# The Fate of Cyclodiene Insecticides Administered to Susceptible and Resistant Houseflies

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Dieldrin and several related cyclodiene insecticides were found to be quite stable following their absorption by susceptible and resistant houseflies. Large quantities of dieldrin absorbed during the larval stage of resistant flies remained unchanged throughout the pupal stage and were excreted during the first few days of adult life. Aldrin was converted to dieldrin as well as to nontoxic degradation products by susceptible and resistant flies. In no instance was detoxification in this group of chlorinated insecticides as pronounced as it is with DDT or lindane. It is concluded that the metabolism of cyclodiene insecticides is a relatively unimportant resistance mechanism in houseflies.

THERE is good evidence that detoxification is the primary cause of resistance to DDT and lindane (*10, 15*) in the housefly (*Musca domestica* L.). However, the importance of detoxification as a resistance mechanism is questionable with regard to the chlorinated cyclodiene insecticides. Resistant houseflies can detoxify the bromine and sulfur analogs of dieldrin, but so can susceptible flies (*19*). Resistant German cockroaches (*Blattella germanica* L.) (*12*) and resistant houseflies (*6, 12*) have been shown to detoxify chlordan, but it is not certain whether susceptible strains were also included in these studies. Resistant houseflies do not detoxify dieldrin, endrin, or heptachlor epoxide (*2, 12, 13*). Apparently, the ability to form toxic oxides from various unsaturated insecticides is not correlated with resistance since resistant as well as susceptible houseflies can metabolize the following insecticides to their corresponding oxides: aldrin (*2, 13*), isodrin (*2*), and heptachlor (*13*).

The work reported here is concerned primarily with evaluation of detoxification as a factor contributing to the resistance of houseflies to dieldrin and seven related insecticides. Closely related analogs were compared to determine whether detoxification could be the cause of resistance to one compound but not to another. Lindane was also studied because its toxic action seems to be similar to that of the cyclodiene group (*18*). Insecticides were administered topically to adults or as suspensions in larval media. The amounts of toxicants recoverable by extraction were determined after intervals that ranged from a few hours to several days.

The results of one experiment, not directly related to detoxification, are included in this report. The level of resistance to vapors of aldrin and lindane was measured to determine whether the resistant flies could tolerate the insecticide vapors which would reach the presumed site of action through the tracheae.

## Materials

**Housefly Strains.** A composite strain of houseflies, distributed by the National Association of Insecticide and Disinfectant Manufacturers in 1948, was used as the source of susceptible flies. The multiple-resistant strain used originated in California. This strain had been selected with a variety of chlorinated hydrocarbon insecticides and when received was highly resistant to DDT as well as to the cyclodiene insecticides. The level of resistance had declined in the strain after it had passed through about 10 generations in the laboratory without selection, which explains some of the relatively high resistant fly mortalities reported in several tables. When this partial reversion was noticed, the strain was then maintained under constant selection pressure by treatment with a mixture of DDT and dieldrin.

**Insecticides.** Purified samples of the following insecticides were used: aldrin, dieldrin, endrin, lindane, a dieldrin analog with an oxygen bridge (5,6,7,8,9,9-hexachloro-1-4, 2-3-diepoxy-1,2,3,4-4*a*,5,8,8,8*a*-octahydro-5-8-methanonaphthalene), an *N*-oxide analog of dieldrin (5,6,7,8,9,9-hexachloro-1,4,4*a*,5,-8,8*a*-hexahydro-1,4,5,8-dimethanophthalazine 2-oxide), nonachlor (*4*) (1,2,3,4,5,-6,7,8,8-nonachloro-2,3,3*a*,4,7,7*a*-hexahydro-4,7-methanoindene), and *beta*-chlordan according to Metcalf (*7*) (the more toxic of the two known isomers of 1,2,4,5,6,7,8,8-octachloro-2,3,3*a*,4,7,7*a*-hexahydro-4,7-methanoindene). It was considered preferable to use two of the toxicants known to occur in chlordan (nonachlor and *beta*-chlordan) rather than the technical material.

