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THE EFFECT OF PYRETHRIN SYNERGISTS,* ESPECIALLY SESAMEX†, ON THE INSECTICIDAL POTENCY OF HEXACHLOROCYCLOPENTADIENE DERIVATIVES ("CYCLODIENE" INSECTICIDES) IN THE ADULT HOUSEFLY, *MUSCA DOMESTICA* L

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Abstract—The toxicity of fifty-one cyclodiene compounds has been examined on strains of houseflies resistant and susceptible to dieldrin. The results demonstrate the marked cross-resistance of houseflies selected only with dieldrin to other compounds having the hexachloronorbornene nucleus (or variants of it) in common, only seven of the compounds tested being noticeably active against the resistant strains.

The toxicities of compounds tested to susceptible flies fall into six groups of which the first two contain the highly toxic cyclodienes which are mostly not markedly synergized by sesamex. The third and fourth groups include compounds of moderate or low toxicity which are synergized and the remaining groups contain compounds which are virtually non-toxic and are not synergized.

With a few exceptions, none of the compounds were synergized by sesamex against resistant flies. Moderately toxic compounds which were strongly synergized in susceptible flies but not in resistant flies were of particular interest since stabilisation of these compounds *in vivo* has been demonstrated in both strains. Assuming that the enhanced poisoning effect in susceptible strains is due to the observed inhibition by sesamex of the breakdown of the poison, then the absence of poisoning effects in resistant strains suggests that the resistance is not dependent solely on the metabolism.

That sesamex was able to delay not only the conversion of aldrin to dieldrin but also the degradation of other members of this series demonstrates its versatility as a metabolic inhibitor. If the assumption concerning the action of sesamex is valid, the synergized toxicity of compounds is an indication of their intrinsic toxicities, which give a truer indication of structure–toxicity relationships than the toxicities normally observed; the difference between toxicities with and without synergist gives a measure of the amount of metabolism occurring. The cyclodiene compounds of low toxicity which were not synergized may have low intrinsic toxicity or ability to reach their site(s) of action.

Houseflies recovered from poisoning by some mildly toxic cyclodiene compounds which were metabolized, and appeared normal except for permanent wing damage. This suggests that cyclodiene poisoning is at least partly reversible with the severity of the effect depending on the persistence of the poison in the tissues.

INTRODUCTION

FOLLOWING studies in this Laboratory on the problem of insect resistance to the insecticide dieldrin[1–3] we undertook the detailed study of a number of derivatives of

* The term synergist is used throughout this paper though the compounds may or may not exhibit synergism with a particular insecticide.

† 2-(3,4-methylenedioxyphenoxy)-3,6,9-trioxaundecane.

hexachlorocyclopentadiene (15, Table 1) and related compounds in an attempt to explain the marked differences in toxicity to the adult housefly between closely related compounds of this type. It seemed likely that the relatively low toxicity of certain compounds closely related to potent insecticides such as heptachlor, aldrin and dieldrin might be a consequence of relatively rapid detoxication of the former *in vivo* and a comparative study of the metabolism of compounds of low or intermediate toxicity would be valuable in assessing the influence of structural changes on their 'intrinsic' toxicities (that is, the toxicity which the molecules would have in the absence of metabolic changes in the organism). Studies of this nature are playing an increasingly important part in the rational development of new and safer insecticides for use against normal or insecticide-resistant insects.[4]

Investigations of the antagonism and synergism of various types of insecticides by pyrethrin synergists[5-10] indicate the potential of these compounds as inhibitors of insecticide metabolism. Metabolism studies briefly reported elsewhere[8, 11] showed that the synergism of certain 'cyclodiene' type insecticides against the housefly by pyrethrin synergists was an indication that these insecticides were detoxified *in vivo*. This paper presents the results of toxicity experiments with fifty-one compounds of the 'cyclodiene' type, alone and in the presence of the pyrethrin synergist 'sesamex', on both normal and dieldrin resistant strains of the housefly. This survey was conducted to determine which compounds might be detoxified by normal houseflies and to give some idea of their intrinsic toxicities. Compounds (32) and (35) are good examples of aldrin and dieldrin analogues whose mild toxicity appears to result from detoxication *in vivo* and these were chosen for detailed investigation. A further purpose was to determine which, if any, of these compounds were effective against dieldrin-resistant houseflies and to ascertain whether such compounds could be synergised against these insects. A discussion of the results in relation to molecular structure and intrinsic toxicity will be presented in a later communication.

EXPERIMENTAL

Insects

The houseflies used were of the susceptible strain of *Musca domestica* in use at this laboratory and of dieldrin-resistant strains of *Musca domestica vicina* called the R and RND strains. These were reared on a larval medium of grass meal, wheat toppings, yeast, stortex and water, and the cultures were kept at 27° and 70% relative humidity. The R strain was exposed to a hundred parts per million of dieldrin in the larval medium and the adults contained dieldrin throughout their lifetime. The RND-strain was a sub-strain obtained from the R- strain by discontinuing the dieldrin exposure, and had a lower resistance to dieldrin and related compounds than the latter strain[2] (Table 1).

Observation of signs of poisoning and estimation of toxicities

The signs of poisoning which will be referred to were:

(1) 'Flight convulsions'. These were the first definite signs of poisoning; normal insects developed wing tremors which became rapid beating as in flight, though the insects usually remained stationary.

(2) 'Complete knock-down'. This was a later stage of poisoning at which insects could no longer remain upright. The wings were held rigidly in abnormal positions. There were characteristic spasmodic leg movements which became weaker with time.

The toxicity tests were carried out at 25° using a range of doses of the insecticide topically applied in acetone (2μl) to the dorsal surface of the thoraces of adult females 4–5 days old (normally in groups of 10 or 20) by means of an 'Agla' micrometer syringe. The insects were then confined under crystallizing dishes standing on filter papers and were provided with aqueous glucose during the observation period which was usually 24 hr. This technique allowed comparison of the behaviour of insects treated with different insecticides, as well as 24-hr mortalities. Most of the tests were triplicated during the investigation and median lethal doses (LD 50's) were estimated from log dosage–probit mortality plots (4 points per line).

