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RETROSPECT ON THE DISCOVERY OF A NEW INSECTICIDE

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Summary Several new insecticides with a favourable combination of properties were developed in the course of fundamental investigations of the relationships between chemical structure and biological activity of pyrethroids. The sequence of compounds synthesized and the factors which, in retrospect, appear to have contributed to the discovery of the new compounds are reviewed.

Résumé Plusieurs insecticides nouveaux avec une combinaison favorable de propriétés ont été développé pendant les recherches fondamentales de la relation des structures chimiques et les activités biologiques des pyréthrinoides. L'on considère ici la suite des composés synthétisés ainsi que tout ce qu'a contribué à la découverte des composés nouveaux.

INTRODUCTION

New insecticides will continue to be needed in the foreseeable future to control pests more effectively and economically and to replace existing compounds which have ceased to give reliable control because of resistance or which have deficiencies such as excessive persistence, harmful effects on non-target organisms or lack of appropriate mobility in soils or plants. We aim to contribute to the discovery of such new insecticides by improving understanding of the fundamental principles determining relationships between chemical structure and biological activity, concentrating on the pyrethroids. The primary object is thus not to develop new products for commerce. Nevertheless in the course of the work several new insecticides with particularly favourable combinations of properties were synthesised; the further development of these materials was supported by the National Research Development Corporation. In this paper we review briefly the sequence of compounds synthesised and discuss the factors which, with hindsight, appear to have contributed most to the discovery of the new pyrethroids, attempting to identify general principles which might enable other new insecticides to be found more effectively. The basic knowledge gained in the course of the work is reviewed more fully elsewhere (Elliott, 1971; Elliott et al., 1974a).

The examples discussed here illustrate only one approach to discovering new compounds: the progressive elucidation of the structural requirements for activity by systematic synthesis and testing starting from a lead compound and with little initial understanding of the nature of the poisoning process at a molecular level. The chemist uses the effects of structural variations on activity to try to characterise the topography of the site of action, but without detailed knowledge of the mode of action, he must rely on purely chemical insight to suggest structures to investigate. This approach to the discovery of new compounds probably remains

the most productive, but complements others such as fundamental investigations of biochemical processes, potentially capable of being disrupted.

We discuss first some very general considerations which apply to this approach and then examine more specific factors, using illustrations from our experience with pyrethroids.

GENERAL CONSIDERATIONS

1. Choice of lead compounds

It is self-evident that it is crucial to choose areas of study and lead compounds which offer real potential for significant advances. While there is clearly no way of ensuring this beforehand, it is often possible to make a reasonable assessment of the possibilities of modifying prospective lead compounds and of the scope for improvement over existing insecticides.

The natural pyrethrins were known to be potent insecticides but appeared to have been eclipsed by the introduction of the organochlorine compounds such as DDT, lindane and later the cyclodienes which were very cheap and had good residual contact activity. Subsequent development of the organophosphates and carbamates seemed likely to fill any remaining gaps in the armoury of insecticides. The deficiencies of some of the newly introduced compounds such as undue persistence, high mammalian toxicity and potential for selecting resistant insects were not then fully appreciated. Superficially therefore there seemed little prospect for the natural pyrethrins which were unstable and relatively expensive. Against this background, it is appropriate to record the foresight of Dr. C. Potter, then Head of the Insecticides and Fungicides Department at Rothamsted Experimental Station, in recognising the potential long term disadvantages of the new synthetic compounds and encouraging and supporting work on the natural pyrethrins and related compounds because of their high activity to insects and safety to mammals.

In addition to their potential as insecticides, the chemical structures of the pyrethroids suggested that detailed study over an extended period would be rewarding. An almost endless number of structural variations was accessible by synthesis and the association of biological activity with individual optical and geometrical isomers provided an additional challenge. These factors, together with their high cost and photoinstability rendered the pyrethroids less immediately attractive than other classes as a basis for industrial research and therefore appropriate for more extended investigation by research groups outside industry, able to undertake sustained exploratory work free from commercial considerations.