## Procedure

**Housefly Rearing.** The standard CSMA larval medium was used except when insecticides were incorporated in the medium. When insecticides were used, the medium consisted of 25 ml. of condensed milk (diluted according to the instructions on the can), 2.5 grams of bleached cellucotton, and 0.1 gram of brewer's yeast. Insecticides were added by mixing not more than 1 ml. of an acetone solution with 100 ml. of the diluted milk before adding the cellucotton. Constituents were mixed in a 4-ounce jar; about 100 eggs were placed on the surface; and the jars were covered with cloth.

**Topical Treatment.** An uncalibrated 0.25-ml. tuberculin syringe, actuated by a micrometer, was used to apply acetone solutions to the dorsal surface of the thorax. The quantity of insecticide delivered was determined by analyzing the extract prepared immediately after treatment of a group of flies of mixed sexes with the insecticide solution. This 0-hour extract was prepared for each insecticide each time flies were treated for the purpose of measuring absorption or detoxification rates.

After treatment, the flies were held at 27 ± 1° C. in groups of 50 in 1-pint jars covered with gauze. A piece of

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cotton soaked in milk was placed on top of the gauze cover.

**Extraction Procedure.** At given intervals, the unabsorbed insecticide was recovered by briefly shaking the groups of flies three times with 15-ml. portions of reagent-grade acetone (external rinse). The rinsed flies were then macerated in a mortar with sand and anhydrous sodium sulfate. The resulting powder was extracted by shaking with one 20-ml. portion and two 15-ml. portions of acetone, 15 minutes each time, by means of a mechanical shaker. The extracts were filtered and combined. These internal extracts contained the toxicant that was absorbed and retained by the flies. Pupae from larvae reared in insecticide-treated media were rinsed with tap water before being macerated and extracted by the above procedure.

Insecticides in feces of adult flies were recovered by rinsing the walls of the jar with two 20-ml. portions of acetone. The acetone was added to the jar, and the feces were loosened from the walls and bottom with a spatula. The cheesecloth cover was moistened with acetone and used to wipe traces of excreta from the glass surface. The jar and cheesecloth were rinsed a second time, and the two extracts were filtered and combined. This jar rinse generally contained only a small amount of insecticide, and was usually combined with the external rinse before analysis.

**Chromatography of Aldrin and Dieldrin.** Aldrin undergoes a biological oxidation to dieldrin in insects (*3*) as well as in higher animals (*7*). The identity of the toxic material in extracts of aldrin-treated flies, therefore, could not be known from a simple bioassay since this method of analysis is nonspecific. To permit the analysis of both aldrin and dieldrin, the extracts were first chromatographed. Acetone extracts of flies were evaporated to dryness with a minimum amount of heat, and the residues were dissolved in petroleum ether, b.p. 66–68° C. Excess moisture was removed with anhydrous sodium sulfate. A small volume of the petroleum ether solution was added to a 1.7-cm. diameter column packed with 15 grams of 200-mesh Florisil. The column was then washed continuously with petroleum ether under slight pressure until 12 25-ml. fractions had been collected. Mixtures containing known amounts of aldrin, dieldrin, and untreated fly extracts were also passed through similar columns. Toxic fractions were identified by exposing flies to residues obtained by evaporating small aliquots of each fraction. Aldrin appeared in the second and third fractions and dieldrin in the fifth through the 11th fractions. The aldrin and dieldrin fractions were each pooled and made up to a constant volume. The identities of the toxicants were verified by the aldrin and dieldrin colorimetric methods (*8*, *9*).

**Bioassay Procedure.** The microbioassay method was essentially the same as that described by Sun and Sun (*17*). The toxicity of each extract was measured by exposing residues deposited on the bottoms of 1-pint jars to groups of 50 houseflies. Six jars were prepared for each sample, and the mortalities were compared with those of a concurrent standard series containing known amounts of insecticides. Untreated check extracts were included in the standard jars to equalize any effects the extractives may have had on the toxicity of the insecticides.

The analysis was repeated, usually on the following day, and the two results were averaged. Analyses of more than 100 recovery extracts of agricultural products containing known amounts of insecticides, have shown that the accuracy of this method is comparable to that of the colorimetric procedures for aldrin and dieldrin (*16*). The average standard deviation for the bioassay method was about 16%. Some of this variation was probably introduced by the chromatographic purification of the extracts. Inconsistent removal of plant extractives among a group of samples will usually lead to differences in the masking of the toxicity of the insecticides. When the amount of extractives is low, as occurred with the fly extracts, there is no need for chromatographic cleanup, and the results obtained can be expected to be more accurate. For example, one sample of untreated soil extract, which contained virtually no extractives, was fortified with 0.333 p.p.m. dieldrin and then bioassayed. Four pairs of assays averaged 0.330 p.p.m. with a standard deviation of only 5.8%.

