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.

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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,

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a degree of specificity was attributed to the Kepone inhibition since several other chlorinated hydrocarbons were observed to be ineffective as inhibitors of this enzyme. Since inhibition of dehydrogenases by Kepone is not restricted to lactate dehydrogenases, it was of interest to examine the effects of Kepone on other types of pyridine nucleotide requiring oxidoreductases. Evidence will be presented for the effective inhibition of malate dehydrogenases (EC 1.1.1.37) from two different sources.

MATERIALS AND METHODS

Crystallized and lyophilized yeast alcohol dehydrogenase and horse liver alcohol dehydrogenase (alcohol:NAD oxidoreductase, EC 1.1.1.1), crystalline 6-phosphogluconate dehydrogenase (6-phospho-D-gluconate:NADP oxidoreductase (decarboxylating), EC 1.1.1.44), NAD, NADH, NADP, L-malic acid, *cis*-oxaloacetic acid, and 6-phosphogluconic acid (trisodium salt) were obtained from Sigma. *m*-Phenanthroline, 7,8-benzoquinoline, and 2-adamantanone were supplied by Aldrich. *N*-Ethylmaleimide was obtained from Eastman Organic Chemicals. Analytical samples of Kepone and mirex were obtained from the Environmental Protection Agency, Research Triangle, N.C. Kepone and mirex were added to reaction mixtures from ethanolic stock solutions with the total percentage of ethanol being adjusted to a constant level.

Purified porcine heart mitochondrial malate dehydrogenase (malate:NAD oxidoreductase, EC 1.1.1.37) and porcine heart cytoplasmic malate dehydrogenase were generously supplied by Dr. J. H. Harrison of the Department of Chemistry, University of North Carolina. Bovine heart mitochondrial malate dehydrogenase was purified according to Gregory (1975).

The reactions catalyzed by the different dehydrogenases were studied under various conditions in 3-mL reaction mixtures at 25 °C. The formation or disappearance of NADH was followed spectrophotometrically at 340 nm. A minimum of ten initial velocity measurements were made for the determination of K_i values through double reciprocal plotting methods. Each determination of K_i value was reproduced a second time with the largest variation in the calculated K_i value being 8%. Spectrophotometric measurements were made on a Beckman Acta M VI recording spectrophotometer. pH measurements were made with a Radiometer PHM 52 pH meter and a type 202 C glass electrode.

RESULTS

Since Kepone was previously shown to be an effective inhibitor of lactate and glutamate dehydrogenases, experiments were designed to examine inhibition by Kepone of other dehydrogenases. For these experiments the NAD-dependent yeast alcohol, horse liver alcohol, porcine malate, and bovine malate dehydrogenases, and the NADP-dependent yeast 6-phosphogluconate dehydrogenase were chosen for study. The yeast alcohol dehydrogenase catalyzed oxidation of ethanol was studied in 3-mL reaction mixtures containing 100 mM sodium pyrophosphate buffer, pH 8.2, 260 μM NAD, 10% ethanol, and 0.25 IUB unit of enzyme. The horse liver alcohol dehydrogenase catalyzed oxidation of ethanol was studied in 3-mL reaction mixtures containing 100 mM sodium phoshate buffer, pH 7.5, 100 μM NAD, 10% ethanol, and 0.05 IUB unit of enzyme. The yeast 6-phosphogluconate dehydrogenase catalyzed oxidation of 6-phosphogluconate was studied in 3-mL reaction mixtures containing 60 mM glycylglycine buffer, pH 7.5, 960 μM 6-phosphogluconate, 32 μM NAD, 10% ethanol, and 0.1 IUB unit of enzyme. In the reactions catalyzed by yeast alcohol dehydrogenase, horse liver alcohol dehydrogenase, and yeast 6-phosphogluconate dehydrogenase, no inhibition by Kepone up to a concentration of 80 μM was observed. In the above studies, ethanol was included in these reaction mixtures to maintain solubility of micromolar concentrations of pesticides and the concentration of ethanol used was shown not to affect the properties of enzymes being investigated. In the case of horse liver alcohol dehydrogenase, when 10% dioxane was used to solubilize the pesticide and concentrations of ethanol were maintained below 1 M again, no inhibition by 80 μM Kepone was observed. Malate dehydrogenases, however, were effectively inhibited by Kepone, and the properties of these reactions are described below.