To determine the time after application of synergist at which its effect became maximal, a pre-determined dose (sub-lethal in the absence of synergist) of compound (35, Table 1) was applied at various times following topical application of 5 μg of synergist per fly (in 1 μl of acetone) to the dorsal thoraces of groups of ten females. The synergists were non-toxic at this dose. Each group of insects corresponded to a different time interval between application of synergist and insecticide and the time of appearance of signs of poisoning (flight convulsions) and time to complete knock-down were observed for each group (Table 2). For sesamex, the time to appearance of flight convulsions was minimal when the application interval was not less than 3 hr and in the toxicity tests of Table 1, sesamex was always applied 3 hr prior to applica- of the insecticides. For direct comparison, toxicity tests using sesamex (or other synergists) were conducted at the same time as those with insecticides only.

Effects of synergists on the conversion of aldrin to dieldrin

Groups of 10 female *Musca domestica* were treated with 1·0 μg of aldrin 3 hr after treatment with 5 μg of the various synergists. The rate of onset of signs of poisoning in these groups was compared with that in control groups treated with the same dose of aldrin only. When the groups treated with aldrin only became completely knocked-down, all groups were rinsed to remove external aldrin, then extracted to recover internal aldrin and dieldrin. The rinsing and extraction medium was a petroleum (b.p. 40–60°)–acetone (4:1) mixture. The aldrin and dieldrin content of extracts from all groups was compared by gas–liquid chromatography following the method of Goodwin *et al.*[12]

Synergists

Technical grades of MGK 264*, piperonyl butoxide (1-(3,4-methylenedioxy-6-propylphenyl)-2,5,8-trioxadodecane), sulfoxide (2-(3,4-methylenedioxyphenyl)-1-methylethylsulphinyloctane) and 'sesamex' (2-(3,4-methylenedioxyphenoxy)-3,6,9-trioxaundecane) were used and a sample of SKF 525A as the free base (2-diethyl-aminoethyl-2,2-diphenylpentanoate) was provided by Dr. P. S. Hewlett.

Hexachlorocyclopentadiene derivatives

1,2,3,4,9,9-Hexachloro-1,4,4a,5,8,8a-hexahydro-5,8-epoxy-1,4-methanonaphthalene (24, Table 1), telodrin† (5) and the tetrachloro analogue (51) of dieldrin‡ were the

* N-(2-ethylhexyl)-norborn-2-ene-5,6-dicarboximide.

† 'Shell' registered trade name for the compound 1,3,4,5,6,7,8,8-octachloro-1,3,3a,4,7,7a-hexahydro-4,7-methanoisobenzofuran.

‡ The common names dieldrin, aldrin, etc., are used for convenience though they normally refer to not quite pure materials; see British Standard 1831:1961.

gift of Shell Research Ltd. Alodan (8), bromodan (9) and α- and β-thiodan (13 and 14) were supplied by Farbwerke-Hoechst, A.G., and heptachlor, its known epoxides and the chlordane isomers by the Velsicol Chemical Corporation. Compounds (47) and (48) were provided by Dr. P. Bruck. Other substances were synthesized by previously described methods or were new compounds. Compounds were examined for purity by paper chromatography, gas–liquid chromatography and infra-red spectroscopy. Gas–liquid chromatography was conducted on a Shandon Universal Chromatograph using an electron capture detector. Infra-red spectra were recorded for carbon tetrachloride and carbon disulphide solutions, on a Perkin–Elmer Infracord spectrophotometer. Compounds prepared from highly toxic precursors by catalytic hydrogenation, for example dihydro-aldrin (20), were treated with potassium permanganate before recrystallization as an additional precaution against the presence of traces of unchanged precursor. Melting points are uncorrected. Hexachlorocyclopentadiene (15) was obtained from L. Light and Co. and redistilled.

1,2,3,4,5,7,7-Heptachloronorbornene (16) was prepared by passing vinyl chloride into (15) at 200° until the theoretical increase in weight was obtained (about 24 hr). The product, recrystallised from methanol (charcoal) had m.p. 145–148°. Bluestone[13] records m.p. 125–136° for the crude product.

1,2,3,4,7,7-Hexachloronorborna-2,5-diene (17) was prepared by dehydrochlorination of the heptachloro-compound (16) by the method of Bluestone.[13] For insect work, the product was freed from traces of 1,2,4,7,7-pentachloro-3-ethoxyborna-2,5-diene by conversion to a solid dibromide which was reconverted to (17) with zinc dust in ethanol.[14] The product had b.p. 126–128°/11 mm, n_D^{20} 1·5531. Bluestone[13] records b.p. 128–145°/18 mm.

1,2,3,4,7,7-Hexachloro-5,6-epoxynorborn-2-ene (19). Hexachloronorbornadiene (17, 10·5 g) in acetic acid (16 ml) was stirred at 45° with 40% peracetic acid (30 ml) containing sodium acetate (0·7 g) for 18 hr, during which time the expected amount of peracetic acid was consumed. The mixture was poured into water, the aqueous solution extracted with ether and the ethereal extract washed with sodium carbonate solution, dried (Na_2SO_4) and evaporated. Fractionation of the liquid product at 0·8 mm gave mostly unchanged hexachloronorbornadiene followed by a small end fraction which partly solidified. This fraction, chromatographed on alkaline alumina (Peter Spence, type H) in petroleum (b.p. 40–60°), gave hexachloronorbornadiene and a solid m.p. 110–112° (100 mg) after recrystallisation from methanol ν_{max} 820, 1248 (epoxide), 1600 cm^{-1}. Kleiman[15] records m.p. 111–112° for this compound.

1,2,3,4,7,7-*Hexachloronorborn-2-ene* (18). Hexachloronorbornadiene (5·0 g) was hydrogenated in ethanol (25 ml) over Adam's platinum oxide catalyst (50 mg) at atmospheric pressure until 1 mol was absorbed. The liquid product solidified in methanol and the recrystallized product (3 g) had m.p. 36–37° (Found: C, 27·6; H, 1·6; Cl, 70·4. $C_7H_4Cl_6$ requires C, 27·9; H, 1·3; Cl, 70·8%). The supernatant contained a liquid (2 g) which was shown to be an impure sample of the same product by infra-red spectrum and gas–liquid chromatography. Mackenzie[14] records b.p. 104–108°/2 mm for a similar product.

4,5,6,7,10,10-Hexachloro-4,7,8,9-tetrahydro-4,7-methanoindene (chlordene; 28), m.p. 153–158°, was prepared by the method of Riemschneider[16] who records that melting of this substance begins at about 155°.