The sensitivity of the bioassay is limited by the toxicity to houseflies of the particular insecticide being analyzed. Usually, one fifth of the $LD_{50}$ of the bioassay standard can be taken as the minimum amount of insecticide that can be detected in each jar (*17*).

## Results

**Absorption Rates.** The rate of absorption of topically applied dieldrin by susceptible and resistant flies was studied on two occasions. Mortality data were not recorded for the first experiment (Table I), but the resistance level was estimated to be about 40-fold. As with all other topical-application re-

**Table I. Comparative Rates of Absorption of Topically Applied Dieldrin by Susceptible (S) and Resistant (R) Houseflies (Experiment 1)**

| Interval between Treatment and Extraction, Hr. | Strain | Dieldrin Recovered, μG. per Fly | |
|---|---|---|---|
| | | External rinse | Internal extract |
| 0 | R | 1.64 | 0 |
| | S | 1.64 | 0 |
| 0.5 | R | 1.28 | 0.10 |
| 0.25 | S | 1.18 | 0.16 |
| 1 | R | 1.18 | 0.18 |
| | S | 1.06 | 0.18 |
| 2 | R | 1.10 | 0.36 |
| | S | 1.02 | 0.46 |
| 4 | R | 0.92 | 0.40 |
| | S | 0.86 | 0.50 |
| 8 | R | 0.38 | 0.60 |
| | S | 0.54 | 0.52 |
| 18 | R | 0.22 | 0.88 |
| | S | 0.20 | 0.94 |
| 24 | R | 0.12 | 0.92 |
| | S | 0.10 | 0.98 |
| Sensitivity | | 0.14 | 0.16 |

**Table II. Comparative Rates of Absorption of Topically Applied Dieldrin by Susceptible (S) and Resistant (R) Houseflies (Experiment 2)**

| Interval between Treatment and Extraction, Hr. | Strain | Mortality % | Dieldrin Recovered, μG. per Fly | |
|---|---|---|---|---|
| | | | External rinse | Internal extract |
| 0 | R | ... | 1.68 | 0.22 |
| | S | ... | 1.74 | 0.16 |
| 2 | R | 0 | 1.32 | 0.34 |
| | S | 82 | 1.20 | 0.32 |
| 4 | R | 2 | 1.08 | 0.58 |
| | S | 100 | 1.00 | 0.58 |
| 6 | R | 8 | 1.00 | 0.56 |
| 5 | S | 100 | 1.06 | 0.68 |
| 24 | R | 28 | 0.06 | 1.00 |
| | S | 100 | 0.32 | 0.98 |
| 24[a] | R | 24 | 0.24 | 1.26 |
| | S | 100 | 0.34 | 1.04 |
| Sensitivity | | | 0.18 | 0.16 |

[a] 10 μg. of oleic acid applied with the dieldrin to enhance absorption and to reduce losses due to volatility.

sults reported here, the 0-hour values were assumed to be the amounts actually applied. Only three of the values for the jar rinse were greater than the calculated sensitivity, the highest being 0.24 μg. The 0.5-hour internal extracts contained amounts about equal to the sensitivity. The only external-rinse samples containing less than the estimated sensitivity were the 24-hour samples.

In the second experiment (Table II), the resistance level was considerably higher. As in the first experiment, there were no marked differences in absorption rates. In both instances, the susceptible strain continued to absorb dieldrin at the same rate after the flies were dead or moribund. The addition of 10 μg. of oleic acid may have enhanced the absorption of dieldrin to a slight extent. Dieldrin recovered from the jar rinse ranged between 0.09 and 0.30 μg. It is unlikely that dieldrin was excreted since the amount did not increase with time.

**Fate of Topically Applied Insecticides.** For the calculation of per cent recoveries in Table III, the values for the external rinses and jar rinses were combined. This sum was subtracted from the amount applied to give the amount absorbed. The values for the amounts found in the internal extracts were then used to calculate the percentages of absorbed toxicant recovered. Any values less than 10% are only approximations.

