Environmental Health Perspectives
Vol. 87, pp. 255–262, 1990

Mechanisms of Toxic Action and Structure-Activity Relationships for Organochlorine and Synthetic Pyrethroid Insecticides

by Joel R. Coats*

The mechanisms and sites of action of organochlorine (DDT-types and chlorinated alicyclics) and synthetic pyrethroid insecticides are presented with discussion of symptoms, physiological effects, and selectivity. The structural requirements for toxicity are assessed, and structure-activity relationships are considered for each subclass. Lipophilicity is important for all the groups because it facilitates delivery of these neurotoxicants to the site of action in the nerve. Steric factors including molecular volume, shape, and isomeric configuration greatly influence toxicity. Electronic parameters also have been demonstrated to affect biological activity in some of the groups of insecticides, e.g., Hammett's σ and Taft's σ* as indicators of electronegativity. New synthetic pyrethroids continue to be developed, with varied structures and different physicochemical and biological properties.

Introduction

The organochlorine and synthetic pyrethroid insecticides are acute neurotoxic chemicals with major uses in public health, agriculture, and forestry. As the usage of many chlorinated hydrocarbons has diminished in many parts of the world, synthetic pyrethroids have often served as replacements. Studies on their insecticidal activity have determined the principal sites of toxicity and, with a lesser degree of confidence, the mechanisms of their toxic action. Two principal groups of organochlorine insecticides are the DDT-type compounds and the chlorinated alicyclics—which include, for the purpose of this paper—the cyclodienes, bornanes, and the cyclohexanes. All the synthetic pyrethroids are analogs developed with pyrethrinlike structures and which elicit pyrethrinlike symptoms. This discussion will include the present state of knowledge on the mechanism of toxic action for each group as well as an overview of known quantitative structure-activity relationships (QSAR) for them. Structural requirements for toxicity will be described as definitively as the current QSAR information will permit.

Organochlorine Insecticides

Some of the most widely used insecticides in history are members of the organochlorine class and include DDT, dieldrin, aldrin, heptachlor, chlordane, lindane, and toxaphene. All have chlorinated hydrocarbon structures and all have some common physical properties such as very low water solubility (0.001–10 ppm) (*1*) and very high lipid solubility (log P of 4–7) (*2*). All are very resistant to degradation and are, therefore, persistent in the environment. Their acute toxic effects in animals are principally due to hyperexcitation in the nervous system and death is frequently ascribed to respiratory failure after the disruption of nervous system function. However, on the basis of distinctly different sites of toxic action, mechanisms, and specific symptomology, the organochlorine class of insecticides must be divided into two subclasses.

DDT-Type Insecticides

The DDT-type insecticides act primarily on the peripheral nervous system. The mechanism of action of DDT-type insecticides has been investigated for several decades and the currently accepted hypothesis was essentially put forth in its present form by Holan (*3*). At the sodium gates of the axon, DDT exerts its toxic action by preventing the deactivation or closing of that gate after activation and membrane depolarization. The result is a lingering leakage of Na^+ ions through the nerve membrane, creating a destabilizing negative afterpotential. The hyperexcitability of the nerve results in trains of repetitive discharges in the neuron after a single stimulus and/or occur spontaneously (*4*).

The basic structure of the DDT class of insecticides is shown in Figures 1 and 2. As illustrated, the fundamental structure of active compounds consists of two *p*-substituted phenyl rings that are connected by a mono-substituted methylene bridge. The *para* position is critical, with any additional or alternative substitution

*Department of Entomology, Insectary Building, Iowa State University, Ames, IA 50011-3140.

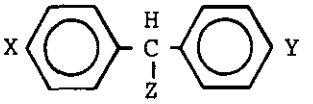


FIGURE 1. Basic skeleton of DDT-type insecticide.

resulting in reduced activity. It is also important that the *para* positions be substituted to prevent the relatively facile detoxification by enzymatic aryl hydroxylation. The fundamental requirements for the X and Y *para* substituents (Fig. 1) are that they be nonpolar groups and be of relatively low molecular volume. Specifically, alkyl, alkyloxy, alkylthio, and halogen groups are suitably lipophilic; and hydroxyl, amino, carboxy, sulfonyl, and nitro groups are too polar to allow penetration of the nerve sheath. The size of the X and Y substituents in active compounds are generally no larger than butyl or butoxy groups (*3–6*).

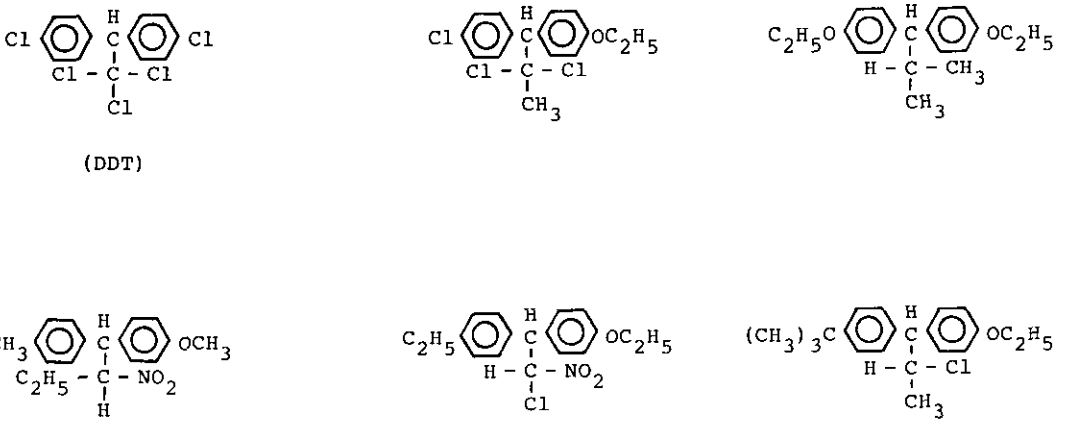
The requisite properties of the Z group are less specific, although there remain some restraints on both its polarity and size. The somewhat polar nitro group in the Z moiety contributes toward a toxic analog, while hydroxyl, ester, and amine groups are too polar to impart potency (*7,8*).

