86.5	93.6

[a] [^{14}C]Parathion metabolism by Orlando Regular, R-Baygon, and R-Diazinon strains was 48.5, 109.5, and 204.5 pmol/15 min per 1 fly abdomen, respectively. Substrate concentration was 1 nmol.

in 0.05 M phosphate buffer (pH 7.4), and a final concentration of 1 mg of whole fly per milliliter of buffer was used as the enzyme source.

The incubation mixture contained 1 mL of enzyme and 1 mL of 10^{-2} M α-naphthyl acetate. Incubation was carried out in a moderately shaking incubator for 5 min at 32 °C before adding 5 mL of 3:97 v/v 1% Diazo Blue B:5% sodium lauryl sulfate mixture. Samples were examined 10 min later at 600 nm.

For measuring anti-ali-E activity in vitro, 0.1-mL aliquots of 10^{-10} to 10^{-2} M insecticides in acetone were pipetted into test tubes, and the solvent was evaporated. Fly homogenate, prepared as before, was incubated with the inhibitor for 15 min before addition of the substrate. The steps of the reaction followed the same procedure as above.

Measurement of ChE Activity. The procedure of Bigley and Plapp (1960) was utilized for measuring cholinesterase activity in houseflies exposed to insecticides in vivo. The enzyme preparation was the same as for in vivo anti-ali-E activity except one whole fly per milliliter of buffer was used. Acetylcholine chloride at 2.5×10^{-3} M was the substrate.

For measuring anti-ChE activity in vitro, the procedure of Hestrin (1949) as modified by Robbins et al. (1958) was utilized. The test tubes were treated with 0.1 mL of 10^{-10} to 10^{-3} M insecticides, and the solvent was evaporated. Fly homogenate was added and the tubes incubated 15 min before adding substrate. The steps of the reaction followed the same procedure as for the in vivo anti-ChE study.

RESULTS

Bioassay Study. The results of bioassays on the different strains of houseflies are summarized in Table II. Methamidophos and acephate are highly toxic insecticides. They are nearly as toxic as methyl parathion to the susceptible Orlando Regular strain and are even more toxic to R-Parathion classic wing (*clw*) flies than to the susceptible strain. Resistance to methamidophos ranged from 0.8- to 2.6-fold in the three resistant strains and resistance to acephate ranged from 0.8- to 4.2-fold. This is much less than the 2.7- to 34.8-fold resistance to methyl parathion.

Measurement of knockdown times for the three insecticides at doses of 100 μg/jar revealed that both methamidophos and acephate cause knockdown more slowly than methyl parathion, at least with the more susceptible strains. This is true even when methamidophos and acephate are more toxic than methyl parathion. Acephate is about half as fast as methamidophos in causing knockdown.

Inhibition of NADPH-Dependent Parathion Metabolism. Data on these studies are shown in Table III. Resistant strains metabolized more [^{14}C]parathion than the susceptible strain by factors of 2 and 4, respectively. Metabolism was highest in R-Diazinon, the most resistant strain, followed by R-Baygon and Orlando Regular, suggesting that the amount of metabolism is proportional to the level of resistance.

Methamidophos and acephate are poor inhibitors of [^{14}C]parathion metabolism at every concentration and in every strain tested, never causing as much as 30% inhibition. Inhibition of [^{14}C]parathion metabolism by methyl parathion increases as the concentration of inhibitor increases. Similarly, the addition of nonradioactive parathion reduced the metabolism of the radioactive substrate. The data indicate that both methyl parathion and parathion are good competitive inhibitors of

Table IV. Inhibition of [^{14}C]Azinphosmethyl GSH-Dependent Metabolism in Houseflies[a]

Fly strain	Inhibitor (500 nmol)	[^{14}C]Azinphosmethyl metabolism pmol/15 min per 10 mg fly	% inhibition
Orlando Regular	None	57.6	0.0
	Methamidophos	47.4	17.9
	Acephate	57.6	0.0
R-Parathion	None	170.2	0.0
	Methamidophos	126.7	25.5
	Acephate	170.2	0.0
	Methyl parathion	58.6	65.7
R-Diazinon	None	229.4	0.0
	Methamidophos	161.8	29.5
	Acephate	174.8	21.5
	Methyl parathion	47.6	79.2

[a] Substrate was 1 nmol of [^{14}C]azinphosmethyl.

Table V. NADPH- and GSH-Dependent Metabolism of ^{14}C-Labeled Insecticides in R-Diazinon Housefly Tissue Preparations

Cofactor	^{14}C-Labeled insecticides	Substrate concn, nmol	Amount of metabolite	% metabolism
NADPH	Methamidophos	1	9.0[a]	0.9
	Parathion	1	204.5[a]	20.5
GSH	Methamidophos	1	27.7[b]	2.8
	Methamidophos	3	73.9[b]	2.5
	Azinphosmethyl	1	229.4[b]	22.9

[a] pmol/15 min per 1 fly abdomen. [b] pmol/30 min per 1 whole fly.