4,5,6,7,10,10-Hexachloro-4,7,8,9-tetrahydro-4,7-methanoindane (dihydrochlordene 27). Chlordene (0·5 g) in ethanol (10 ml) containing Adam's catalyst (2 mg) rapidly absorbed hydrogen at atmospheric pressure to give the dihydro-derivative (27). The product, recrystallized from methanol, had m.p. about 170° (0·47 g) and was identical in melting point behaviour and infra-red spectrum with the product of direct condensation of equivalent quantities of hexachlorocyclopentadiene and cyclopentene in a sealed evacuated tube at 130° (5 hr). Riemschneider and Grabitz[17] record m.p. 172° and Riemschneider[18] discusses the melting point interval observed with chlordene-type compounds.

4,5,6,7,10,10-Hexachloro-4,7,8,9-tetrahydro-2,3-epoxy-4,7-methanoindane (chlordene-epoxide; 29). Chlordene (2 g) in chloroform (30 ml) containing perbenzoic acid (1·7 g) was kept at 0° for 4 days. After being washed with 10% aqueous sodium carbonate and dried (Na_2SO_4), the chloroform solution was evaporated and a solution of the crude solid in petroleum (b.p. 40–60°), chromatographed on alkaline alumina (Peter Spence, type H) gave the epoxide, m.p. 232–234° (1·8 g) after recrystallization (methanol). Peri[19] records m.p. 235°.

1,2,3,4,9,9-*Hexachloro*-1,4,4a,5,6,8*a*-*hexahydro*-1,4-*methanonaphthalene* (32). Hexachlorocyclopentadiene (1·4 g) cyclohexa-1,3-diene (0·4 g) and a little quinol were heated under reflux for 15 min at 120° (oil bath temp). The cooled mixture solidified and the solid (1·12 g) had m.p. 115–116° after recrystallization from methanol (Found: C, 37·0; H, 2·3; Cl, 59·0. $C_{11}H_8Cl_6$ requires C, 37·4; H, 2·3; Cl, 60·3%). Riemschneider and Grabitz[17] record m.p. 94–96° for a product obtained by prolonged heating of these reactants in a sealed tube. The compound m.p. 115–116°, hydrogenated in ethanol at atmospheric pressure over Adam's catalyst, gave a dihydro-derivative m.p. 77–78°, identical in all respects with 1,2,3,4,9,9-hexachloro-1,4,4a,5,6,7,8,8a-octahydro-1,4-methanonaphthalene (31), m.p. 78°, prepared by direct condensation of hexachlorocyclopentadiene and cyclohexene in a sealed tube at 130° (7 hr). Riemschneider and Grabitz[17] record m.p. 78–79° for this compound.

1,2,3,4,9,9-*Hexachloro*-1,4,4a,5,8,8a-*hexahydro*-1,4-*methanonaphthalene**(33). (a) Hexachloronorbornadiene (2·5 g), butadiene (0·5 g) and a trace of quinol, shaken in an evacuated, sealed tube at 120° (7 hr), gave an oil which crystallized in methanol. Recrystallization (methanol) gave a solid m.p. 94–95° (1·4 g). (b) Hexachlorocyclopentadiene (1·4 g), cyclohexa-1,4-diene (0·93 ml; 100% excess) and a little quinol were heated in an evacuated, sealed tube at 130° (7 hr). The cold reaction mixture deposited a solid, m.p. about 334° (decomp.) on recrystallization (chloroform), which appeared to be a bis adduct (Found: C, 31·2; H, 1·24; Cl, 67·6. $C_{16}H_8Cl_{12}$ requires C, 30·7; H, 1·3; Cl, 68·0). The residual oil, treated as in (a), gave a compound m.p. 94–95° (0·5 g), identical with that prepared in method (a) (Found: C, 37·7; H, 2·3; Cl, 59·1. $C_{11}H_8Cl_6$ requires C, 37·4; H, 2·3; Cl, 60·3%). The product absorbed 1 mol of hydrogen to give the 1,2,3,4,9,9-hexachloro-1,4,4a,5,6,7,8,8a-octahydro-1,4-methanonaphthalene (31), m.p. 77–78°, previously described.

1,2,3,4,9,9-*Hexachloro*-1,4,4a,5,6,7,8,8a-*octahydro* -7,8 -*epoxy* -1,4 -*methanonaphthalene* (35). The hexachlorocyclopentadiene/cyclohexa-1,3-diene adduct (32) (1·5 g) was oxidised in chloroform (25 ml) containing perbenzoic acid (1.3 g) for 4 days at 18°. The epoxide, isolated as for chlordene epoxide, had m.p. 222–224° and ν_{max} 833, 1250

* Originally prepared by Professor C. W. Kearns; personal communication.

(epoxide) and 1600 cm^{-1} (Found: C, 36·2; H, 2·2; Cl, 56·4. $C_{11}H_8Cl_6O$ requires C, 35·8; H, 2·2; Cl, 57·7%).

1,2,3,4,9,9-Hexachloro-1,4,4a,5,6,7,8,8a-octahydro-6,7-epoxy-1,4-methanonaphthalene (34). The hexachlorocyclopentadiene/cyclohexa-1,4-diene adduct was oxidised as in the previous example to an epoxide m.p. 93–94°. ν_{max} 827, 1250 (epoxide) and 1600 cm^{-1} (Found: C, 36·3; H, 2·5; Cl, 56·2. $C_{11}H_8Cl_6O$ requires C, 35·8; H, 2·2; Cl, 57·7%).

1,2,3,4,9,9-Hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-methanophthalene-5,8-dione(43) m.p. 189° was prepared according to the method of Lidov *et al.*[20] by the reaction between hexachlorocyclopentadiene and *p*-benzoquinone in boiling xylene. Rearrangement of this adduct (0·25 g) in boiling methanol (2 ml) containing sodium hydroxide (0·02 g) following the method of Segel *et al.*[21] gave 1,2,3,4,9,9-hexachloro-1,4-dihydro-1,4-methanonaphthalene-5,8-diol (44) m.p. 183–185°.

1,2,3,4,9,9-Hexachloro-1,4,4a,5,8,8a-hexahydro-6,7-dimethyl-1,4-methanonaphthalene (39). 2,3-Dimethyl-1,3-butadiene (0·9 g), from catalytic dehydration of anhydrous pinacol with aqueous hydrobromic acid[22], and hexachloronorbornadiene (3 g) were heated together in an evacuated sealed tube at 130° (8 hr). The decolourized solution of the liquid product in methanol deposited a solid which had m.p. 94–95° (1·8 g) after further recrystallization from methanol. (Found: C, 40·8; H, 3·3; Cl, 54·5. $C_{13}H_{12}Cl_6$ requires C, 41·0; H, 3·2; Cl, 55·8%). The product, hydrogenated in ethanol over Adam's catalyst, gave a dihydro-derivative, *1,2,3,4,9,9-hexachloro-1,4,4a,5,6,7,8,8a-octahydro-6,7-dimethyl-1,4-methanonaphthalene* (40), m.p. 47–49°.