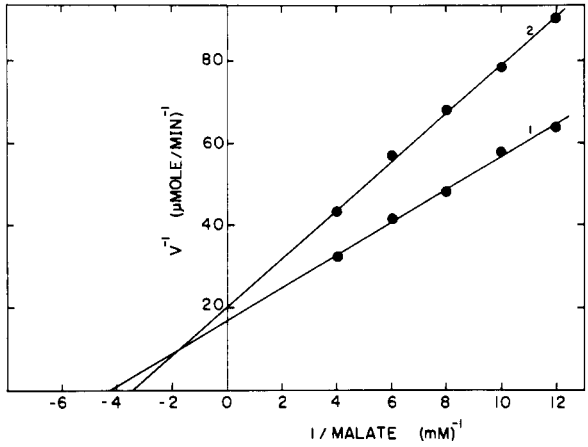

Figure 1. Inhibition of bovine heart mitochondrial malate dehydrogenase by Kepone. Malate was varied from 0.83 to 2.5 mM. Reaction mixtures contained 66 mM sodium pyrophosphate buffer, pH 9.0, 2.0 mM NAD, 0.17 unit of bovine heart mitochondrial malate dehydrogenase, malate as indicated, 5% ethanol, and Kepone at concentrations of 0 (line 1) and 20 μM (line 2), in a total volume of 3.0 mL.

The malate dehydrogenase catalyzed oxidation of malate to oxalacetate was studied at 25 °C in 3-mL reaction mixtures containing 66 mM sodium pyrophosphate buffer, pH 9.0, 5.0% ethanol, 2.0 mM NAD, and concentrations of sodium malate varying from 0.83 to 2.5 mM. Reactions were initiated by the addition of 0.17 IUB unit of bovine mitochondrial malate dehydrogenase. Inhibition of this reaction by 20 μM Kepone was observed, and the data obtained, plotted according to Lineweaver and Burk (1934), are shown in Figure 1. The inhibition was indicated to be mixed with respect to malate, and a K_i of 51 μM was calculated from these data. Kepone inhibition of malate oxidation to oxalacetate was also studied under identical conditions with malate maintained at a saturating concentration of 16.6 mM and NAD varying from 100 to 500 μM. A double reciprocal plot of the data obtained in these reactions is shown in Figure 2. The inhibition by Kepone was also shown to be mixed with respect to coenzyme, and K_i of 56 μM was calculated from these data.

Using the same concentrations of sodium pyrophosphate buffer, pH 9.0, bovine malate dehydrogenase, and ethanol, Kepone inhibition of the reverse reaction, the reduction of oxalacetate to malate, was studied, both with respect to varying substrate and varying coenzyme. In the first case, NADH was held constant at 600 μM and oxalacetate was varied from 85 to 330 μM. In the second set of experiments, oxalacetate was held constant at 1.0 mM and NADH was varied from 20 to 250 μM. Double reciprocal plots of the data obtained in these experiments again indicated effective inhibition by Kepone, and the inhibition was observed to be mixed with respect to both substrate

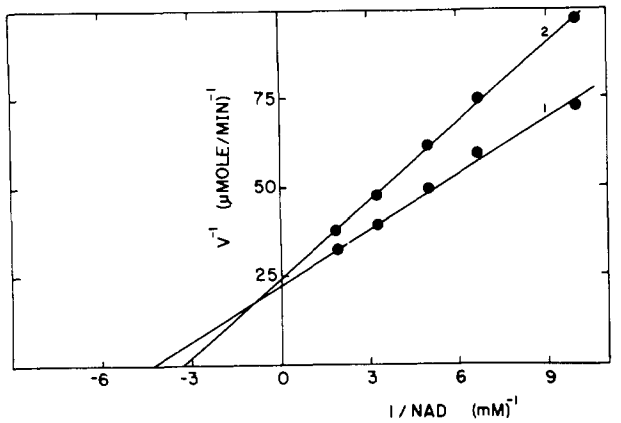

Figure 2. Inhibition of bovine heart mitochondrial malate dehydrogenase by Kepone. NAD concentration was varied from 100 to 500 μM. Reaction mixtures contained 66 mM sodium pyrophosphate buffer, pH 9.0, 16.6 mM malate, 0.17 unit of bovine heart mitochondrial malate dehydrogenase, NAD as indicated, 5% ethanol, and Kepone at concentrations of 0 (line 1) and 20 μM (line 2), in a total volume of 3.0 mL.