Quantitative structure-activity relationships for DDT-type insecticides have been developed to address the importance of lipophilicity, steric parameters, and electronic character for the entire substituted diphenylalkane structure and for certain portions of the molecule.

It has long been recognized that neuroactive-contact insecticides must be highly lipophilic to penetrate the insect cuticle as well as the nerve sheath. The use of hydrophobicity parameters in QSAR analyses has attempted to confirm and describe that importance. It should be noted that in the following studies, that within sets of relatively lipophilic analogs, the π or log P indicator was not the most important determinant for toxic action, but rather it was a parameter that demonstrated a lesser but significant contribution to explaining toxicity in multiple regression analyses. Lee et al. (*9*) determined that π of 1.5 to 2.0 for the aromatic substituents in a large series of prolan (2-nitropropanes) analogs conferred optimal toxicity (LD_{50} to flies). A study of DDT analogs on cockroach nerve preparations indicated that higher values for the X and Y substituents resulted in higher neurotoxicity thresholds (less bioactivity), but all analogs tested were relatively lipophilic, with π-values from −0.04 to 3.14 (*10*). The relevance of hydrophobicity of the aliphatic Z group to neurotoxic activity has also been investigated. Nishimura and Fujita (*11*) found an optimum of zero for the π value of Z in the aliphatic moiety by using excised cockroach nerve cords.

All the reports mentioned determined steric descriptors to be of the greatest value in quantifying toxicity differences among DDT and prolan-type compounds. The van der Waals volume V_w (*5,10,11*) and the Taft E_s value (*9,12*) have been used to describe steric relationships of analogs (*5*). The STERIMOL constants have also been successfully employed in some QSAR analyses (*10,11*). Optimal bulk values exist for the X, Y, and Z substituents (the V_w for X + Y should be 40–65 cm^3/mole and for Z should be 32–40 cm^3/mole), and analogs that fail these steric requirements have little effect on the nervous system (*5,6*). In multiple regression analyses, care has been taken to assess co-correlation between π and steric factors.

Within a series of sterically acceptable analogs, some electronic influences have also been detected. The field (F) and resonance (R) components of the Hammet σ parameter for the aromatic substituents (X and Y) have been quantified (*10,11*). Using an *in vivo* cockroach crural nerve assay, the electronegativity of the Z group was also illustrated to be important in a paired ANOVA test with 14 pairs of isosteres. Analogs with chloro, bromo, or nitro groups were more potent than their paired isosteres with less electronegative substitutions (e.g., methyl groups) (*10*). Chloronitro analogs have been demonstrated to be quite active, but dichloronitroethanes are less effective (*8*). Polyfluoroalkane analogs were relatively inactive, probably due to excessive electronegativity in the Z group (*13*). An optimum σ^* is apparently 0.4 to 2.6. The σ^* value has been used to



(DDT)

FIGURE 2. Active analogs of DDT.

demonstrate the importance of the aliphatic moiety electronegativity in mammalian toxicity QSAR as well with an optimum σ^* apparent at 1.5–2.5 (*14*).

In summary, the current QSAR status entails a very general hydrophobicity requirement, relatively strict steric conditions, and an optimum for electronic character, especially in the aliphatic moiety.

Chlorinated Alicyclic Insecticides

This diverse group of compounds includes several types of polychlorinated ring structures, most with bicyclic or cagelike carbon skeletons (Fig. 3). Chlorinated cyclodienes include aldrin, dieldrin, heptachlor, endrin, chlordane, and endosulfan. Toxaphene consists of 177 different chlorinated bornane derivatives. Chlorination patterns vary in both the number of chloro-substituents and their positions. Additionally, conformational isomers exist for some of the chemicals. Certain structural features are seemingly critical to the neurotoxic activity of the molecules (see later material), but the exact requisite features are not as well-defined as in the case of DDT-type organochlorines.

Although signs of intoxication are not completely uniform among this diverse group of compounds, some general trends are apparent. Usually following a 2- to 8-hr delay in the onset of symptoms, major neural effects are observed, which include depressed activity, followed by hyperexcitability and tremors, and finally, convulsions. Neurophysiological observations have revealed hyperexcitation and repetitive discharges in insect nerves. At sublethal concentrations, tonic spasms and discharges have been reported for cyclodienes as well as reductions in conduction velocities and amplitudes of potentials (*15*). Rates of respiration also have been observed to increase dramatically for lindane and the cyclodienes (*16*).