1,2,3,4,9,9-Hexachloro-1,4,4a,5,8,8a-hexahydro-6-methyl-1,4-methanonaphthalene (41). Isoprene (0·9 g) and hexachloronorbornadiene (3·7 g), heated in an evacuated sealed tube at 130° (10 hr), gave an oil which solidified when diluted with methanol. The recrystallized (methanol) solid had m.p. 37–40°. (Found: C, 39·3; H, 2·6; Cl, 57·5. $C_{12}H_{10}Cl_6$ requires C, 39·3; H, 2·9; Cl, 58·0%).

This absorbed 1 mol of hydrogen over Adam's catalyst to give the 1,4,4a,5,6,7,8,8a-octahydro compound (42), m.p. 66–67°.

5,6,7,8,9,9-Hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-ethano-5,8-methanonaphthalene (36). Hexachloronorbornadiene (1·6 g), cyclohexa-1,3-diene (0·5 ml) and a little quinol were heated in an evacuated sealed tube at 130° (5 hr). The product, recrystallized from methanol (charcoal) had m.p. 194–195° (1 g) (Found: C, 41·45; H, 2·6; Cl, 55·1. $C_{13}H_{10}Cl_6$ requires C, 41·2; H, 2·6; Cl, 56·2%). It absorbed 1 mol. of hydrogen over Adam's catalyst in ethanol to give the *1,2,3,4,4a,5,8,8a-octahydro* compound (37), m.p. 171–172° (Found: C, 40·8; H, 3·4; Cl, 55·5. $C_{13}H_{12}Cl_6$ requires C, 41·0; H, 3·2; Cl, 55·8%).

5,6,7,8,9,9-Hexachloro-1,2,3,4,4a,5,8,8a-octahydro-2,3-epoxy-1,4-ethano-5,8-methanonaphthalene (38). The above hexahydro-compound (0·5 g) was kept at 18° for 6 days in chloroform (12 ml) containing perbenzoic acid (0·36 g). The crude product, chromatographed in petroleum (b.p. 40–60°) on alkaline alumina (Peter Spence, type H) and recrystallized (methanol) gave the epoxide (0·4 g) m.p. 201–203°; ν_{max} 855, 1270 (epoxide), 1600 cm^{-1}. (Found: C, 39·2; H, 2·85; Cl, 53·5. $C_{13}H_{10}Cl_6O$ requires C, 39·5; H, 2·6; Cl, 53·8%).

6,7-Dihydro-aldrin (20)[23], m.p. 78° and 6,7-dihydro-isodrin (21)[24] m.p. 219° were prepared by hydrogenation of aldrin and isodrin respectively over Adam's catalyst in ethanol.

1,2,3,4,9,9-Hexachloro-1,4,4*a*,5,6,7,8,8*a*-octahydro-5,8-epoxy-1,4-methano-naphthalene (25), m.p. 127–129°, was similarly prepared by catalytic reduction of the precursor 1,2,3,4,9,9-hexachloro-1,4,4a,5,8,8a-hexahydro-5,8-epoxy-1,4-methanonaphthalene (24), m.p. 139°.

1,2,3,3,4,11-Hexachlorohexacyclo[5,4,1,$0^{2,6}$, $0^{4,11}$, $0^{5,9}$, $0^{10,12}$]dodecane (22; called photodrin for convenience by Cookson and Crundwell[25]) was prepared by heating under reflux for 7 hr a mixture of isodrin (0·5 g) and acetic acid (8·5 ml) containing sulphuric acid (1·5 ml), according to the method of Bruck *et al.*[26] The product (0·4 g) which crystallized from the cold reaction mixture, had m.p. 298° (decomp).

4,5,6,7,8,8-Hexachloro-1,3,3a,4,7,7a-hexahydro-4,7-methanoisobenzofuran (26). Hexachlorocyclopentadiene (2·73 g) and 2,5-dihydrofuran (0·8 ml), heated in an evacuated sealed tube at 130° (5 hr) gave a dark oil which solidified when cooled. Repeated recrystallisation from methanol (charcoal) gave the above compound (2 g) m.p. 225–230° (sublimed and decomp). Feichtinger and Tummes[27] record m.p. 236°.

1,2,3,4,10,10-Hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-exo-1,4-endo-5,8-dimethanonaphthalene* (23) was prepared by the two stage process of Soloway[28].

(1) 1,2,3,4,10,10-Hexachloro-6-iodo-1,4,4a,5,6,7,8,8a-octahydro-exo-1,4-endo-5,8-dimethanonaphthalene-7-ol acetate (aldrin iodo-acetate). Iodine (8·9 g) was added to a stirred solution of aldrin (11·0 g) in ether (25 ml). Silver acetate (5·84 g) was then introduced gradually to maintain a reaction temperature of 20°. After 2 hr, aqueous potassium cyanide was added to dissolve silver iodide, the ether was evaporated and benzene added to dissolve the ether insoluble organic material. The benzene solution, washed with aqueous potassium cyanide, dried (Na_2SO_4) and concentrated, afforded the iodo-acetate, m.p. 195–196° (5·9 g).

(2) Conversion of the iodo-acetate to aldrin endo-epoxide. A mixture of 85% potassium hydroxide (2 g), dioxan (7 ml), *tert*-butyl alcohol (7 ml) and water (1·4 ml) was heated under reflux for 30 min to dissolve the alkali. The iodo-acetate (4 g) was then added portionwise to the cooled solution to give a two-phase system which was stirred and heated under reflux for 18 hr. The mixture, poured into ice-cold water, gave a solid which was recrystallized from acetone–methanol (charcoal) to give the endo-epoxide (2 g), m.p. 138–140°. ν_{max} 855, 1240 cm^{-1} (epoxide) and 1600 cm^{-1}. Soloway[28] records m.p. 138–140° for this epoxide.