2. Assessment of insecticidal activity

A detailed structure-activity study for groups of compounds such as the pyrethroids needs precise bioassay data on carefully maintained strains of insect whose response remains constant over long periods to permit detection of small effects of changes in chemical structure. Broad conclusions from the results of conventional industrial screening are not adequate. Furthermore, as Potter recognised and emphasised from his experience as a pioneer of techniques for precise insecticidal assay, it is most important to investigate activity against as many species of insect as possible following various routes of administration. Thus, although an initial observation very important for the development of the present series of compounds was the high activity of 4-allylbenzyl chrysanthemate against houseflies (see development section), had this been the only species studied, as in many early investigations reported in the literature, some key observations leading to the later more active compounds could not have been made.

3. Recognition of broad requirements for activity

In this type of approach the chemist must always have some kind of hypothesis about the structural requirements for activity to guide synthesis. Initially conjectural and vague, this only becomes more precise as knowledge accumulates. However, the efficiency of the search can be greatly increased, and whole categories of compounds which would prove inactive avoided if generalised characteristics of the active compounds and the nature of the factors influencing activity are recognised at an early stage. Polarity is an example of such a general property. With the pyrethroids, it was realised at an early stage that modifications likely to lead to significantly more polar compounds, for example the introduction of hydroxyl groups, should be avoided as they greatly diminished or eliminated activity. Such conclusions give very valuable general guidelines, but any attempt to specify too precisely the required physical properties at an early stage would probably have restricted constructive speculation. Thus, although detailed examination of the oil/water partition coefficients of the synthetic pyrethroids shows some tendency to a parabaloid relationship with activity, and an optimum polarity (Briggs et al., 1974, 1976), as would be predicted by the Hansch approach, too much emphasis on this property would probably have restricted rather than assisted the progress of the project. The value of recognising the nature of the factors influencing activity is illustrated by the importance of stereochemistry for pyrethroids. For example (-)-trans chrysanthemates are virtually inactive compared with the (+)-trans isomers, although their physical properties are identical. Stereochemistry is thus a vital consideration in structure/activity studies of pyrethroids, a situation which may be contrasted with, for example, the organophosphates where electron density, as influenced by variation in the substituents affecting the phosphorylating properties, is particularly important.

DEVELOPMENT OF SYNTHETIC PYRETHROIDS

In the light of these general considerations, we shall now outline the sequence by which recent active synthetic pyrethroids evolved, indicating what proved to be the more important advances in understanding of the structural requirements. The constituents of natural pyrethrum extract having been identified (Crombie & Elliott, 1961; Elliott & Janes, 1973), the problem of discovering the structural requirements for activity was approached by attempting to determine what features could be varied without loss of insecticidal activity. The developments relevant to our work are set out in the accompanying figure with reference to the structure of pyrethrin I, the most active of the six natural esters. The activity of the different constituents of the natural extract, and of allethrin, the first synthetic analogue of major practical importance, indicated that various side chains on the alcoholic component could be effective. Initially the essential common feature was considered to be a methylene group, preferably activated by unsaturated groups on each flank. We interpreted American work (Barthel, 1961) showing that 2,4- and 3,4- dimethylbenzyl esters were active insecticides as indicating that the 4-methyl group was equivalent to the methylene group of the natural ester side chain. 4-Allylbenzylchrysanthemate (ABC) was synthesised to combine the structural features of allethrin and of the methylbenzyl chrsanthemates and was considerably more active against houseflies. A detailed study of the influence of methylation on the activity of benzylchrysanthemates by synthesis and testing of the remaining 18 isomers showed that 2,3,6- and 2,4,6-trimethyl substitution was particularly effective. The 2,6- dimethyl pattern was therefore incorporated into ABC to give 4-allyl 2,6-dimethylbenzylchrysanthemate (DMABC) which had a broader spectrum of activity than ABC (Elliott et al., 1965).