Table I. Kepone Inhibition of Malate Dehydrogenases

Enzyme	Inhibition Type	Inhibition (Varied substrate)	pH	K_i μM
Bovine mitochondrial MDH	Mixed	(Malate)	9.0	51
	Mixed	(NAD)	9.0	56
	Mixed	(Oxalacetate)	9.0	45
	Mixed	(NADH)	9.0	39
Porcine mitochondrial MDH	Mixed	(Malate)	9.0	49
	Mixed	(NAD)	9.0	53
	Mixed	(Oxalacetate)	9.0	50
	Mixed	(NADH)	9.0	57
Porcine cytoplasmic MDH	Mixed	(Malate)	9.0	53
	Mixed	(NAD)	9.0	64
	Mixed	(Oxalacetate)	9.0	120
	Mixed	(NADH)	9.0	95
Bovine mitochondrial MDH	Mixed	(Oxalacetate)	7.4	36
Porcine mitochondrial MDH	Mixed	(Oxalacetate)	7.4	24
Porcine cytoplasmic MDH	Mixed	(Oxalacetate)	7.4	37

and coenzyme. K_i values obtained in these experiments are listed in Table I with those obtained in the studies of the oxidation of malate. The K_i values obtained agreed well with one another regardless of the direction in which the reaction was studied and whether coenzyme or substrate was the varied reaction component.

The above-described set of four malate dehydrogenase catalyzed reactions, which can be defined as NAD_{sat}–$malate_{var}$, $malate_{sat}$–NAD_{var}, $oxalacetate_{sat}$–$NADH_{var}$, and $NADH_{sat}$–$oxalacetate_{var}$ (sat, saturating; var, variable) were studied using porcine mitochondrial and porcine cytoplasmic malate dehydrogenases as catalysts. Conditions of pH, temperature, buffer concentration, and ethanol concentrations were the same as those described for the reactions catalyzed by the bovine malate dehydrogenase. Reactions were initiated by the addition of 0.20 IUB unit of porcine cytoplasmic malate dehydrogenase. The data obtained were plotted according to Lineweaver and Burk (1934) to determine the type of inhibition and values for inhibitor dissociation constants. The reactions catalyzed by the two porcine malate dehydrogenases were inhibited by Kepone, and regardless of the reaction component varied, mixed inhibition was observed. The K_i values for the four sets of reactions studied with each porcine enzyme are shown in Table I.

In order to provide information concerning Kepone inhibition of malate dehydrogenases under more physiological conditions, the reduction of oxalacetate as catalyzed by bovine and porcine malate dehydrogenases was studied at pH 7.4 in 3-mL reaction mixtures containing 66 mM sodium phosphate buffer, 5.0% ethanol, 0.60 mM NADH, and oxalacetate varying from 25 to 210 μM. Again, Kepone inhibited all three malate dehydrogenases, the inhibition was mixed (double reciprocal plots), and the K_i values obtained (Table I) were slightly lower than those obtained at pH 9.0. Under the conditions described above for the reactions carried out at pH 7.4, possible inhibition of the bovine malate dehydrogenases by mirex and selected nonhalogenated hydrocarbons was investigated. Mirex inhibited this reaction, and the inhibition was mixed with respect to oxalacetate, the reaction component varied. The K_i value obtained was 63 μM. On the other hand, no inhibition was observed with 50 μM adamantanone, 50 μM 7,8-benzoquinoline, and 50 μM *m*-phenanthroline.

Since previously reported Kepone inhibition of dehydrogenases was attributed to reversible inhibitory processes, it was felt necessary to investigate the reversibility of the Kepone inhibition of malate dehydrogenases. Bovine malate dehydrogenase (1.4 IUB units) was incubated at 25 °C in a reaction mixture containing 66 mM sodium phosphate, pH 7.4, 5% ethanol, and 20 μM Kepone. At timed intervals, 0.14 unit of enzyme was transferred to an assay mixture containing 66 mM sodium phosphate, pH 7.4, 5% ethanol, 0.6 mM NADH, and 0.67 mM oxalacetate. Initial velocities were recorded as a function of time of enzyme incubation. At zero time, the initial velocity resulting from the transfer of 0.14 IUB unit of enzyme from the incubation mixture was identical with that obtained with 0.14 IUB unit of enzyme incubated in the absence of Kepone. The same was true for aliquots of enzyme tested after 60 min of incubation.