The mechanism of action has been only recently elucidated for these insecticides and is purported to involve binding at the picrotoxinin site in the γ-aminobutyric acid (GABA) chloride ionophore complex (*17*). This binding inhibits Cl^- flux into the nerve (*18*). Another site at the receptor-ionophore complex is instrumental in the pharmacological actions of barbiturates and benzodiazepines (*19*). With the function of the GABA-ergic inhibitory neurons impaired, hyperexcitation results. Several chlorinated insecticides have been shown to bind at the picrotoxinin site at which t-butylbicyclophosphorothionate (TBPS) also binds (*20–22*). Currently the inhibition of Cl^- uptake by brain vesicles (*16,23*) and the competitive binding for the TBPS site (*24*) are two valuable assays for comparing neurotoxic potencies among chlorinated cyclodienes, cyclohexanes, and bornanes. Gant et al. (*25*) have also examined the effect of cyclodienes on TBPS binding and Cl^- transport, as well as the antagonistic effects of pentobarbital and diazepam.

Structure-activity knowledge for the chlorinated alicyclics is not well developed. The degree of similarity between a compound and picrotoxinin (Fig. 4) seems to be an indicator of its potency at the GABA-ionophore complex, but the similarity is based only on the superimposability of these structures (*22*). The similarity to the specific binding ligand TBPS (Fig. 5) can also be an indication of potency in preparations from mammalian brains (*22*). Comparisons of polychlorocyclodienes' activities as inhibitors of TBPS binding in mouse brain revealed the epoxide forms to be 5-fold to 9-fold more potent than the parent cyclodiene insecticides (*24*). The epoxides were also noted to be more bioactive than their corresponding parent cyclodienes in the chloride influx

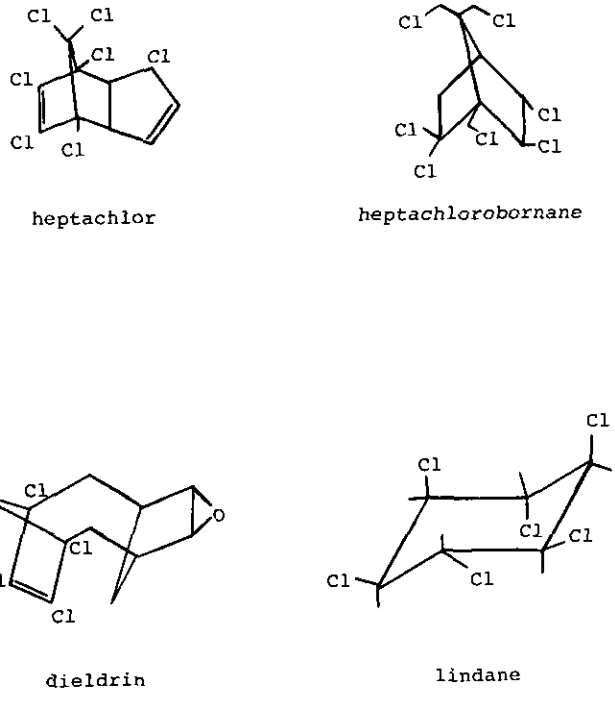


FIGURE 3. Structures of four polychlorocycloalkane insecticides.

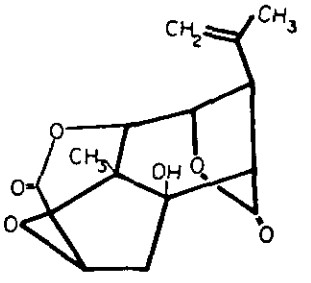


FIGURE 4. Structure of picrotoxinin.

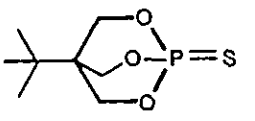


FIGURE 5. Structure of *t*-butyl bicyclophosphorothionate (TBPS).

assay (*23*). The noninsecticidal isomers of hexachlorocyclohexane were less active than the active (γ) isomer, lindane, in the TBPS biding assay (*24*) and the Cl^- flux assay (*23*). Cyclodiene effects on Cl^- flux into rat brain vesicles correlated well with TBPS binding studies (*25*). Mammalian acute oral LD_{50} values have also been correlated with these two biochemical assays (*24,25*).

In the recent work of Brooks and Mace (*26*) the biological activity of reductively dechlorinated analogs of dieldrin, endrin, endosulfan, isobenzan, and photodieldrin were investigated. Insecticidal potency against flies was the toxicity indicator used. Structural similarity to the carbon skeleton of picrotoxinin and its centers of electronegativity seemed to account reasonably well for insecticidal activity in this very systematic series of deschloro derivatives. The end-on perspective (chlorinated cyclopentadiene ring) of the cyclodienes provided the best analysis of the steric and electronic features requisite to toxicity. Lindane, in the end-on orientation (similar to the hexachlorocyclopentadiene ring of the cyclodienes), also fulfilled the appropriate spatial pattern. While quantitation of the most important parameters has not been realized, significant progress has been made toward elucidation of the steric and electronic character of potent GABA-inhibitors.

TBPS analogs with GABA antagonism effects have been reported as potent insecticides (*27,28*). These trioxabicyclooctanes have been compared to the cyclodienes for their efficacy (*29*) (Fig. 6). The unrelated insecticides/parasiticides in the avermectin class (Fig. 7) also exert their effect at the GABA-ionophore complex (*16*). The evidence currently indicates that these compounds, derived from *Streptomyces avermitilis*, act as GABA agonists, resulting in excessive Cl^- influx into the nerve (*23*). The mechanisms and structure-activity of avermectins have been reviewed recently (*30*).