Having established that the benzylic system could replace the cyclopentenone ring of the natural compounds without loss of activity, examination was extended to other aromatic groups, but the synthetic difficulties deterred an immediate attempt at direct replacement. However, other results had shown that the allyl or pentadienyl side chain could be substituted by benzyl in benzylrethrin and its demethyl analogue, benzyl northrin (Elliott et al., 1971a), so that benzyl-furylmethyl derivatives were examined. The results with 5-benzyl-furfuryl chrysanthemate were sufficiently encouraging to justify investigating all accessible benzylfurfuryl and furylmethyl esters, including 5-benzyl-3-furylmethyl derivatives, for which new syntheses were developed (Elliott et al., 1971b). These proved extremely active: bioresmethrin for example has been developed as a commercial product. The corresponding 5-allyl derivatives, originally envisaged have subsequently been synthesised and found to be much less active. Such results illustrate the capricious nature of investigations in this field and the need to maintain an open mind !

The activity of the furan derivatives called into question again some of the original assumptions. The substituents on the furan ring in bioresmethrin are approximated better by a meta than by a para substituted benzene; 3-benzylbenzyl-chrysanthemate was therefore examined. Although less active than the 3,5- substituted furan, it was more potent than the corresponding para- substituted benzene. This enabled the importance of the bridging methylene group, present in all the more active compounds to be reassessed. Its replacement by oxygen (3-phenoxy-benzyl chrysanthemate for 3-benzylbenzyl chrysanthemate) actually increased insecticidal potency, thus requiring revision of the original working hypothesis of the structural requirements for activity (Elliott, 1971).

The possibilities of increasing activity by modifying the acid side chain were revealed by the structures of other known compounds and by our own parallel investigations in which over 50 analogues were synthesised and tested (Elliott, et al., 1974b). The monochlorovinyl analogue made as an intermediate in the synthesis of the ethynyl derivative was itself found to give active esters; the obvious next step was to examine the dichlorovinyl acid, an isostere of chrysanthemic acid. Esterification with one of the most effective of the recently discovered alcohols led to permethrin (NRDC 143) a pyrethroid combining many favourable features: great potency to a wide range of insects, much increased photostability, low mammalian toxicity and relative ease of synthesis (Elliott et al. 1973). It is of interest that the dihalovinyl acids were suggested by the sequential development described, independently of earlier work on their esters with other alcohols (Farkas et al., 1958). This illustrates an important principle: to ensure fastest progress it is necessary to re-examine all aspects of the work continually in the light of advances on any particular front, as these may greatly modify existing assumptions. Thus the potential of the dihalovinyl acids was not revealed by the earlier alcohols and only emerged on esterification with 5-benzyl-3-furylmethyl and 3-phenoxybenzyl alcohols.

Further exploration of the dihalovinyl series demonstrated the effectiveness of bromine rather than chlorine, especially with side chains cis to the ester function. Other workers (Matsuo et al., 1973) had shown the important effect of introducing an α-cyano group in the 3-phenoxybenzyl esters. Incorporation of this improvement with the recently developed dihaloacids resulted in NRDC 161 which was found to have quite outstanding insecticidal activity (Elliott et al., 1974c). We have been concerned here with the way in which development of knowledge of the insecticidal activity of the successive compounds influenced the programme of synthesis. More recently, information on the relative mammalian toxicity of some of the compounds has been considered. This aspect cannot be discussed in the present brief survey but has been reviewed elsewhere (Elliott, 1976).

CONCLUSIONS

In summarising the development of recent pyrethroid insecticides, we have attempted to identify the key steps in advancing understanding of the structural requirements for activity, which were progressively incorporated into successive compounds. However, selecting these for illustration inevitably gives a misleadingly favourable picture of the logicality of the process. It should be stressed that many unsuccessful variations were investigated for each step forward. At the time, progress is usually hesitant and it is only with hindsight that the full significance of each development can be recognised and the process represented rationally.