The Kepone inhibition of bovine malate dehydrogenase was further studied with respect to the effects of the inhibition on the maleimide inactivation of this enzyme. A reaction mixture containing 1.4 IUB units of enzyme, 66 mM sodium phosphate, pH 7.4, 5% ethanol, and 10 mM *N*-ethylmaleimide was incubated at 25 °C, and aliquots (0.14 unit) were removed at timed intervals and assayed as described above for the reversibility experiment. The loss of enzyme activity over a 60-min period is shown in Figure 3 to be a slow reaction with a $t_{1/2}$ of 90 min. When the same reaction was studied in the presence of 6.6 μM Kepone and 20 μM Kepone, the half-life values decreased to 70 and 54 min, respectively (Figure 3). As indicated also in Figure 3, there is a slight loss of enzyme activity in the absence of *N*-ethylmaleimide; however, this rate was not affected by added Kepone, as demonstrated in the above reversibility experiment. The enhancement of *N*-ethylmaleimide inactivation of bovine mitochondrial malate dehydrogenase was also observed with the fully chlorinated analogue, mirex. At a concentration of 20 μM, mirex caused a decrease in the half-life of inactivation from 90 to 45 min.

DISCUSSION

The malate dehydrogenase catalyzed oxidation of malate to oxalacetate was observed to be inhibited effectively at pH 9.0 by low concentrations of the polychlorinated hydrocarbon pesticide, Kepone. This inhibition was shown to be of a mixed type of inhibition with respect to the

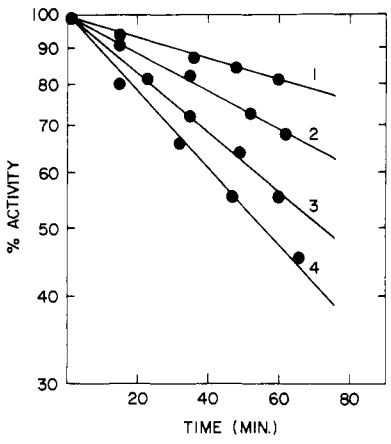

Figure 3. Enhancement by Kepone of the rate of *N*-ethylmaleimide inactivation of bovine heart malate dehydrogenase. The reaction mixture contained 1.4 units of enzyme, 66 mM sodium phosphate buffer, pH 7.4, 5% ethanol, and other compounds as follows: line 1, none; line 2, 10 mM *N*-ethylmaleimide; line 3, 10 mM *N*-ethylmaleimide and 6.6 μM Kepone; line 4, 10 mM *N*-ethylmaleimide and 20 μM Kepone. Total volume was 3.0 mL.

substrate, malate (Figure 1), and with respect to the coenzyme, NAD (Figure 2). When studied in the reverse reaction, the malate dehydrogenase catalyzed reduction of oxalacetate to malate was likewise inhibited by Kepone, and the inhibition was again mixed with respect to the substrate, oxalacetate, and with respect to the coenzyme, NADH. The observed mixed inhibition with respect to both substrates and coenzymes can be interpreted to mean that the binding of inhibitor interferes with both the catalytic process and the specific binding of the reaction components (Webb, 1963). This pattern of inhibition by Kepone was observed in reactions catalyzed by bovine mitochondrial malate dehydrogenase, porcine mitochondrial malate dehydrogenase, and porcine cytoplasmic malate dehydrogenase. Values for the inhibitor dissociation constants obtained for the three malate dehydrogenases studied are listed in Table I. The porcine cytoplasmic malate dehydrogenase appeared to be slightly less sensitive to Kepone inhibition than the two mitochondrial malate dehydrogenases. All three malate dehydrogenases were more sensitive to Kepone inhibition at the more physiological pH of 7.4 (Table I).