Another mechanism of action put forth for cyclodiene insecticides is the inhibition of Ca + Mg-ATPase and Ca-ATPase (*32*). Insect brain symaptosomes have been used to demonstrate the effects of cyclodienes and toxaphene on Ca + Mg-ATPase (*31*).

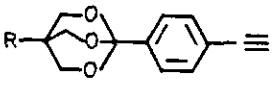


FIGURE 6. Structure of a trioxabicyclooctane insecticide.

Synthetic Pyrethroids

The pyrethroid insecticides have their origin in the natural insecticide pyrethrum, an extract of *Chrysanthemum cinerariaefolium*. The six insecticidal esters in the extract are all toxic to insects but relatively safe to mammals and birds, primarily due to poor gut uptake and rapid detoxification by endotherms. Synthetic analogs have been developed through directed synthesis efforts to retain or enhance insecticidal activity while reducing the extreme photolability of the natural products. Early analogs such as allethrin are very similar to the naturally occurring esters, but resmethrin (Fig. 8) incorporates the more stable phenyl ring into the alcohol half of the molecule, while still retaining the same acid moiety. Phenothrin contains the *m*-phenoxybenzyl alcohol moiety, which confers additional photostability. Substitution of the isobutenyl methyl groups with chlorine atoms then produces permethrin, the first commercial product with major field uses. Deployment of a cyano group at the benzylic carbon yields cypermethrin (Fig. 9). A *p*-fluoro substituent on the benzyl ring of cypermethrin gives cyfluthrin. Replacement of the dichlorovinyl group with dibromovinyl generates deltamethrin, whereas one chlorine and one trifluoromethyl as vinyl substituents result in cyhalothrin. The use of a phenylisovaleric acid moiety creates another series of analogs, which includes fenvalerate (Fig. 9), cyfluthrinate, and the phenylamino acid derivative fluvalinate. Halogenation of both the alcohol and acid moieties results in more stable esters; e.g., tefluthrin (Fig. 9). Addition of Br_2 across the vinylic double bond of deltamethrin or cypermethrin yields tralomethrin or

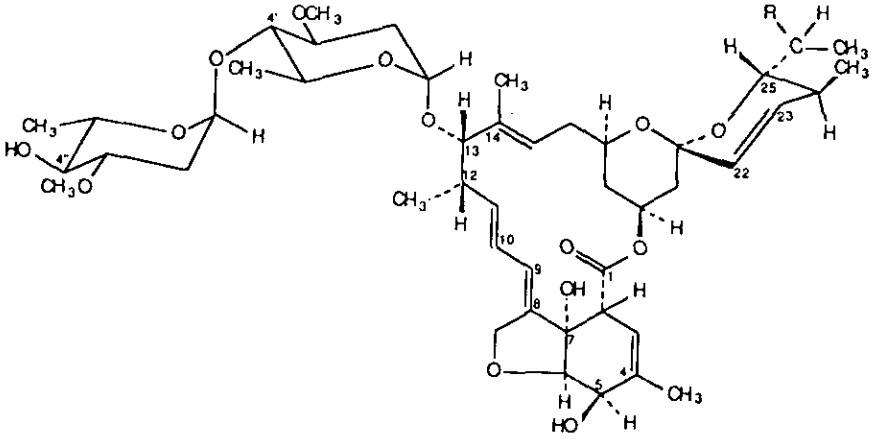


FIGURE 7. Structure of an avermectin.

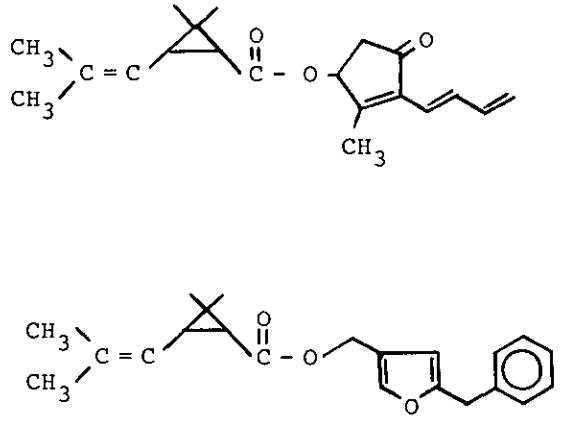


FIGURE 8. Pyrethrin I (above) and resmethrin (below).

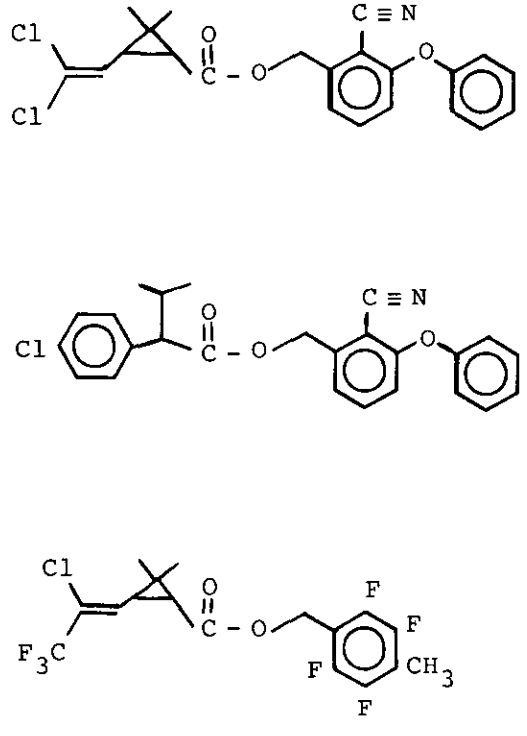


FIGURE 9. Photostable synthetic pyrethroids: cypermethrin (top), fenvalerate (middle), and tefluthrin (bottom).

tralocythrin, respectively. When debrominated these propyrethroids are converted to the parent molecules. A detailed discussion of the historical development of structural variations of pyrethroids has been published by Davies (*33*).