There were no consistent differences in the dieldrin recoveries from susceptible and resistant flies. All but one of the recovery values were greater than 60%. There was evidence for only a slight, if any, detoxification of *beta*-chlordan. There may have been some detoxification of nonachlor and the dieldrin analog with an oxygen bridge. Only 41% of the absorbed *N*-oxide analog of dieldrin was recovered from resistant flies, which would indicate some detoxification of this compound. There was no simple relation between the toxicity of the insecticides to susceptible flies and rate of absorption. The nitrogen analog of dieldrin is the most toxic, and nonachlor is the least toxic in this group of insecticides, yet they are both absorbed at about the same rate by resistant flies.

Aldrin was apparently detoxified to a greater extent than dieldrin (Table IV). Resistant flies absorbed and metabolized more aldrin to dieldrin and to nontoxic materials than susceptible flies, perhaps as a result of the higher survival rate of resistant flies. Substantial losses due to the volatility of unabsorbed aldrin seem unlikely, especially when oleic acid was present. Sternburg and Kearns (*14*) reported only a 10% loss of the highly volatile insecticide, lindane. However, Perry (*11*) found greater losses due to the volatility of aldrin.

**Table III. Recovery of Topically Applied Insecticides 24 Hours after Treatment**

| Insecticide | μG. per Fly | Strain | Mortality, % | % of Toxicant Recovered: External rinse | Internal extract | Jar rinse | % of Absorbed Toxicant Recovered |
|---|---|---|---|---|---|---|---|
| Dieldrin | 1.6 | S | 100 | 7.0 | 56 | 15 | 72 |
| | 1.6 | R | .. | 7.0 | 60 | 7.0 | 70 |
| | 1.9 | S | 100 | 17 | 52 | 6 | 68 |
| | 1.9 | R | 28 | 3 | 53 | 16 | 65 |
| | 2.4 | S | 100 | 43 | 18 | 11 | 39 |
| | 2.4 | R | 10 | 18 | 46 | 6.7 | 61 |
| | 8.8 | S | 100 | 75 | 15 | 8.0 | 88 |
| | 8.8 | R | 22 | 65 | 33 | 11 | 140 |
| Dieldrin + oleic acid | 1.9+10 | S | 100 | 18 | 55 | 6 | 72 |
| | 1.9+10 | R | 24 | 13 | 66 | 6 | 82 |
| *beta*-Chlordan | 8.9 | S | 100 | 61 | 29 | [a] | 74 |
| | 8.9 | R | 90 | 63 | 28 | [a] | 76 |
| | 8.2 | R | 14 | 65 | 38 | [a] | 109 |
| Nonachlor | 11 | R | 0 | 80 | 8.2 | [a] | 41 |
| Dieldrin analog with oxygen bridge | 8.7 | S | 100 | 99 | 8.4 | [a] | 100 |
| | 8.7 | R | 94[b] | 97 | 9.8 | [a] | 100 |
| | 8.0 | R | 0 | 65 | 4.0 | [a] | 10 |
| *N*-Oxide analog of dieldrin | 6.7 | R | 44[b] | 78 | 9 | [a] | 41 |

[a] Jar rinse combined with external rinse and analyzed together.
[b] Cotton soaked in milk became dry.

**Table IV. Metabolism of Topically Applied Aldrin by Resistant and Susceptible Houseflies by Bioassay (*B*) and Colorimetric (*C*) Analysis**

| μG. Applied | Strain | 24-Hour Mortality, % | % of Toxicant Recovered after 24 Hours: External rinse B | External rinse C | Internal Extract: Aldrin B | Aldrin C | Dieldrin B | Dieldrin C | % of Absorbed Toxicant Recovered |
|---|---|---|---|---|---|---|---|---|---|
| 5.0 | S | 100 | 13 | ... | 22 | 24 | 24 | 20 | 53 |
| | R | 44 | 4.2 | ... | 4.6 | 4.8 | 28 | 25 | 34 |
| 10 + 50 oleic acid | S | 100 | 53 | 44 | 39[a] | 28 | ...[a] | 11 | 70 |
| | R | 36 | 36 | 33 | 33[a] | 12 | ...[a] | 20 | 48 |
| | R | 6 | 15 | 22 | 33[a] | 6 | ...[a] | 21 | 35 |

[a] Aldrin and dieldrin were bioassayed as a mixture without prior chromatographic separation. When the housefly was the bioassay organism, aldrin and dieldrin were about equally toxic.