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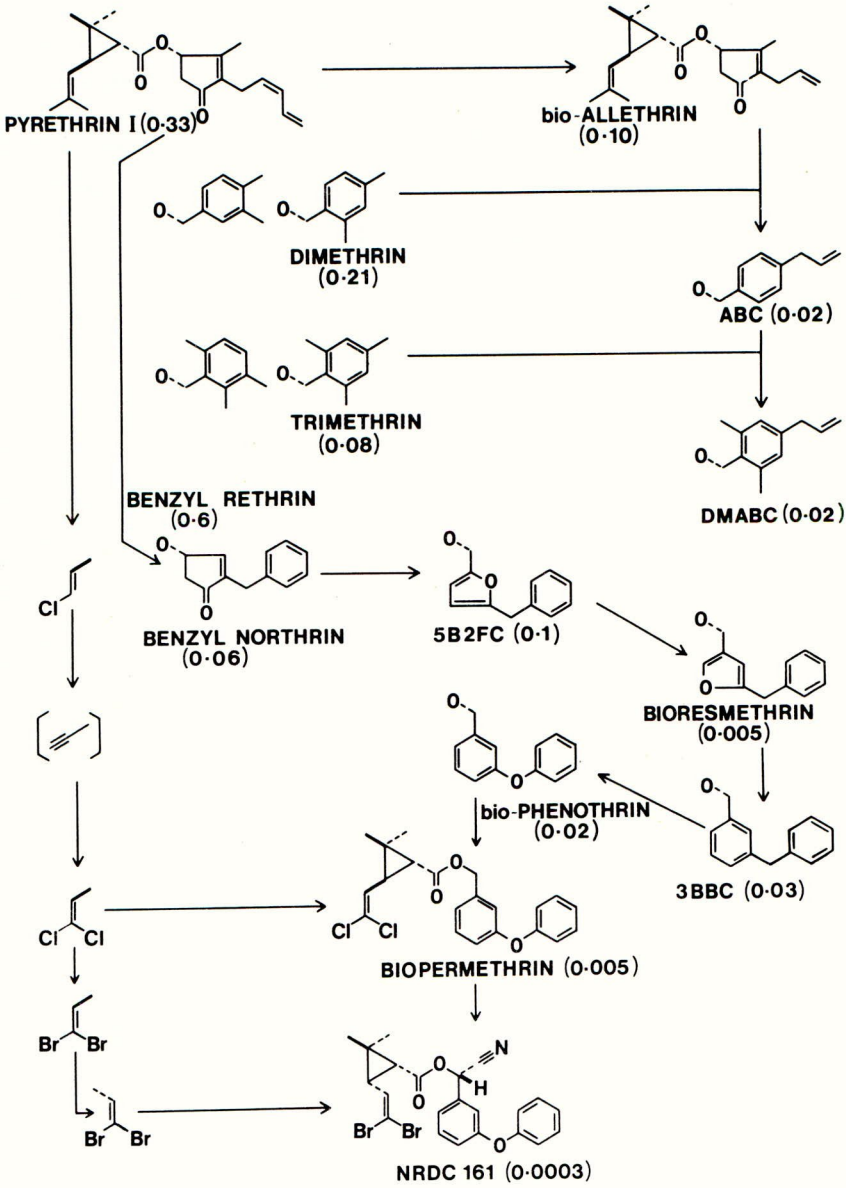
SYNTHETIC PYRETHROIDS

(approximate LD50, μg. insect $^{-1}$) by topical application to normal susceptible musca domestica



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THE INSECT TOXICITIES OF BIODEGRADABLE DERIVATIVES OF CHLORINATED NORBORNENES

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Summary The effect has been examined of the replacement of chlorine atoms by hydrogen on the toxicities of five cyclodiene insecticides to the blowfly (Calliphora erythrocephala). The toxicities are greatly reduced, little changed, or even increased by this process, depending on the parent insecticide and the pattern of replacement. The results are discussed in relation to biodegradability and selective toxicity.

Resume On examine l'effet du remplacement des atomes du chlore par l'hydrogene sur les toxicités de cinq insecticides cyclodiéniques à la mouche à viande (C. erythrocephala). Les toxicités sont grandement réduites, peu changées, ou même augementées par ce procédé, suivant l'insecticide original et la mode de remplacement. Ce qui en resulte concerne la relation entre la dégradation biologique et la toxicité sélective.