Symptomology is somewhat dissimilar for the Type I and Type II pyrethroids. Type II analogs have the α-cyano group at the benzylic carbon of the *m*-phenoxybenzyl alcohol moiety, e.g., cypermethrin and fenvalerate (*34*). Type I pyrethroid toxicity is characterized by aggressive behavior, fine tremors, prostration, and high body temperature. Type II symptoms include salivation and chewing, burrowing, choreoathetosis, and clonic and tonic seizures (*35*). The differential symptomology extends throughout the several levels of animal species that have been tested (*35*).

The primary mechanism of toxic action of pyrethroids has generally been considered to be interference with the sodium gate in the nerve membrane (*36*). The marked similarity of the first generation pyrethroids to the effects of DDT led to investigations using similar neurophysiological approaches (*37*). The hyperexcitation that results from prolongation of the open phase of sodium gate function results in neurotoxic effects such as tremors and convulsions (*38*). Both Type I and II pyrethroids have effects on the sodium gate, but the gating kinetics are affected in different ways (*37*). Type I pyrethroids primarily produce bursts of repetitive discharges due to the increased afterpotential; Type II pyrethroids cause lower amplitude of the action potentials and, eventually, total blocking of neural activity because of a marked depolarization of the membrane. Mouse brain preparations have also been used to evaluate the effects of pyrethroids on sodium channels (*39,40*).

One mechanism of action that has been proposed for the pyrethroids is that of inhibition of normal Cl^- channel function at the GABA receptor-ionophore complex (*17*). Binding studies have used the ligand TBPS to assess the effects on GABA site function (*41,42*). Only the Type II pyrethroids have a potent inhibitory effect at the TBPS binding site (*22*). Chloride flux assays have also demonstrated the effect of pyrethroids on the GABA receptor (*23*). Diazepam, which exerts its anticonvulsant effect at the GABA site, was observed to diminish and delay the effects of the Type II (but not Type I) pyrethroids (*43*).

Finally, it has been hypothesized that the observed neural action of pyrethroids is related to interference with calcium regulation. Both Ca-ATPase and Ca-Mg ATPase are inhibited by some pyrethroids, the former by Type I analogs and the latter by Type II molecules (*20*). Direct effects on neurotransmitter release have been observed (*44*), as well as the inhibition of Ca^{++} uptake (*45*). The pyrethroids have been implicated in causing effects at nanomolar concentrations in the calcium regulation mechanisms (*46*).

Structure-activity relationships have been of great importance throughout the evolution of the synthetic pyrethroids. Most analogs have been synthesized and studied as mixtures of geometric and/or optical isomers. Investigation of the bioactivity of the individual isomers, or pairs of isomers, has yielded valuable knowledge about the site(s) of action and afforded predictive capability for further synthetic endeavors. Physicochemical parameters have also been analyzed to assess their influence on pyrethroid potency.

Stereospecific toxicity is exhibited for most pyreth-

roids. Among the cyclopropanecarboxylate pyrethroids, the *1R,cis* isomers are more toxic to mammals than ones with the 1S configuration at the 1-carbon of the cyclopropane ring or with the trans geometry across the plane of the cyclopropane ring. A halovinyl group replacing the isobutenyl group in the acid moiety also imparts greater potency (*34*). The *trans* isomers of halovinyl pyrethroids are significantly neurotoxic, unlike the chrysanthemic acid ester trans isomers. For pyrethroids that are branch alkanoic acid esters, the 2*S* isomers are toxic, and the 2*R* ones are not. The addition of the α-cyano group at the benzlic position of the alcohol moiety confers optical activity at the α carbon as well. The α-*S* isomers are the potent neurotoxicants (*34*). Thus, a pyrethroid such as racemic cypermethrin is composed of eight isomers, the most potent of which is *cis* at the cyclopropane ring, *R* at the 1-carbon, and *S* at the α-carbon. The trans 1*R*, *S* isomer also demonstrates some toxicity (*22*). In fish, unlike mammals, there is less strict stereoselectivity across the cyclopropane ring, but the chiral specificity is similar to that for mammals and insects in the fenvalerate-type pyrethroid (*47,48*). In this case the 2*S*,α*S* isomer is the most active, with some minor toxicity contribution apparent from the 2*S*,α*R* isomer. Stereospecific relationships also extend to mechanistic studies. For example, the inhibition at the TBPS receptor has been exhibited by the isomers in a pattern consistent with mammalian and insect toxicities (*22*).