The Kepone inhibition of bovine mitochondrial malate dehydrogenase was shown to be reversible upon dilution. Incubation of this enzyme with 20 μM Kepone resulted in no observable inhibition upon immediate dilution to 0.67 μM Kepone and dilution after a 60-min incubation at the higher Kepone concentration. Thus, no time-dependent irreversible modification of the malate dehydrogenase was indicated. Since under identical conditions the bovine mitochondrial malate dehydrogenase was effectively inhibited by the fully chlorinated derivative, mirex, less attention was given to reactions of the carbonyl group of Kepone playing a role in the inhibition of malate dehydrogenases by Kepone.

It is of interest that Kepone inhibition is not a general feature of dehydrogenases. Although Kepone inhibition has been observed with malate dehydrogenases, lactate dehydrogenases (Hendrickson and Bowden, 1975; Anderson and Noble, 1977), and glutamic dehydrogenase (Freedland and McFarland, 1965), the present study indicates no inhibition by Kepone of yeast alcohol dehydrogenase, horse liver alcohol dehydrogenase, and yeast 6-phosphogluconate dehydrogenase. The lack of inhibition of the two alcohol dehydrogenases is of particular interest since these two enzymes are known to be sensitive to inhibition by relatively nonpolar compounds. For example, in the case of yeast alcohol dehydrogenase, hydrophobic interactions play a role in the binding of N^1-alkylnicotinamide chlorides (Anderson et al., 1965) and alkyl ammonium chlorides (Anderson and Reynolds, 1965), and this enzyme is inhibited effectively by benzoquinolines, quinoline, and phenanthroline derivatives (Anderson et al., 1966). With respect to horse liver alcohol dehydrogenase, hydrophobic interactions were indicated in the binding of dye molecules, such as rose bengal and auramine O, to this enzyme (Brand et al., 1967; Turner and Brand, 1968; Conrad et al.,1970). If one presents the argument that Kepone inhibits dehydrogenases through the formation of enzyme–Kepone complexes stabilized by simple nonpolar interactions, one is confronted with the need to explain the lack of inhibition of alcohol dehydrogenases. Furthermore, 7,8-benzoquinoline and *m*-phenanthroline which inhibit alcohol dehydrogenases were shown in the present study not to inhibit bovine mitochondrial malate dehydrogenase at concentrations above those shown to be effective for Kepone and mirex. Both of these compounds are polycyclic aromatic derivatives, and therefore it is important to note that the nonaromatic adamantanone used in the present study also did not inhibit the bovine malate dehydrogenase.

It was of interest to consider the possibility that the binding of Kepone and mirex to malate dehydrogenase may induce structural changes at either the tertiary or quaternary level. Evidence for structural changes was obtained in the studies of the enhancement by Kepone and mirex of the *N*-ethylmaleimide inactivation of bovine mitochondrial malate dehydrogenase (Figure 3). It has been noted in previous studies (Gregory et al., 1971; Gregory, 1975) that inactivation of malate dehydrogenases through the modification of sulfhydryl groups does not occur readily at neutral pH but can be facilitated by conditions that lead to unfolding or dissociation of these enzymes. The inactivation of bovine mitochondrial malate dehydrogenase (Figure 3) induced by the presence of Kepone or mirex may result from the induction of an unfolding or dissociation process that exposes a relatively buried sulfhydryl group, thereby facilitating irreversible modification by *N*-ethylmaleimide. Such an unfolding or dissociation process may well be an important factor in the inhibition of dehydrogenases by polychlorinated hydrocarbon pesticides.

The observation that mitochondrial malate dehydrogenases from heart tissue can be inhibited by Kepone and mirex is of greater importance if one considers the fact that these two pesticides are also effective inhibitors of lactate producing M_4 lactate dehydrogenase isozymes (Anderson and Noble, 1977; Hendrickson and Bowden, 1976). One can envision a combined effect of these pesticides involving a lower availability of lactate, a substrate needed for a constant supply of energy for aerobic tissue, and a decreased capability for Krebs cycle oxidation of substrates in aerobic tissues. Studies are underway to determine if the activities of other dehydrogenases are similarly affected by the polychlorinated hydrocarbon pesticides.