INTRODUCTION

Previous investigations with certain metabolically labile cyclodiene insecticides revealed marked differences in the ability of both insects and vertebrates to detoxify them, and such differences provide a basis for selective toxicity (Brooks 1969, Brooks et al. 1970, El Zorgani et al. 1970, Walker and El Zorgani 1974). Some of these compounds have only moderate or low insect toxicity unless applied with an appropriate metabolic inhibitor (synergist). Nevertheless, it seemed possible that they might be useful as environmentally acceptable replacements for dieldrin against insects such as tsetse fly or stable fly (Stomoxys calcitrans) which have only a limited capacity to detoxify them (Brooks, 1972).

The results of tests with experimental formulations (F. Barlow, personal communication) posed the question whether the intrinsic toxicity of compounds such as 1,2,3,4,9,9-hexachloro-5,8-epoxy-exo-1,4,4a,5,6,7,8,8a-octahydro-endo-1,4-methanonaphthalene (ODA, Figure 1) and the isomeric 5,6-epoxide (HCE, Figure 1) could be increased without reducing their general biodegradability, which arises from susceptibility to enzymatic oxidation and/or epoxide ring hydration. Specific dechlorination might accomplish this end, since it is known (Brooks 1964a, Busvine 1964, Soloway 1965) that replacement of the two vinylic chlorine atoms of dieldrin by hydrogen results in a significant increase in the insect toxicity of the product (BD, Table 1).

However, changes in the structure and stereochemistry of the non-chlorinated portion of cyclodiene molecules might also influence the effect of reductive dechlorination on insect toxicity. Accordingly, the present purpose was to examine the effect of equivalent dechlorinations on the toxicities of the representative hexachloronorbornene derivatives shown in Figure 1. The present toxicity tests have been conducted with blowflies as a representative dipteran species.

Figure 1. Hexachloronorbornene derivatives referred to in text

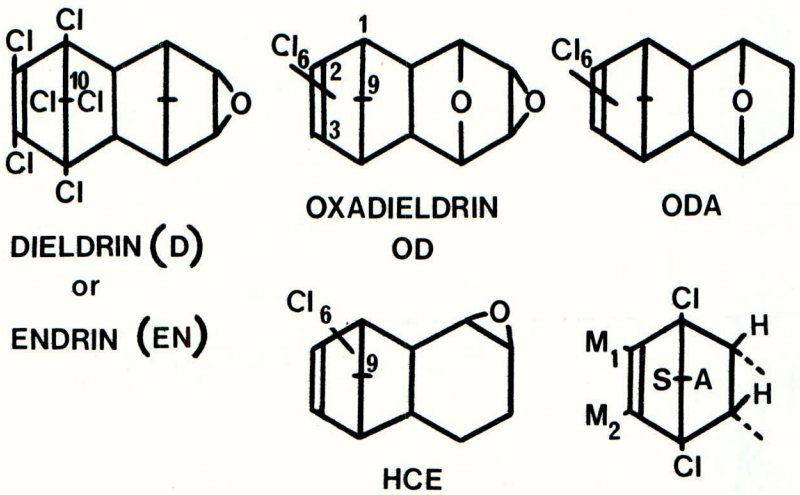

Table 1

Toxicity of dechlorinated analogues to the blowfly - Dieldrin series

Compound	Dechlorination pattern (see Figure 1)				LD50(±SE):µg/fly	
	M_1	M_2	S	A	Alone*	Synergism**
Dieldrin (D)	Cl	Cl	Cl	Cl	0.017 ±0.0011[a,c]	-
MD	H	Cl	Cl	Cl	0.022 ±0.0013[a,b]	-
SD	Cl	Cl	H	Cl	0.046 ±0.0024[b,d]	-
AD	Cl	Cl	Cl	H	1.047 ±0.073	-
BD	H	H	Cl	Cl	0.0049±0.0006[c]	-
MSD	H	Cl	H	Cl	0.10 ±0.0086[d]	-

* similar superscripts denote significant difference at 95% probability level.