Several physicochemical parameters have been evaluated for their influence on the toxicity of pyrethroids to insects. Nishimura et al. (*49*) evaluated the lethality and knockdown potential for a series of substituted benzyl chrysanthemates. For the meta-substituted derivatives, high lipophilicity and steric bulk (ΔV_w = 1.8–2.0, e.g., Et or I) were important, whereas only the steric factor influenced bioactivity of the para-substituted compounds in knockdown assays. Lethality depended on an optimum log P of 4.9–5.2. The properties of the α-substituent on the acid moiety have been assessed for substituted cyclopropanecarboxylates and branched alkanoic acid esters. The π value of the substituent and the length, expressed as the STERIMOL parameter ΔL, were both important in determining activity in neurotoxicity assays. The parabolic relationships indicated an optimum of 5.36 ΔL for length and an optimum π of 2.61 for lipophilicity (*50*). Research in crayfish axon preparations revealed that an optimal log P of 4.6 for chrysanthemates and pyrethrates generated residual and tail Na^+ currents most effectively (*51*). Substituted benzyl chrysanthemates caused two different effects in the axon, depending on position of the substituent. The *ortho* position caused greater inhibition of Na^+ channel inactivation, while the *para* position substitution led to greater depolarizing of the afterpotential (*52*). A comparison of tetramethrin analogs having a variety of heterocyclic alcohol moieties have also been examined for knockdown potency. Substituents with greater Π values resulted in the most intense effect (Π = 3.7 to 5.1), owing to increased facility of transport

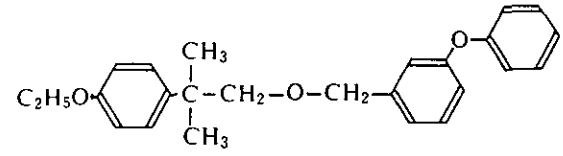


FIGURE 10. Structure of ethofenprox (a pyrethroid).

and penetration of the neural membrane. There also existed a parabolic relationship for molecular volume, with V_w optimally being between 7.40 and 8.02 for *p*-substituted benzyl chrysanthemates and tetramethrin analogs (*53*).

In summary, the potency of the pyrethroid at the site of action is markedly affected by lipophilicity and steric parameters. Differential toxicity at the site of action is responsible, in part, for the notable selectivity of these compounds. Differences in target-site sensitivity have been noted for different classes of vertebrates (*54*). Differences in metabolism also account for part of the differential toxicity (*38,54*).

Other newer variations in pyrethroid chemistry include linkages other than esters (e.g., ethers, alkanes, and alkenes (*55,56*) (Fig. 10) as well as DDT-pyrethroid hybrids (*57*). A continuously changing spectrum of analogs will steadily afford a better knowledge of the mechanisms and structure-activity relationships of this class of chemicals while demonstrating the need for thorough toxicological evaluations of new insecticides as they are developed.

This is journal paper number 13469 of the Iowa Agriculture and Home Economics Experiment Station, Ames, IA, Project No. 2306.

REFERENCES

1. Hartley, D., and Kidd, H., Ed. The Agrochemicals Handbook. The Royal Society of Chemistry, Nottingham, England, 1987.
2. National Research Council of Canada. Pyrethroids: Their Effects on Aquatic and Terrestrial Ecosystems. Publ. No. 24376 of the Environmental Secretariat, Ottawa, 1986.
3. Holan, G. New halocyclopropane insecticides and the mode of action of DDT. Nature 221: 1025–1029 (1969).
4. Narahashi, T. Nerve membrane as a target for pyrethroids. Pestic. Sci. 7: 267–272 (1976).
5. Coats, J. R., Metcalf, R. L., and Kapoor, I. P. Effective DDT analogues with altered aliphatic moieties. Isobutanes and chloropropanes. J. Agric. Food Chem. 25: 859–868 (1977).
6. Coats, J. R. Structure-activity relationships in DDT analogs. In: Insecticide Mode of Action (J. R. Coats, Ed.), Academic Press, New York, 1982, pp. 29–43.
7. Coats, J. R. Structure-activity relationships among DDT derivatives. J. Environ. Sci. Health B18: 173–188 (1983).
8. Coats, J. R., Karr, L. L., Fryer, R. L., and Beard, H. S. Diphenylchloronitroethane insecticides. In: Synthesis and Chemistry of Agrochemicals, ACS Symp. Ser. 355 (D. R. Baker, J. G. Feynes, W. K. Moberg, and B. Cross, Eds.), American Chemical Society, Washington, DC, 1987, pp. 217–225.
9. Lee, A., Metcalf, R. L., Williams, J. W., Hirwe, A. S., Sanborn, J. R., Coats, J. R., and Fukuto, T. R. Structure-activity correlations of the insecticide prolan and its analogs. Pestic. Biochem. Physiol. 7: 426–436 (1977).
10. Brown, D. D., Metcalf, R. L., Sternburg, J. G., and Coats, J. R. Structure-activity relationships of DDT-type analogs based on *in vivo* toxicity to the sensory nerves of the cockroach, *Periplaneta americana* L. Pestic. Biochem. Physiol. 15: 43–57 (1981).