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Azadiene Chemistry. 4. Insecticidal Activities and Chemical Reactivities of Azaaldrin and Azadieldrin. Comparison with Aldrin and Dieldrin

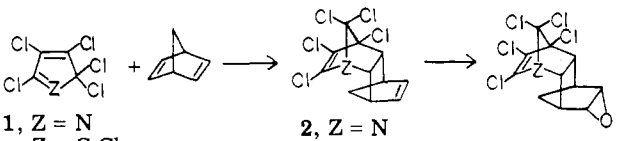
Charles M. Gladstone and John L. Wong*

This paper relates to azaaldrin (**2**) and azadieldrin (**3**) which are bridgehead nitrogen analogues of the once-popular insecticides aldrin (**2a**) and dieldrin (**3a**) whose use has been stringently regulated because of the residue problem. In Scheme I is shown the conversion of 2,3,4,5,5-pentachloro-1-azacyclopentadiene (**1**) to **2** and **3**. The hydrogen-bonding capacity of **2** and **3** was demonstrated by a gas chromatographic technique on a Carbowax 20M column relative to an SE-30 column. This and the enhanced hydrophilicity of the aza compounds, as measured by their solubility properties, are the results of the nitrogen lone pair in **2** and **3**. Another favorable impact of the bridgehead nitrogen can be seen in the hydrolytic behavior of these chlorinated insecticides in an alkaline medium (homogeneous or heterogeneous) in the presence of visible light at 26 °C, e.g. the hydrolysis of **3** proceeds 2.3 times faster than **3a**, and **2** is 1.7 times faster than **2a**. Both aza compounds, when tested on ten common insect pests and rated against standards such as diazinon, methoxychlor, and methylparathion, have shown some very useful activities. However, the aza compounds are not superior to the carbocyclic analogues in several tests, indicating that the lack of a chlorine atom at the bridgehead may have caused some loss of spatial fit into the neuron sites. Also interesting is the fact that azadieldrin (**3**) is ~12 times less toxic than dieldrin (**3a**) on dermal application to rats, thus reducing hazard to users and wildlife.

Aldrin and dieldrin are well-known insecticides conventionally used for the control of soil insects and a variety of household, vegetable, and field crop pests. However, these hydrocarbon insecticides have been found to be disadvantageous due to the accumulation of their residues in the environment, thus posing a threat to the general health. As a consequence, the use of these compounds has been stringently regulated.

Both aldrin and dieldrin belong to the family of chlorinated hydrocarbon insecticides considered to be neurotoxins. They may enter the system of the insect either by mouth or by penetration of the cuticle or lipophilic outer covering of the insect to bind to the axonic membrane of the nervous system (Telford and Matsumura, 1970). It is therefore desirable to have the insecticide lipophilic enough to penetrate the cuticle but also hydrophilic enough to be transported to the locus of activity. However, the structure–activity relationship of these insecticides appears to be very complicated; no definitive rule other than structural similarity of active compounds is available (Matsumura, 1975). This paper relates to azaaldrin and azadieldrin which are bridgehead nitrogen analogues of aldrin and dieldrin. The similar molecular topography of these compounds allows reasonable prediction of insecticidal activity for the new aza compounds. Several favorable effects are plausible: (1) the nitrogen lone pair may enhance hydrophilicity as well as complexation equilibrium constant to the ganglia receptor sites via hydrogen bonding, (2) the nitrogen, in conjunction with the vicinal dichloromethano bridge, constitutes a new functional group for hydrolysis in the otherwise resistant carbocyclic system $-NCCl_2- \rightarrow -NH + -CO_2H$, and (3) the nitrogen itself as well as the adjacent dichlorovinyl π bond being polarized by the inductively withdrawing nitrogen may provide new sites for microbial degradation. Unlike an enamine, the nitrogen is restricted by the bridgehead configuration. We report herein the chemical reactivities and insecticidal activities of azaaldrin and azadieldrin. Comparisons with the carbocyclic analogues will also be presented.

Scheme I



1, Z = N
1a, Z = C-Cl
2, Z = N
2a, Z = C-Cl
3, Z = N
3a, Z = C-Cl

RESULTS AND DISCUSSION

Conversion of 2,3,4,5,5-Pentachloro-1-azacyclopentadiene (1) to Azaaldrin (2) and Azadieldrin (3). As shown in Scheme I, the conversion of **1** → **2** → **3** is well grounded in the format of the Diels–Alder reaction of hexachlorocyclopentadiene (**1a**) with norbornadiene to

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