Analyses of the external rinses by bioassay as well as by the aldrin colorimetric proccdure agreed fairly well—an indication that only small amounts of dieldrin could have been present. Also, there was a close agreement between the aldrin and dieldrin analyses of internal extracts by the colorimetric and bioassay procedures.

Recovery experiments showed that negligible amounts of aldrin and dieldrin were lost during the handling of the extracts. Two mixtures, each consisting of an extract of untreated flies and known amounts of aldrin and dieldrin, were carried through the described evaporation and chromatographic procedures. The percentage recoveries were aldrin, 77 and 98, and dieldrin, 84 and 103.

**Dieldrin in Larvae, Pupae, and Adults.** Preliminary experiments showed that as much as 800 p.p.m. dieldrin in a milk-cellucotton larval medium did not delay the rate of growth or percentage pupation of resistant houseflies, although many adults died soon after emergence. Bioassay of surviving adults from treated larvae revealed high concentrations of dieldrin. These observations suggested a method for introducing large amounts of insecticides into resistant flies where they might be subjected to the action of detoxifying enzymes. By rearing larvae in dieldrin-treated media, pupae and adults could be obtained which would contain large amounts of insecticides. There would be no losses by volatilization or by excretion during the pupal stage, so that any decline in the toxicity of extracts of pupae prepared after different intervals would be due to detoxification. The analysis of extracts of adults and their excreta would supply information concerning detoxification during that stage.

The milk-cellucotton medium described earlier was treated by adding 0.75 ml. of a 1.0% acetone solution of dieldrin to 75 ml. of milk, to give a final concentration of 100 p.p.m. of toxicant. Groups of about 150 eggs from a resistant strain of flies were added to this

**Table V. Recovery of Dieldrin from Houseflies Reared in Larval Media Containing 100 P.P.M. Dieldrin**

| Sample Description | No. of Insects Extracted | Dieldrin per Fly, μG. | | |
|---|---|---|---|---|
| | | Extract of flies | Cumulative Recoveries in Excreta | Cumulative Recoveries Adults + excreta |
| Larvae, 5 days after eggs introduced | 50 | 2.7 | .. | . |
| Empty puparia | 48 | 0.09 | .. | . |
| Pupae, 7 days after eggs introduced | 25 | 7.9 | .. | . |
| 10 days after eggs introduced | 25 | 9.1 | .. | . |
| Adults (av. age, days) | | | | |
| 1/2 | 20 | 10.5 | 0.07 | 10.6 |
| 1 1/2 | 24 | 7.05 | 1.2 | 8.25 |
| 5 1/2 | 19 | 4.23 | 3.52 | 7.75 |
| 8 1/2 | 19 | 2.55 | 6.65 | 9.20 |

**Table VI. Dieldrin Recovered from Pupae and Adults Reared from Treated Larval Media**

| Stage of Development | Days after 1st Sample Taken | No. of Insects | Dieldrin per Insect, μG. |
|---|---|---|---|
| Pupae, immediately after formation of puparia | 0 | 86 | 14.3 |
| Pupae, just before beginning of adult emergence | 4 | 84 | 14.4 |
| Mixture of newly emerged, and incompletely developed adults | 6 | 89 | 14.5 |

quantity of medium in each of several wide-mouthed, 1-pint jars.

Fifty late-instar larvae were removed from the media 5 days after the eggs were introduced. After being rinsed with running tap water, they were macerated and extracted. On the seventh day, pupation was nearly complete. At this time, pupae were collected from two jars, washed with tap water, and dried. A group of 25 was extracted immediately, and another 25 was extracted 3 days later. The remaining pupae were allowed to develop into adults. About 70% of the adults emerged within a 24-hour period, and these flies were divided into four groups. One group was extracted immediately (0.5-day-old flies), and the remaining three were confined to 1-pint jars and provided with milk for various time intervals. Acetone extracts of the adults as well as of the empty puparia were prepared in the usual manner. The analytical results are summarized in Table V.