1-*Bromo*-4,5,6,7,10,10-*hexachloro*-4,7,8,9-*tetrahydro*-4,7-*methanoindene* (30). Chlordene (0·4 g) was heated under reflux for 30 min in anhydrous carbon tetrachloride (10 ml) containing N-bromosuccinimide (0·19 g). The cooled solution was filtered to remove succinimide and evaporated to give an oil which solidified. Recrystallized from methanol, the product (0·3 g) had m.p. 68–70° (Found: C, 29·7; H, 1·0; Cl, 47·8 Br, 21·5. Calc. for $C_{10}H_5Cl_6Br$: C, 28·8; H, 1·2; Cl, 50·9; Br, 19·1). The infra-red spectrum of the product closely resembled that of the analogous heptachloro- compound (heptachlor). Herzfeld and Ordas[29] report this compound b.p. 130–132°/ 0·05 mm.

Aldrin exo-acetate (45), m.p. 144–145° was prepared by heating aldrin under reflux with 5% sulphuric acid in acetic acid for 1 hr.[30]

1,2,3,4,7,7-Hexachloro-5-(3,4-methylenedioxybenzyl)norborn-2-ene (46), m.p. 110–112° was prepared from hexachlorocyclopentadiene and safrole by the method of Riemschneider and Grabitz.[17]

* This compound, which differs from dieldrin only in the stereochemistry of the epoxide ring, is the endo-epoxide of aldrin.

1,2,3,4,9,9-*Hexachloro*-1,4, ?, ?,-*tetrahydro*-1,4-*methanonaphthalene* (50) and 1,2,3,4,9,9-*hexachloro*-1,4-*dihydro*-1,4-*methanonaphthalene* (49). The hexachlorocyclopentadiene/cyclohexa-1,4-diene adduct (33; 19 g) in dry carbon tetrachloride (200 ml), heated under reflux with N-bromosuccinimide (19 g; 2 equiv.) for $1\frac{1}{2}$ hr, gave, after removal of succinimide (filtration) and solvent (reduced pressure) an oil (28 g; corresponding to addition of two bromine atoms) which was dehydrobrominated in ethanol (300 ml) containing potassium hydroxide (32 g). The mixture was stirred and heated under reflux in nitrogen for 1 hr, filtered to remove potassium bromide, the ethanol evaporated under reduced pressure and the residual oil dissolved in chloroform. The oil recovered from the washed (water) and dried (Na_2SO_4) chloroform solution was distilled under reduced pressure and gave a fraction b.p. 127–135°/0·1 mm which solidified (4 g). This solid, m.p. 62–65°, repeatedly chromatographed in petroleum (b.p. 40–60°) on neutral alumina (Woelm, grade 1), gave approximately equal amounts of *tetrahydro-compound* (50), m.p. 72–74° (Found: C, 38·8; H, 1·6; Cl, 60·6. $C_{11}H_6Cl_6$ requires C, 37·7; H, 1·7; Cl, 60·6%); λ_{max} 231 mμ (ϵ 4,864) and 287 mμ (ϵ 8,540) and the dihydro-compound (49) m.p. 94–95° (Found: C, 38·0; H, 1·7; Cl, 59·9. $C_{11}H_4Cl_6$ requires C, 37·9; H, 1·2; Cl, 61·0%); λ_{max} 220 mμ (ϵ 11220), $\lambda_{inflection}$ 250 mμ (ϵ 1000), ν_{max} 3050, 1960, 1920, 1880, 1850, 1800, 1600 cm^{-1}. The ultra-violet spectrum of the tetrahydro-compound and its catalytic reduction (2 molecules of hydrogen absorbed) to the octahydro-compound (31) support its formulation as a cyclohexa-1,3-diene derivative. The infra-red spectrum of the dihydro-compound is consistent with the ortho-disubstituted benzene structure and the ultra-violet spectrum resembles that of the analogous unchlorinated compound described by Wittig and Knaus.[31]

RESULTS

Comparison of groups of insects which received aldrin only with groups treated with aldrin plus any of the synergists showed that onset of signs of poisoning was delayed in the presence of the synergist. Gas-chromatographic examination of the tissue extracts showed that, while all groups contained considerable amounts of unchanged aldrin, the amounts of dieldrin present were 3–4 times greater in insects treated only with aldrin. Since similar amounts of aldrin were recovered from the tissues of insects treated with the pyrethrin synergists plus aldrin, or aldrin only, reduced dieldrin formation in the presence of these synergists could not be attributed to retarded penetration of aldrin.

All the substances synergised compounds (32) and (35) but sesamex was most effective in this respect and was therefore tested for synergism with the remaining hexachlorocyclopentadiene derivatives, which are listed in Table 1.

With compounds (32) and (35) reversible knock-down effects were observed with doses approaching the LD_{50}. Although the progress of poisoning with these compounds was typical of 'cyclodiene' insecticides, recoveries were sometimes observed in insects which had been prostrate for 24 hr. Insects which recovered appeared to survive as long as untreated flies, though their wings remained in abnormal positions and they could not fly. The results of experiments on the duration of the synergism of compound (35) by various synergists are summarized in Table 2.

The time pattern of poisoning was constant when compound (35) was applied between 3 and 10 hr after sesamex and under these conditions the insecticide was

TABLE 1. THE TOXICITIES OF SOME 'CYCLODIENE' AND RELATED COMPOUNDS (ALONE AND IN THE PRESENCE OF 5 μg/FLY OF SESAMEX) TO A SUSCEPTIBLE (S) STRAIN OF *Musca domestica L.* AND TO RESISTANT STRAINS (RND AND R) OF *Musca domestica* VICINA

COMPOUND‡	COMMON NAME	LD50 ALONE† (μg/fly) STRAIN: S	RND	R	LD50 WITH SESAMEX (μg/fly) S	RND	R
	(1) Aldrin	0·03	*	*	0·03	*	*
	(2) Isodrin	0·06	0·90	2·0	0·06	0·90	2·0
	(3) Dieldrin	0·02	16·0	>20	0·02	16·0	>20
	(4) Endrin	0·04	1·1	1·9	0·04 +	1·1 +	1·9 +
	(5) Telodrin	0·02	0·50	0·90	0·02	0·50	0·90
	(6) α-Chlordane	0·20	*	*	0·20	*	*
	(7) β-Chlordane	0·06	*	*	0·06 +	*	*
	(8) Alodan	0·31	*	*	0·05	*	*
	(9) Bromodan	0·23	*	*	0·01	*	*
	(10) Heptachlor	0·02	*	*	0·02	*	*
	(11) Heptachlor epoxide m.p. 161°	0·02	*	*	0·02 +	*	*
	(12) Heptachlor epoxide m.p. 95°	0·12	*	*	0·12	*	*
	(13) α-Thiodan	0·10	2·9	7·0	0·02	1·1	3·3
	(14) β-Thiodan	0·10	7·0	*	0·02	6·7	*
	(15) Hexachloro-cyclopentadiene	*	*	*	*	N	N
	(16) Heptachloro-norbornene	*	*	*	*	*	*
	(17) Hexachloro-norbornadiene	*	*	*	*	*	*