** 5ug sesamex applied in acetone (1µl) 2h before the insecticide.

Table 2

Toxicity of dechlorinated analogues to the blowfly - Endrin series

Compound	Dechlorination pattern				LD50(±SE):µg/fly	
	M_1	M_2	S	A	Alone*	Synergism*
Endrin (EN)	Cl	Cl	Cl	Cl	$0.074 \pm 0.0036^{a,b}$	+
MEN	H	Cl	Cl	Cl	$0.174 \pm 0.007^{a,c}$	+
SEN	Cl	Cl	H	Cl	$0.030 \pm 0.0021^{b,c,d}$	+
SEN (old flies)	Cl	Cl	H	Cl	0.068 ± 0.0067^{d}	+
AEN	Cl	Cl	Cl	H	1.0 ± 0.078	+
MSEN	H	Cl	H	Cl	0.191 ± 0.016	+

* see footnotes in Table 1.

Table 3

Toxicity of dechlorinated analogues to the blowfly - Oxadieldrin (OD) series

Compound	Dechlorination pattern				LD50(±SE):µg/fly	
	M_1	M_2	S	A	Alone**	Synergism*
Oxadieldrin (OD)	Cl	Cl	Cl	Cl	0.015 ± 0.0007^{a}	-
MOD	H	Cl	Cl	Cl	$0.079 \pm 0.0043^{a,b,c}$	+
SOD	Cl	Cl	H	Cl	0.060 ± 0.0056^{b}	+
AOD	Cl	Cl	Cl	H	approximately 3.3	+
BOD	H	H	Cl	Cl	0.112 ± 0.013^{c}	+

* see footnotes in Table 1.

Table 4

Toxicity of dechlorinated analogues to the blowfly - Oxadihydroaldrin series

Compound	Dechlorination pattern				LD50(±SE):µg/fly	
	M_1	M_2	S	A	Alone*	Synergism*
Oxadihydroaldrin (ODA)	Cl	Cl	Cl	Cl	1.82 ± 0.168^{a}	0.072 ± 0.008**
MODA	H	Cl	Cl	Cl	2.88 ± 0.186^{a}	0.062 ± 0.011**
SODA	Cl	Cl	H	Cl	1.32 ± 0.272†	+
AODA	Cl	Cl	Cl	H	>10	

* see footnotes in Table 1.

** not significantly different at 95% probability level.

† not significantly different from ODA at 95% probability level.

Table 5

Toxicity of dechlorinated analogues to the blowfly - HCE series

Compound	Dechlorination pattern				LD50:µg/fly*	
	M_1	M_2	S	A	Alone	Synergism**
HCE	Cl	Cl	Cl	Cl	12.0	1.7
MHCE(1)	H	Cl	Cl	Cl	15.0	3.3
MHCE(2)	Cl	H	Cl	Cl	>10.0	1.8
SHCE	Cl	Cl	H	Cl	8.4	1.8
AHCE	Cl	Cl	Cl	H	>20.0	5.0

* Approximate values estimated from log dose v. probit mortality plots with lines fitted by eye.

** Synergist, 10µg/fly.

METHOD AND MATERIALS

Dieldrin, endrin and BD were provided by Shell Research Ltd., and authentic specimens of 1,2,3,4-syn-10-pentachloro-6,7-exo-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo,exo-5,8-dimethanonaphthalene (anti-10-dechlorodieldrin; AD, Table 1) and its anti-10-pentachloro-isomer (syn-10-dechlorodieldrin; SD, Table 1) by Dr. K. McKenzie and Dr. J. D. McKinney, respectively. The fully chlorinated cyclodienes 1,2,3,4,9,9-hexachloro-5,8:6,7-diepoxy-exo-1,4,4a,5,6,7,8,8a-octahydro-endo-1,4-methanonaphthalene (oxadieldrin; OD, Figure 1), ODA and HCE (Figure 1) were synthesised as described before (Brooks and Harrison 1964). The synergist sesamex was the gift of Shulton Incorporated, Clifton, New Jersey.