11. Nishimura, K., and Fujita, T. Quantitative structure-activity relationships of DDT and its related compounds. J. Pestic. Sci. 8: 69–80 (1983).
12. Fahmy, M. A. H., Fukuto, T. R., Metcalf, R. L., and Holmstead, R. L. Structure-activity correlations in DDT analogs. J. Agric. Food Chem. 21: 585–591 (1973).
13. Abu-El-Haj, S., Fahmy, M. A. H., and Fukuto, T. R. Insecticidal activity of 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl) ethane (DDT) analogues. J. Agric. Food Chem. 27: 258–261 (1979).
14. Coats, J. R., Williams, J. W., Chang, C., Lee, A., and Metcalf, R. L. Structure-activity for uptake and toxicity of DDT-type insecticides utilizing an NMR method for estimating σ^*. Environ. Toxicol. Chem. 8: 45–52 (1989).
15. Drewes, C. D., and Vining, E. P. *In vivo* neurotoxic effects of dieldrin on giant nerve fibers and escape reflex function in the earthworm, *Eisenia foetida*. Pestic. Biochem. Physiol. 22: 93–103 (1984).
16. Matsumura, F. Toxicology of Insecticides, Second Ed. Plenum Press, New York, 1985.
17. Lawrence, L. J., and Casida, J. E. Stereospecific action of pyrethroid insecticides on the γ-aminobutyric acid receptor-ionophore complex. Science 221: 1399–1401 (1983).
18. Bloomquist, J. R., and Soderlund, D. M. Neurotoxic insecticides inhibit GABA-dependent chloride uptake by mouse brain vesicles. Biochem. Biophys. Res. Commun. 133: 37–43 (1985).
19. Leeb-Lundberg, F., Snowman, A., and Olsen, R. W. Barbiturate receptors are coupled to benzodiazepine receptors. Proc. Natl. Acad. Sci. USA 77: 7486–7472 (1980).
20. Matsumura, F., and Ghiasuddin, S. M. Evidence for similarities between cyclodiene type insecticides and picrotoxinin in the action mechanisms. J. Environ. Sci. Health B18: 1–14 (1983).
21. Tanaka, K., Scott, J. G., and Matsumura, F. Picrotoxinin receptor in the central nervous system of the American cockroach: its role in the action of cyclodiene-type insecticides. Pestic. Biochem. Physiol. 22: 117–127 (1984).
22. Casida, J. E., and Lawrence, L. J. Structure-activity correlations for interactions of bicyclophosphorus esters and some polychlorocycloalkane and pyrethroid insecticides with the brain-specific *t*-butylbicyclophosphorothionate receptor. Environ. Health Perspect. 61: 123–132 (1985).
23. Abalis, I. M., Eldefrawi, M. E., and Eldefrawi, A. T. Effects of insecticides on GABA-induced chloride influx into rat brain microsacs. J. Toxicol. Environ. Health 18: 13–23 (1986).
24. Cole, L. M., and Casida, J. E. Polychlorocyloalkane insecticide-induced convulsions in mice in relation to disruption of the GABA-regulated chloride ionophore. Life Sci. 39: 1855–1862 (1986).
25. Gant, D. B., Eldefrawi, M. E., and Eldefrawi, A. T. Cyclodiene insecticides inhibit $GABA_A$ receptor-regulated chloride transport. Toxicol. Appl. Pharmacol. 88: 313–321 (1987).
26. Brooks, G. T., and Mace, D. W. Toxicity and mode of action of reductively dechlorinated cyclodiene insecticide analogues on houseflies (*Musca domestica* L.) and other Diptera. Pestic. Sci. 21: 129–142 (1987).
27. Palmer, C. J., and Casida, J. E. 1,4-Disubstituted 2,6,7-trioxabicyclo[2.2.2]octanes: a new class of insecticides. J. Agric. Food Chem. 33: 976–980 (1985).
28. Palmer, C. J., and Casida, J. E. 1-(4-Ethynylphenyl)-2,6,7-trioxabicyclo[2.2.2] octanes: a new order of potency for insecticides acting at the GABA-gated chloride channel. J. Agric. Food Chem. 37: 213–216 (1989).
29. Obata, T., Yamamura, H. I., Malatynski, E., Ikeda, M., Laird, H., Palmer, C. J., and Casida, J. E. Modulation of γ-aminobutyric acid-stimulated chloride influx by bicycloorthocarboxylates, bicyclophosphorus esters, polychlorocycloalkanes and other cage convulsants. J. Pharmacol. Exp. Ther. 244: 802–806 (1988).
30. Wright, D. J. Biological activity and mode of action of avermectins. In: Neuropharmacology and Pesticide Action (M. G. Ford, G. G. Lunt, R. C. Reay, and P. N. R. Usherwood, Eds.), Ellis-Horwood, Chichester, England, 1986, pp. 174–202.
31. Luo, M., and Bodnaryk, R. P. The effect of insecticides on (Ca^{2+} + Mg^{2+})-ATPase and the ATP-dependent calcium pump in moth brain synaptosomes and synaptosome membrane vesicles from the bertha armyworm, *Mamestra configurato* Wlk. Pestic. Biochem. Physiol. 30: 155–165 (1988).
32. Matsumura, F. Influence of chlorinated and pyrethroid insecticides on cellular calcium regulatory mechanisms. Pesticide Chemistry: Human Welfare and the Environment, Vol. 3 (J. Miyamoto and P. C. Kearney, Eds.), Pergamon Press, Oxford, 1983, pp. 3–13.
33. Davies, J. H. The pyrethroids: an historical introduction. In: The Pyrethroid Insecticides (J. P. Leahey, Ed.), Taylor and Francis, Philadelphia, PA, 1985, pp. 1–41.
34. Gray, A. J., and Soderlund, D. M. Mammalian toxicology of pyrethroids. In: Insecticides (D. H. Hutson and T. R. Roberts, Eds.), John Wiley and Sons, New York, 1985, pp. 194–248.
35. Bradbury, S. P., and Coats, J. R. Comparative toxicology of the pyrethroid insecticides. Rev. Environ. Contam. Toxicol. 108: 133–177 (1989).
36. Narahashi, T. Effect of the insecticide allethrin on membrane potentials of cockroach giant axons. J. Cell. Comp. Physiol. 59: 61–65 (1962).
37. Narahashi, T. Mode of action of DDT and allethrin on nerve: cellular and molecular mechanisms. Residue Rev. 25: 275–288 (1969).
38. Narahashi, T. Mechanisms of action of pyrethroids on sodium and calcium channel gating. In: Neuropharmacology and Pesticide Action (M. G. Ford, G. G. Lunt, R. C. Reay, and P. N. R. Usherwood, Eds.), Ellis Horwood, Chichester, England, 1986, pp. 36–60.
39. Soderlund, D. M. Pyrethroid-receptor interactions: stereospecific binding and effects on sodium channels in mouse brain preparations. Neurotoxicology 6: 35–46 (1985).
40. Ghiasuddin, S. M., and Soderlund, D. M. Pyrethroid insecticides: potent stereospecific enhancers of mouse brain sodium channel activation. Pestic. Biochem. Physiol. 24: 200–206 (1985).
41. Seifert, J., and Casida, J. E. Regulation of [^{35}S]*t*-butylbicyclophosphorothionate binding sites in rat brain by GABA, pyrethroid and barbiturate. Eur. J. Pharmacol. 115: 191–198 (1985).
42. Lummis, S. C. R., Chow, C. S., Holan, G., and Johnston, G. A. R. γ-Aminobutyric acid receptor ionophore complexes: differential effects of deltamethrin, dichlorodiphenyltrichloroethanes and some novel insecticides in a rat brain membrane preparation. Neurochemistry 48: 689–694 (1987).
43. Gammon, D. W., Lawrence, L. J., and Casida, J. E. Pyrethroid toxicology: protective effects of diazepam and phenobarbital in the mouse and the cockroach. Toxicol. Appl. Pharmacol. 66: 290–296 (1982).
44. Brooks, M. W., and Clark, J. M. Enhancement of norepinephrine release from rat brain synaptosomes by alpha cyano pyrethroids. Pestic. Biochem. Physiol. 28: 127–139 (1987).
45. Doherty, J. D., Nishimura, K., Kurihara, N., and Fujita, T. Promotion of norepinephrine release and inhibition of calcium uptake by pyrethroids in rat brain synaptosomes. Pestic. Biochem. Physiol. 29: 187–196 (1987).
46. Clark, J. M., and Brooks, M. W. Neurotoxicology of pyrethroids: single or multiple mechanisms of action? Environ. Toxicol. Chem. 8: 361–372 (1989).
47. Bradbury, S. P., Symonik, D. M., Coats, J. R., and Atchison, G. J. Toxicity of fenvalerate and its constituent isomers to the fathead minnow (*Pimphales promelas*) and bluegill (*Lepomis macrochirus*). Bull. Environ. Contam. Toxicol. 38: 727–735 (1987).
48. Nakayama, T., Ohno, N., Aketa, K., Suzuki, Y., Kato, J., and Yoshioka, M. Chemistry, absolute structure and biological aspects of the most active isomers of fenvalerate and other pyrethroids. In: Advances in Pesticide Science (H. Geissbühler, Ed.), Pergamon Press, Oxford, 1979, pp. 174–181.
49. Nishimura, K., Kitahaba, T., Okajima, N., and Fujta, T. Quantitative structure-activity studies of substituted benzyl chrysanthemates: 5. Physicochemical substituent factors and neurophysiological effects in knockdown and lethal activities against the house fly. Pestic. Biochem. Physiol. 23: 314–327 (1985).
50. Yang, H., Nishimura, M., Nishimura, K., Kuroda, S., and Fujita, T. Quantitative structure-activity studies of pyrethroids: 12. Physicochemical substituent effects of *meta*-phenoxybenzyl α,α-