COMPOUND‡	COMMON NAME	LD50 ALONE† (μg/fly) STRAIN: S	RND	R	LD50 WITH SESAMEX (μg/fly) S	RND	R
	(18) Hexachloro-norbornene	*	*	*	*	N	N
	(19)	*	*	*	*	N	N
	(20) 6,7-Dihydro-aldrin	0·80	*	*	0·06	*	*
	(21) 6,7-Dihydro-isodrin	0·78	*	*	0·08	*	*
	(22) Photodrin	0·30	*	*	0·06	*	*
	(23) Aldrin endo-epoxide	0·25	*	*	0·08	*	*
	(24)	0·02	*	*	0·01	*	*
	(25)	0·60	*	*	0·03	*	*
	(26)	>10	*	*	>10 +	N	N
	(27) Dihydro-chlordene	2·0	*	*	0·70	N	N
	(28) Chlordene	1·0	*	*	0·40	N	N
	(29) Chlordene epoxide	0·70	*	*	0·08	*	*
	(30)	0·25	*	*	0·25	N	N
	(31)	2·9	*	*	0·21	N	N
	(32)	2·5	*	*	0·20	*	*
	(33)	2·6	*	*	0·30	N	N
	(34)	>10	*	*	>10 +	*	*

COMPOUND‡	COMMON NAME	LD50 ALONE† (μg/fly) STRAIN: S	RND	R	LD50 WITH SESAMEX (μg/fly) S	RND	R
	(35)	1·8	*	*	0·04	*	*
	(36)	0·49	>10 §	*	0·04	5·0 §	*
	(37)	1·9	*	*	0·16	N	N
	(38)	0·74	4·6	>10 §	0·02	0·73	10-3·3 §
	(39)	>10	*	*	3·3	N	N
	(40)	>10	*	*	1·0	N	N
	(41)	4·2	*	*	0·70	N	N
	(42)	5·7	*	*	0·70	N	N
	(43)	*	*	*	*	N	N
	(44)	*	*	*	*	N	N
	(45) Acetoxy-aldrin	*	*	*	*	N	N
	(46)	*	*	*	*	N	N
	(47)	*	*	*	*	N	N
	(48)	*	*	*	*	N	N
	(49)	*	*	*	*	N	N
	(50)	>10	*	*	2·5	N	N
	(51) Tetrachloro-dieldrin	0·004	*	*	0·004	*	*

* Indicates no toxic effect at the 10 μg dose.

† > indicates less than 50% of flies killed at this dose; + indicates progress of poisoning appeared more rapid with sesamex but LD_{50} was unaltered; N indicates compound not tested.

§ Strain appears heterogeneous to this compound or synergist/compound combination.

‡ The symbol or is an abbreviation for

more rapidly effective than the same dose of dieldrin applied alone. Following application intervals greater than 10 hr, flight convulsions appeared in approximately the same time but the time for all flies in a group to be knocked-down increased and in each group a number of flies recovered. With all the synergists the time required for onset of flight convulsions appeared to remain approximately constant even when the remaining course of poisoning became obscured by recoveries from knock-down. This was particularly noticeable with MGK 264 which appeared to be least persistent of all the synergists tested.

TABLE 2. THE EFFECT OF SYNERGISTS (5 μg/FLY) APPLIED AT DIFFERENT INTERVALS PRIOR TO A SUBLETHAL DOSE (0·4 μg/FLY) OF COMPOUND (35) ON ITS INSECTICIDAL POTENCY

Synergist	Minimum interval required between application of synergist and insecticide for most rapid appearance of flight convulsions (hr)	Interval following application of synergist within which synergistic effect remained maximal* (hr)	Interval following application of synergist during which insecticide (0·4 μg) produced irreversible knock-down (hr)
Sesamex	3	3–10	0–10
Piperonyl butoxide	1	1–3	0–3
Sulfoxide	2	2–10	0–10
MGK 264	1	Time to complete knock-down was variable for all intervals and a large proportion of insects in all groups recovered	
SKF 525A (free base)	2	2–25	0–25

* As indicated by the time after dosing with the insecticide at which signs of poisoning (flight convulsions) appeared and the time to complete knock-down. These times remained approximately constant for application intervals within the range shown.

DISCUSSION

The synergism of compounds (32) and (35) by sesamex has been shown to be accompanied by inhibition of their breakdown *in vivo* in *M. domestica*.[8] Sun and Johnson[5] showed that sesamex reduced the conversion of aldrin to dieldrin in houseflies and the present results show that this is true of the other synergists tested. The results of Sun and Johnson[5] indicated that inhibition of dieldrin formation resulted in lowered toxicity of aldrin to the housefly and in the present experiments all the substances producing this inhibition slowed the course of aldrin poisoning. These results are in accord with the observation of Brooks *et al.*,[32] that the course of aldrin or isodrin poisoning was greatly retarded when dieldrin or endrin formation was suppressed in houseflies deprived of oxygen. The other synergists were less effective than sesamex in synergizing compounds (32) and (35) and their effect on the insecticide concentration *in vivo* was not examined but there is little doubt that, as with sesamex, the synergistic effects are associated with inhibition of metabolic breakdown of the insecticides.

The precursor (32) produces little epoxide *in vivo* in the housefly* and may be metabolized by mechanisms involving direct hydroxylation of the double bond or oxidation

* Unpublished results.

at carbon atoms adjacent to the double bond as with, for example, camphene, the ionones, and cyclohexenyl barbiturates in mammals.[33] The synergism by sesamex of dihydro-aldrin (20), dihydro-isodrin[8] (21) and the cyclohexane derivative* (31) is accompanied by stabilization of these substances *in vivo*, so that pyrethrin synergists appear to be capable of inhibiting not only epoxide formation (as with aldrin) and breakdown (as with compound 35) but also a variety of other metabolic mechanisms, some probably involving direct attack on reduced alicyclic rings. As with other types of insecticides, therefore, the observation of synergistic (or occasionally antagonistic) effects with 'cyclodienes' appears generally to be evidence of the existence of metabolic pathways *in vivo*.