There was no decline in the amount of toxicant recovered from pupae of two ages. Nor was there any evidence of detoxification over an 8-day period during adult life. The small variations observed were probably due to the relatively small numbers of insects involved.

A second series of extracts was prepared involving larger numbers of insects. The pupae were separated from the media a little earlier than before at a time when only about 60% of the larvae had formed puparia. After being washed with tap water, the pupae were divided into three nearly equal groups. One group was extracted at this time. A second group was held for 4 days before extraction, and the remaining group was held for an additional 2 days. Of this last group, 40% had reached the adult stage. The analytical results which are summarized in Table VI show that there was no loss of toxicant over a 6-day period beginning with the time of pupation.

**Table VII. Recovery of Various Insecticides from Pupae of Different Ages Obtained from Larvae Reared in Treated Media**

| Insecticide in Media | Concn., P.P.M. | No. of Pupae per Group | Toxicant Found in Pupae of Different Ages (μg. per pupa) | | | |
|---|---|---|---|---|---|---|
| | | | 0 day | 2 days | 3 days | 5 days |
| Endrin | 25 | 25 | 1.64 | 1.69 | ... | ... |
| | 25 | 25 | 2.38 | 2.76 | ... | 2.65 |
| *N*-Oxide analog of dieldrin | 25 | 25 | 4.35 | ... | ... | 4.48 |
| Aldrin | 100 | 75 | 0.39 (aldrin) | ... | 0.22 (aldrin) | ... |
| | | | 9.8 (dieldrin) | ... | 8.9 (dieldrin) | ... |
| Lindane | 50 | 25 | 0.07 | ... | ... | 0.07 |
| | 100 | 25 | 0.23 | ... | ... | 0.07 |

Some variation was noted in the amounts of toxicant found in various groups of pupae reared from larval media containing the same concentration of dieldrin. This was caused, in part, by the failure to collect samples when the percentage pupation was the same. Analyses showed that the flies that pupated first contained the greatest amount of toxicant. For example, a group of 25 dark brown pupae contained an average of 16.4 μg. of dieldrin, whereas a group of 25 light-colored pupae from the same jar, which had pupated at a later time, contained an average of 12.1 μg. per pupa.

**Aldrin, Endrin, *N*-oxide Analog of Dieldrin, and Lindane in Pupae.** Several insecticides other than dieldrin were added to the larvel media. Pupae were then isolated and extracted at different intervals to determine the extent of metabolism (Table VII). Neither endrin nor the *N*-oxide analog of dieldrin was detoxified over a 5-day period. A smaller amount of endrin was accumulated by the larvae than the *N*-oxide analog. Lindane was apparently detoxified soon after ingestion by the larvae, and even with 100 p.p.m. lindane in the medium, the newly formed pupae contained very little toxicant. Five days later, the pupae contained a negligible amount of toxic material. Most of the aldrin had been metabolized to dieldrin before the first sample of pupae was collected. There was a slight, but probably insignificant, decline in the amount of aldrin during the 3-day period. It is significant that lindane was the only insecticide included in this series of tests that was detoxified.

Apparently there was very little conversion of aldrin to dieldrin in the larval medium itself. A jar of aldrin-treated larval medium without flies was allowed to stand for several days, and was then extracted with dimethylformamide (DMF). A relatively large volume of water was added to the DMF extract, and this mixture was then extracted several times with petroleum ether. Chromatography of the petroleum ether solution revealed no trace of dieldrin.

**Dieldrin Concentration in Internal Organs.** The results in Table VII show that relatively large amounts of dieldrin ingested and stored by resistant larvae remained undetoxified throughout the pupal stage. This lack of detoxification would have significance only if it were demonstrated that the toxicant passed into the body cavity where it could be carried to potential sites of detoxification. To study the distribution of dieldrin among the various tissues, adults of two ages that had developed from treated media were dis-

sected, and various parts were extracted and analyzed. Flies from a similar group were simply divided into the three body regions before analysis. Results are summarized in Table VIII.

Dieldrin appeared in all three body regions; however, the abdomen contained the largest amount. It is not known whether the toxicant was associated with the fat body, hypodermal cells, or cuticle. Very little remained in the alimentary canal. Thus, dieldrin that was ingested during the larval stage was eventually distributed throughout the body by the time the adult stage was reached. The dieldrin presumably would have come in contact with any detoxifying enzymes that might have been present in the insect.