Table VI. In Vitro Inhibition of Housefly ChE and ali-E Activity by Methyl Parathion (MP), Methamidophos (MM), and Acephate

Strain	Molar concn	% inhibition of ChE by			% inhibition of ali-E by		
		MP	MM	Acephate	MP	MM	Acephate
Orlando Regular	10^{-5}	3.8	1.9	0.0	15.9	0.0	0.0
	10^{-4}	85.3	51.3	0.0	45.1	0.0	0.0
	10^{-3}	100.0	100.0	0.0	62.2	32.9	0.0
	10^{-2}				76.9	58.6	0.0
R-Diazinon	10^{-5}	8.6	4.3	0.0	4.9	0.0	0.0
	10^{-4}	78.4	25.3	0.0	26.8	0.0	0.0
	10^{-3}	100.0	93.8	0.0	56.1	9.8	0.0
	10^{-2}				65.9	53.7	0.0

NADPH-dependent [^{14}C]parathion metabolism, while methamidophos and acephate are poor inhibitors of the reaction.

Inhibition of GSH-Dependent Azinphosmethyl Metabolism. Data on inhibition of GSH-dependent azinphosmethyl metabolism in several housefly strains are shown in Table IV. The results show that R-Parathion and R-Diazinon strains metabolized [^{14}C]azinphosmethyl about three and four times faster than the susceptible Orlando Regular strain.

In every strain of housefly, methamidophos and acephate inhibit [^{14}C]azinphosmethyl metabolism only slightly or not at all. In contrast, methyl parathion strongly inhibits GSH-dependent azinphosmethyl metabolism.

NADPH- and GSH-Dependent Metabolism of [^{14}C]Methamidophos in R-Diazinon Houseflies. Previously described tests clearly showed that methamidophos and acephate were ineffective as competitive inhibitors of NADPH-dependent parathion metabolism or GSH-dependent azinphosmethyl metabolism in houseflies. The present experiment was set up to measure [^{14}C]methamidophos metabolism and to determine if its metabolism could be inhibited by other OP insecticides.

The results in Table V clearly show that parathion and azinphosmethyl are better substrates for NADPH- and GSH-dependent enzymes than methamidophos. At a 1 nmol concentration, only 0.9 or 2.7% of [^{14}C]methamidophos was metabolized by NADPH- or GSH-dependent enzymes compared with 20.4% of [^{14}C]parathion metabolized by NADPH-dependent enzymes and 22.9% of [^{14}C]azinphosmethyl metabolized by GSH-dependent enzymes.

Further experiments designed to measure competitive inhibition were done by incubating 1, 2, 4, or 8 nmol of [^{14}C]methamidophos with housefly homogenate in the presence or absence of nonradioactive parathion, methyl parathion, and methamidophos. Again, there was no or very little breakdown of [^{14}C]methamidophos in most of the treatments and little effect upon addition of nonradioactive insecticide substrates (data not shown). Methamidophos is such a poor substrate for the detoxification reactions tested that it was not possible to demonstrate inhibition by other OP insecticides.

In Vitro Inhibition of ChE and ali-E Activities. Data on in vitro inhibition of ChE and ali-E activity in Orlando Regular and R-Diazinon houseflies are shown in Table VI. Acephate caused no inhibition of either ChE or ali-E. Methamidophos and methyl parathion were poor inhibitors of both enzymes. Methamidophos caused partial inhibition of ali-E at 10^{-3} M and of ChE at 10^{-4} M. Methyl parathion was slightly more inhibitory than methamidophos against both enzymes.

In Vivo Inhibition of ChE and ali-E Activities. Data on in vivo inhibition of ChE and ali-E activity in Orlando Regular and R-Diazinon houseflies are shown in Table VII. Methamidophos and acephate inhibit ChE and ali-E activity in both strains of housefly as does methyl parathion. The percent of inhibition was very high under the

Table VII. In Vivo Inhibition of Housefly ChE and ali-E by Methyl Parathion (MP), Methamidophos (MM), and Acephate[a]

Strain	% inhibition of ChE by			% inhibition of ali-E by		
	MP	MM	Acephate	MP	MM	Acephate
Orlando Regular	100.0	95.7	87.1	90.8	82.3	76.1
R-Diazinon	96.8	100.0	94.8	83.3	77.7	76.0

[a] Flies were exposed to ten times the LC_{90} of insecticides until knockdown.

test conditions by the three insecticides used.

DISCUSSION

Rapid degradation in vivo of insecticides by insects is one explanation for insecticide resistance. Conversely, a lack of metabolism of an insecticide can explain insecticide susceptibility.