In order to use sesamex as an indicator of metabolism it was desirable to use it at optimum effectiveness and the conditions for this were determined for compound (35) which was markedly synergized by sesamex, by the experiments of Table 2. The lag (about 3 hr) between application of sesamex and attainment of optimum effect was probably related to the time required for its penetration into and distribution throughout the insect and in the toxicity experiments of Table 1 this interval was always allowed between application of synergist and insecticides. However, in toxicity experiments using shorter time intervals with compound (35) and other strongly synergized compounds, the ultimate result was the same although flight convulsions were later in appearing. From Table 2, the period during which knock-down was irreversible with SKF 525A and compound (35) was longer than the corresponding period with sesamex. This suggests that although sesamex is a better synergist according to toxicity tests, it may be inactivated more rapidly than SKF 525A in the housefly.

The recovery of insects from knock-down has been observed with DDT and γ-BHC poisoning but in the author's experience, poisoning with the better known 'cyclodiene' insecticides such as aldrin, dieldrin, heptachlor etc., is irreversible. The reversibility of the knock-down effect observed with compound (35) is interesting in relation to the mode-of-action of dieldrin. The structural resemblances between the two compounds and the characteristically similar behaviour of insects poisoned with them suggests that their mode-of-action is similar. The reversible knock-down with the epoxide which can be metabolized by the housefly suggests that dieldrin poisoning would be reversible were it not for the persistence of dieldrin in the tissues. Experiments *in vivo** showed that at the doses used, sesamex did not entirely suppress metabolism so that if the mechanism of poisoning with compound (35) involved a reversible narcosis, insects which were able to survive desiccation and starvation while they were knocked-down might be expected to recover when they had reduced their internal level of insecticide below the threshold required for narcosis. Since insects which recovered had apparently suffered permanent damage to the flight muscles or their nervous control mechanisms, irreversible effects *are* involved but in all other respects insects recovering appeared normal.

Dieldrin resistant houseflies

The results of Table 1, together with those of Metcalf and Georghiou,[34] demonstrate the striking cross resistance of dieldrin-resistant houseflies to a range of compounds having the hexachloronorbornene system, or variants of it, in common and to which

* Unpublished results.

the insects have never been exposed. Of the compounds tested against the dieldrin-resistant strains only isodrin, endrin, telodrin, α-thiodan, β-thiodan, compound (38) and the related compound (36) were noticeably active. Only in the case of α-thiodan and compounds (36) and (38) were there indications of increased toxicity to these strains in the presence of sesamex. Since sesamex inhibits metabolism of compound (35) it might be expected to inhibit any dieldrin metabolism occurring in the housefly. If, therefore, dieldrin resistance is due to metabolism, sesamex applied to the R-strain (containing inbred dieldrin) might be expected to produce toxic effects. This was not the case and moreover, with the exception of compounds (36) and (38), mildly toxic compounds which were synergized against susceptible flies were not synergized against insects of either resistant strain. In contrast is the well known example of the synergism of DDT against DDT resistant houseflies by piperonyl cylconene. In this case the increased toxicity of DDT to the resistant insects was shown to be correlated with decreased production of the detoxication product DDE, a finding which provided evidence for the role of metabolism in their resistance to DDT.[35] The toxicity of compounds (36) and (38) to the resistant strains accords with their stereochemical relationship with isodrin and endrin, respectively, and they had been shown to be stabilized by sesamex *in vivo* so that their increased toxicities to these strains were not unexpected. The synergistic effect observed with the resistant strains was, however, much less than that found with the susceptible strains, especially in the case of epoxide (38), so there was clearly a high residual level of resistance to these compounds even when the metabolism was suppressed. The results obtained with 'cyclodiene' insecticides therefore tend to support the view [1, 36–38] that metabolism is not a major factor in dieldrin resistance. Of particular interest in the resistance study was the lower tolerance, reported earlier by Busvine,[39] of dieldrin resistant houseflies to endrin and isodrin (Table 1). The latter is converted into endrin in the housefly[3] and the difference in tolerance appeared to be peculiar to the epoxides and not to be associated with the general structural difference between compounds belonging to the different stereochemical series represented by dieldrin and endrin. Thus, dihydro-isodrin (21) was no more toxic to the resistant strains than dihydro-aldrin (20) so that the difference between the tolerances to endrin and dieldrin was not reflected in the corresponding dihydro-compounds, which have the same stereochemical difference. In this context it is noteworthy that α-thiodan (13) was more toxic than β-thiodan (14) to resistant houseflies and the structure indicated for this substance by Riemschneider and Wuscherpfennig[40] gives it a stereochemical resemblance to endrin, while β-thiodan resembles dieldrin structurally.

Normal houseflies

This investigation has been confined mainly to those compounds in which the hexachloronorbornene nucleus (18) is retained while variations are introduced in the attached groups or ring systems. Compounds (47), (48) and (51), each containing a ring system substituted by only four chlorine atoms, illustrate the effect on toxicity to the normal housefly of changes in the chlorinated ring system. Thus, toxicity disappeared in the aldrin analogue (47) in which a tetrachlorobenzene nucleus replaces the hexachloronorbornene system and in the 6,7-dihydroaldrin analogue (48) in which tetrachlorocyclohexa-1,3-diene replaces hexachloronorbornene. On the other hand, the dieldrin analogue (51), in which tetrachlororonorbornene replaces

hexachloronorbornene, was more toxic than dieldrin, so that limited changes in the chlorinated ring are permissible.

In view of the synergistic effects shown by sesamex with various 'cyclodiene' compounds, it was of interest to examine compound (46), combining the non-toxic and non-synergized hexachloronorbornene system (18) and the methylenedioxyphenyl group, which appears to be involved in the synergistic activity of many pyrethrin synergists. Although compound (46) was not toxic (Table 1), insects treated with it became hyperactive and there was some evidence that it synergized compound (35).

Considering normal flies only, the remaining compounds of Table 1 may be divided into groups according to their LD_{50}'s:

Group A. Not synergized. LD_{50} 0·004 to 0·12 μg/fly. Comprising compounds (1), (2), (3), (4), (5), (10), (11), (12), (7) and (51).