disubstituted acetates on insecticidal activity. Pestic. Biochem. Physiol. 29: 217–232 (1987).
51. Kobayashi, T., Nishimura, K., and Fujita, T. Quantitative structure-activity studies of pyrethroids: 13. Physicochemical properties and the rate of development of the residual and tail sodium currents in crayfish giant axon. Pestic. Biochem. Physiol. 30: 251–261 (1988).
52. Omatsu, M., Murayama, K., Kitasato, H., Nishimura, K., and Fujita, T. Effect of substituted benzyl chrysanthemates on sodium and potassium currents in the crayfish giant axon. Pestic. Biochem. Physiol. 30: 125–135 (1988).
53. Nishimura, K., Kitahaba, T., Ikemoto, Y., and Fujita, T. Quantitative structure-activity studies of pyrethroids: 14. Physicochemical structural effects of tetramethrin and its related compounds on knockdown activity against house flies. Pestic. Biochem. Physiol. 31: 155–165 (1988).
54. Edwards, R., Millburn, P., and Hutson, D. H. Comparative toxicity of *cis*-cypermethrin in rainbow trout, frog, mouse and quail. Toxicol. Appl. Pharmacol. 84: 512–522 (1986).
55. Class, T. J., Casida, J. E., and Ruzo, L. O. Photochemistry of ethofenprox and three related pyrethroids with ether, alkane, and alkene central linkages. J. Agric. Food Chem. 37: 216–222 (1989).
56. Udagawa, T. Trebon (etofenprox), a new insecticide. Jpn. Pestic. Inf. 53: 9–13 (1988).
57. Kirihara, S., and Sakurai, Y. Cycloprothrin, a new insecticide. Jpn. Pestic. Inf. 53: 22–26 (1988).