Our bioassay and enzymatic experiments were designed to compare a susceptible housefly strain with resistant strains known to possess altered ali-E or high levels of microsomal oxidases and GSH-dependent transferases. All of these are important detoxifying enzymes in insects, and all are thought to be important in conferring resistance. We found that methamidophos and acephate reacted with ali-E in susceptible and resistant strains, but did not inhibit the other detoxifying enzymes. This lack of reactivity seems sufficient to explain the lack of resistance to methamidophos and acephate in housefly strains resistant to other OP insecticides.

Metamidophos, acephate, and methyl parathion inhibit ChE activity in vivo, but they are poor inhibitors of ChE activity in vitro. It is known that to be toxic to insects, methyl parathion must be converted to its phosphate analogue, methyl paraoxon, a strong ChE inhibitor. Methamidophos and acephate may also be converted to toxic analogues. If this is so, however, the active metabolites have not yet been isolated or identified.

LITERATURE CITED

Bigley, W. S., Plapp, F. W., *Ann. Entomol. Soc. Am.* **53**, 360 (1960).
Chevron Chemical Company, Orthene insecticide, Technical Information, 1973a.
Chevron Chemical Company, Monitor insecticide, Technical Information, 1973b.
Gomori, G., *J. Lab. Clin. Med.* **42**, 445 (1953).
Hestrin, B., *J. Biol. Chem.* **180**, 249 (1949).
Hoyer, R. F., Plapp, F. W., Orchard, R. D., *Entomol. Exp. Appl.* **8**, 65 (1965).
Khasawinah, A. M. A., Dissertation, University of California, Riverside, Calif., 1970.
Motoyama, N., Dauterman, W. C., *Pestic. Biochem. Physiol.* **2**, 113 (1972).
Plapp, F. W., *J. Econ. Entomol.* **65**, 903 (1972).
Plapp, F. W., Casida, J. E., *J. Econ. Entomol.* **62**, 1174 (1969).
Quistad, G. B., Fukuto, T. R., Metcalf, R. L., *J. Agric. Food Chem.* **18**, 189 (1970).
Rhee, K. S., Plapp, F. W., *J. Agric. Food Chem.* **22**, 261 (1974).
Robbins, W. E., Hopkins, T. L., Roth, A. R., *J. Econ. Entomol.* **51**, 326 (1958).

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Kepone Inhibition of Malate Dehydrogenases

Bruce M. Anderson,* Carter Noble, Jr., and E. M. Gregory

Malate dehydrogenase catalyzed reactions, both the oxidation of malate and the reduction of oxalacetate, are effectively inhibited by the polychlorinated hydrocarbon pesticide, Kepone. Mixed inhibition with respect to substrates and coenzymes was observed in reactions catalyzed by bovine mitochondrial malate dehydrogenase, porcine mitochondrial malate dehydrogenase, and porcine cytoplasmic malate dehydrogenase. With the bovine enzyme, the Kepone inhibition was shown to be reversible upon dilution, better at pH 7.4 than at pH 9.0, and not to involve a time-dependent inactivation process. At concentrations inhibitory to the bovine enzyme, Kepone induces an accelerated rate of inactivation of this enzyme by *N*-ethylmaleimide, suggesting the possible importance of an unfolding or dissociation process in the inhibition by Kepone. The fully chlorinated pesticide, mirex, was also shown to be inhibitory and to facilitate *N*-ethylmaleimide inactivation of bovine mitochondrial malate dehydrogenase.

The polychlorinated hydrocarbon pesticide, Kepone (decachloro-1,3,4-metheno-2*H*-cyclobuta[6*d*]pentalen-2-one), has been shown in in vitro studies to be an effective inhibitor of certain pyridine nucleotide requiring dehydrogenases. The in vitro inhibition of lactate dehydrogenase (EC 1.1.1.27) was first observed by Hendrickson and Bowden (1973, 1975). A physiological significance was suggested for this inhibitory process since the low concentrations of Kepone causing inhibition compare favorably with concentrations readily attainable through tissue accumulation of this pesticide. Evidence was presented indicating that the structurally related, fully chlorinated pesticide mirex also inhibits lactate dehydrogenase in the same fashion (Hendrickson and Bowden, 1975). In continuing studies (Hendrickson and Bowden, 1976), a mechanism for Mirex inhibition of lactate dehydrogenase involving pyridine nucleotide–mirex complexes was proposed. The inhibition of lactate dehydrogenases by Kepone was recently studied (Anderson and Noble, 1977) with homologous isozymes from rabbit, beef, pig, and chicken. In these studies, Kepone was observed to be an effective inhibitor of several M_4 isozymes while within the limits of solubility, no inhibition of H_4 isozymes was detected.

In earlier studies (Freedland and McFarland, 1965), Kepone was reported to be a potent inhibitor of beef liver glutamate dehydrogenase (EC 1.4.1.3). In these studies,

Department of Biochemistry and Nutrition, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061.