Group B. Synergized. LD_{50} without synergist 0·01 to 0·12 μg/fly; with synergist 0·01 to 0·12 μg/fly. Comprising compounds (13), (14) and (24).

Group C. Synergized. LD_{50} without synergist $\geqslant$ 0·25 μg/fly; with synergist 0·01 to 0·12 μg/fly. Comprising compounds (8), (9), (20), (21), (22), (23), (25), (29), (35), (36) and (38).

Group D. Synergized. LD_{50} without synergist $\geqslant$ 1·0 μg/fly; with synergist $>$ 0·12 μg/fly. Comprising compounds (27), (28), (31), (32), (33), (37), (39), (40), (41), (42) and (50).

Group E. Compounds (26) and (34) giving less than 50% knock-down at 10 μg/fly either with or without synergist, but showing initially accelerated signs of poisoning in the presence of synergist.

Group F. Compounds (15), (16), (17), (18), (19), (43), (44), (45), (46), (47), (48) producing no signs of poisoning at 10 μg/fly either with or without synergist.

Groups A and B contain most of the 'cyclodiene' insecticides used commercially and these compounds appear to be characterized by a relatively high stability *in vivo*. The compounds of groups C and D were of major importance in this study since synergistic effects, especially pronounced with compounds (25), (35) and (38), have been associated with stabilization *in vivo* of all the compounds so far examined in detail. The intrinsic toxicities of such compounds were thus greater than was apparent from simple tests of toxicity.

Groups E and F contain, in addition to compounds already discussed, hexachlorocyclopentadiene, hexachloronorbornene (and its simple derivatives) and several compounds containing polar groups. Compounds (15) to (19) were not synergized and the reasons for their low toxicity are not apparent; they are being examined in detail. The low toxicity of compounds (43), (44) and (45) may be associated with poor penetration through the insect integument.

A detailed consideration of the relationships between structure, metabolism, and toxicity of these compounds clearly involves their stereochemical relationships and these matters will be discussed elsewhere. The authors acknowledge the invaluable assistance of Miss J. T. Cox with the toxicity determinations. This investigation was supported in part by a grant from the World Health Organization.

REFERENCES

1. F. P. W. Winteringham and A. Harrison, *Nature, Lond.* **184**, 608 (1959).
2. R. G. Bridges and J. T. Cox, *Nature, Lond.* **184**, 1740 (1959).
3. G. T. Brooks, *Nature, Lond.* **186**, 96 (1960).

4. F. P. W. WINTERINGHAM, *J. roy. Soc. Arts*, **110**, 719 (1962).
5. Y. SUN and E. R. JOHNSON, *J. Agric. Fd Chem.* **8**, 261 (1960).
6. G. P. GEORGHIOU and R. L. METCALF, *J. econ. Ent.* **55**, 125 (1962).
7. A. B. HADAWAY, F. BARLOW and J. DUNCAN, *Bull. ent. Res.* **53**, 769 (1963).
8. G. T. BROOKS and A. HARRISON, *Nature, Lond.* **198**, 1169 (1963).
9. R. D. O'BRIEN, *Biochem. J.* **79**, 229 (1961).
10. P. S. HEWLETT, C. J. LLOYD and ANGELA N. BATES, *Nature, Lond.* **192**, 1273 (1961).
11. G. T. BROOKS and A. HARRISON, *Biochem. J.* **87**, 5P (1963).
12. E. S. GOODWIN, R. GOULDEN and J. G. REYNOLDS, *Analyst*, **86**, 697 (1961).
13. H. BLUESTONE, U.S. Patent 2,676,132 (1954).
14. K. MACKENZIE, *J. chem. Soc.* 457 (1962).
15. M. KLEIMAN, U.S. Patent 2,736,730 (1956).
16. R. RIEMSCHNEIDER, *Mh. Chem.*. **83**, 802 (1952).
17. R. RIEMSCHNEIDER and B. E. GRABITZ, *Mh. Chem.* **91**, 22 (1960).
18. R. RIEMSCHNEIDER, *Z. Naturf.* **17**b, 723 (1962).
19. C. A. PERI, *Gazz. chim. ital.* **85**, 1118 (1955).
20. R. E. LIDOV, J. HYMAN and E. SEGEL, U.S. Patent 2,584,139 (1952).
21. E. SEGEL, R. E. LIDOV and J. HYMAN, U.S. Patent 2,584,140 (1952).
22. A. I. VOGEL, *Practical Organic Chemistry*, p. 454. Longmans, Green and Co., London (1951).
23. R. E. LIDOV, U.S. Patent, 2,635,979 (1953).
24. R. E. LIDOV, U.S. Patent, 2,717,851 (1956).
25. R. C. COOKSON and E. CRUNDWELL, *Chem. and Ind., Lond.* 1004 (1958).
26. P. BRUCK, D. THOMPSON and S. WINSTEIN, *Chem. and Ind., Lond.* 405 (1960).
27. H. FEICHTINGER and H. TUMMES, German Patent 960,284 (1957).
28. S. B. SOLOWAY, Ph.D. Thesis, p. 121, University of Colorado (1955).
29. S. H. HERZFELD and E. P. ORDAS, U.S. Patent 2,528,656 (1950).
30. C. W. BIRD, R. C. COOKSON and E. CRUNDWELL, *J. chem. Soc.* 4809 (1961).
31. G. WITTIG and E. KNAUS, *Chem. Ber.* **91**, 895 (1958).
32. G. T. BROOKS, A. HARRISON and J. T. COX, *Nature, Lond.* **197**, 311 (1962).
33. R. T. WILLIAMS, *Detoxication Mechanisms*, pp. 539, 536, 610. Chapman and Hall, London (1959).
34. R. L. METCALF and G. P. GEORGHIOU, *Bull. Wld. Hlth. Org.* **27**, 251 (1962).
35. A. S. PERRY and W. M. HOSKINS, *J. econ. Ent.* **44**, 839 (1951).
36. F. P. W. WINTERINGHAM, A. HARRISON and G. DAVIDSON, Inform Circ. World Hlth. Org., **21**, item 55 (1959).
37. J. RAY, *Nature, Lond.* **197**, 1226 (1963).
38. N. W. EARLE, *J. Agric. Fd. Chem.* **11**, 281 (1963).
39. J. R. BUSVINE, *Nature, Lond.* **174**, 783 (1954).
40. R. RIEMSCHNEIDER and V. WUSCHERPFENNIG, *Z. Naturf.* **17b**, 585 (1962).