**Vapor Toxicity of Aldrin and Lindane.** One way in which resistant insects may handle an insecticide is by storing it at an insensitive site. If this presumed storage site could be bypassed in any way, an increase in mortality would result. This might be done by treating resistant flies to insecticide vapors, since the toxicant would then be transported directly to the central nervous system which is probably the site of action of aldrin (*5*) and possibly lindane.

Two of the more highly volatile insecticides, aldrin and lindane, were chosen to treat groups of susceptible and resistant housefly adults. One hundred-milligram quantities of the insecticides were deposited on the bottoms of 1-liter Erlenmeyer flasks. The stoppered flasks were immersed in a water bath maintained at 40° C. and allowed to equilibrate for 30 minutes. Cylindrical screen cages, each containing 10 flies, were then lowered into the flasks by means of strings. After various exposure periods, the flies were transferred to 4-ounce jars with milk, and mortalities were recorded on the following day.

An exposure of only 1 minute to aldrin vapors killed 90% of the susceptible flies, and longer exposures killed all of the flies. Exposure periods of 1, 2, 4, and 8 minutes to lindane vapors resulted in mortalities of 80, 90, 80 and 100%, respectively, of susceptible flies. Exposure of resistant flies for periods up to 120 minutes to either aldrin or lindane resulted in mortalities no greater than the control group. There was 0 mortality in the groups exposed for only 45 minutes. Thus, fewer of the resistant flies that were exposed for over 100 minutes to either aldrin or lindane were killed than were the susceptible flies that were exposed for only 1 minute. It seems unlikely, then, that resistance is due to the protective effect of storage or detoxification at sites removed from the site of toxic action. The possibility that the insecticide is detoxified, or otherwise rendered inactive, at or near the site of action must still be considered, however.

**Table VIII. Toxicant Content of Tissues from Resistant Adults Obtained from Larvae Reared in Media Containing 100 P.P.M. Dieldrin**

| Tissue | ½-Day-Old Flies | | 2-Day-Old Flies | |
|---|---|---|---|---|
| | No. | Av. μg. per fly | No. | Av. μg. per fly |
| Abdominal tissue including remainder of abdomen after removal of intestine and gonads | 9 | 9.2 | 10 | 5.9 |
| Alimentary canal from thorax and abdomen, and gonads, including some body fat | 9 | 1.5 | 10 | 2.0 |
| Head | 18 | 2.0 | ... | ... |
| Thorax | 18 | 2.2 | ... | ... |
| Abdomen | 18 | 10.3 | ... | ... |

## Discussion

Most studies of detoxification have been based on analyses by colorimetric or radiotracer methods. Specificity is often increased by applying both methods, or by using one or the other in combination with chromatography. In the present study, the measurement of detoxification was based primarily on results by bioassay, with a few confirmatory chromatographic and colorimetric analyses. It might be said that studies of this type that rely so much on bioassay alone are open to question since the identities of the toxicants in the insect extracts are not proved. However, complete identification of the toxicants is of little consequence when the object is simply to determine the importance of detoxification as a resistance mechanism. It is sufficient to be able to measure accurately how toxic the extracts of treated resistant insects are when applied to susceptible individuals of the same species—houseflies in these tests. The data, of course, would mean much less if another species were used as the bioassay organism.

When considered as a group, the cyclodiene insecticides are remarkably stable when administered to resistant flies. This is especially evident from the results of this study where the levels of dieldrin, endrin, and the *N*-oxide analog of dieldrin in pupae remained unchanged over a period of several days. High recoveries were also obtained following topical application of aldrin, dieldrin, and *beta*-chlordan. Variable results were obtained with topically applied dosages of nonachlor and with two experimental dieldrin analogs.

The data indicate that detoxification does not contribute a great deal to housefly resistance to the chlorinated cyclodienes. Of course, it is conceivable that the resistant flies have developed a unique detoxification system at the site of toxic action that the susceptible flies do not possess (*18*). Improved analytical methods would be necessary to detect such a difference. In any event, it seems reasonable to suppose that the resistance mechanism residues close to the site of toxic action in view of the ability of resistant flies to tolerate vapors of aldrin or lindane.


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