Reductive dechlorination of the dichloromethano-bridge of cyclodienes was effected by the general method (J. D. McKinney, personal communication) of heating the hexachloro-compounds (0.25mmole) under reflux in benzene (0.5 ml) containing tri-n-butyl tin hydride (80µl) and a free radical initiator (α,α'-azobisisobutyronitrile; AIBN, 0.5mg) until thin-layer chromatography (TLC) on aluminium oxide (0.2mm plates, Merck F 254) with hexane/ether (3:1) as mobile phase indicated complete conversion. Spot detection (aqueous MeOH/$AgNO_3$/phenoxyethanol spray, then UV light) revealed the formation of both the syn-(often predominant) and anti-monodechloro-isomers. These were usually precipitated from the concentrate with light petroleum (b.p.40-60°) and separated by recrystallisation, preparative TLC or column chromatography on alkaline alumina (Woelm; deactivated to Grade II or III) using various combinations of ether/light petroleum as eluant.

Replacement of one or both of the vinylic chlorines by hydrogen was achieved by reductive photodechlorination. Batches of the cyclodiene (up to 40mg) were irradiated in hexane in silica spectrophotometer cells (1 or 2 cm path) using a Camag Universal UV lamp Type TL-900 (254 nm) or a Hanovia U.V.S. 500 medium pressure arc as a more energetic source. Endrin and its relatives were irradiated in the presence of powdered sodium hyroxide to suppress acid catalysed rearrangements (Burton 1972, McBee and Burton 1972). Optimal conditions for conversion were determined by TLC and/or gas-liquid chromatography (GLC) with electron-capture detection (Perkin-Elmer F11; 3ft., $\frac{1}{8}$in i.d. glass column; 2.5% SE30 on 80-100 mesh Chromosorb W; carrier gas N_2).

Reaction mixtures were passed through short columns of deactivated alumina (Woelm; Grade III) to remove coloured decomposition products, then separated by a combination of the methods used for the organotin hydride reductions. Chemical structures were assigned by behaviour patterns on TLC and/or GLC, mass spectrometry (molecular wt., chlorine pattern) and NMR spectrometry.

Toxicities were determined by topically applying serial dilutions of the compounds in acetone (2µl) to the dorsum of the thorax of adult (2-4 day old) female blowflies (Calliphora erythrocephala), which were then held at 25°C under glass dishes and supplied with sugar and water for 24-36h. When tests for synergism by sesamex were made, this (5µg) was applied in acetone (1µl) about 2h before the test compound. Log dose v. mortality regression lines and LD50s were derived from the 24h mortalities using the Rothamsted probit analysis programme adapted for a Varian 12K 620/L computer.

RESULTS AND DISCUSSION

Tables 1 to 5 summarise blowfly toxicity data for various dechlorinated analogues of the five types of cyclodiene insecticides shown in Figure 1. It should be noted that the prefixes applied to the parent compound refer to chlorine atoms that have been replaced (e.g. AD, anti-10-dechlorodieldrin; SD, syn-10-dechlorodieldrin; MD, mono (vinylic)-dechlorodieldrin; BD, bis (vinylic)-dechlorodieldrin, etc.). In Figure 1, M_1 and M_2 are equivalent for symmetrical parent cyclodienes but non-equivalent for asymetrical ones such as HCE. Thus, the individual replacement of chlorines M_1 or M_2 by hydrogen in HCE gives different molecules (Table 5).

Busvine (1964) recognised similarities in the action of lindane and the cyclodienes and pointed to their common insect resistance patterns and common pentagonal arrangement of chlorine atoms. However, this pentagonal arrangement disappears in tetrachlorodieldrin (BD, Table 1), which nevertheless has a three to four-fold greater insect toxicity than dieldrin (Busvine 1964, Soloway 1965).

Compared with the cyclodienes, lindane is a smaller molecule which exerts its toxic effect more rapidly. Since many living organisms actually detoxify it fairly readily, its intrinsic toxicity is probably greater than that of most cyclodienes. Considering lindane as the 'ideal' cyclodiene, it would seem that the structural resemblance between dieldrin and lindane could be improved by replacing the S-chlorine atom (Figure 1) but not the A-chlorine by hydrogen. Further, it can be argued (Brooks 1973) on the basis of the superimposition of Soloway's (1965) electronegative centres and fit into a 'receptor pore' that removal of both of the vinylic chlorines M_1 and M_2 from a cyclodiene may actually improve the 'fit' if lindane is the ideal measure of pore size.

For the five cyclodienes examined here, the S-derivatives are considerably more toxic than the A-derivatives (Tables 1 to 5). Thus, as indicated earlier by Soloway (1965) for the corresponding two derivatives of aldrin, the anti-chlorine atoms are more important for toxicity than the syn-chlorines, in accordance with the above view of steric resemblance to lindane. When considering structure-activity relationships some measure of intrinsic toxicity is needed and this has always been difficult for chlorinated insecticides. Metabolic detoxication can result in large differences between intrinsic and observed toxicity and the replacement of chlorine by hydrogen in these molecules may be expected to expose them to increased attack by microsomal oxidases. Tests for synergism with the microsomal oxidase inhibitor sesamex may, if positive, indicate the presence of detoxicative metabolism and such tests were

applied routinely in these experiments. Negative results in the dieldrin series indicate that intrinsic toxicities were probably being measured but detoxicative metabolism is doubtless occurring in the other series and is certainly to be expected for the ODA and HCE types. Although there was evidence of synergism with the A-compounds, except AD (Table 1), the general pattern of least toxicity for this chlorine arrangement remained unchanged.

In the dieldrin series, SD was less toxic than MD and the replacement of both chlorines resulted in a cumulative decrease in toxicity in MSD (Table 1). In the endrin series, syn-dechlorination (SEN, Table 2) of endrin increased toxicity while the replacement of one vinylic chlorine reduced it (MEN, Table 2). Replacement of the S- and one M-chlorine gives MSEN (Table 2) which was not significantly different in toxicity from MEN. With synergist, the range of toxicities was 0.02-0.08µg/fly, with AEN at the upper limit, so that thereplacement of up to two chlorines in this manner has a rather small effect on the toxicity of endrin to blowflies. In the oxadieldrin series (Table 3) synergism was evidenced by the more rapid onset and progress of poisoning and some reduction in the range of toxicities observed in the absence of synergist.

In contrast to the situation in the dieldrin series the replacement of both vinylic chlorine atoms of oxadieldrin by hydrogen to give BOD (Table 3) reduced toxicity to blowflies, although the difference was smaller with the synergist present. More information is needed before any general conclusions can be reached regarding the toxicity trends among B-type compounds.

The ODA and HCE series of compounds (Tables 4 and 5) have an epoxy-cyclohexane ring which is known to be vulnerable to detoxicative attack by microsomal oxidases in some insects (Brooks 1969, 1972, Brooks and Harrison 1964b). The epoxy-cyclohexane ring confers biodegradability and selectivity. Thus, ODA and HCE were poorly toxic to blowflies; both were synergised by sesamex but HCE was still a poor toxicant (Table 4). HCE is also a poor housefly toxicant but in this case approaches dieldrin in toxicity when synergised (Brooks 1972). The apparent reduction in blowfly toxicity when ODA is converted into MODA disappeared in the presence of sesamex, indicating similar intrinsic toxicities for these compounds; SODA was as toxic as ODA, but, suprisingly, was not greatly synergised. Dechlorination in the HCE series gives similar trends in toxicity although these compounds are poor blowfly toxicants, even when synergised.

For biodegradable insecticides intrinsic toxicity can also be measured by using insects which have poor ability to metabolise them, and this approach may have a practical value. Thus, unsynergised ODA approaches dieldrin in toxicity to tsetse flies and stable flies (S. calcitrans); HCE is two to four-fold less toxic than dieldrin to tsetse flies and there is a small synergistic factor with sesamex (A. Hadaway and F. Barlow, personal communication). Dechlorinated analogues have not so far been tested against these more specialised insects. However, the trends in toxicity observed with blowflies (and houseflies; unpublished results) suggest that the replacement of up to two chlorine atoms by hydrogen may give molecules which retain useful toxicity to these other insects.

The removal of chlorine also has environmental advantages, since the dechlorinated analogues should be more vulnerable to enzymatic detoxication in the tissues of higher animals and their terminal residues more amenable to bacterial degradation.

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