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LABELED ENDOSULFAN IN THE HOUSEFLY.

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THE ABSORPTION AND METABOLISM OF C¹⁴-LABELED
ENDOSULFAN IN THE HOUSEFLY

DISSERTATION

Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate
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By

William Wayne Barnes, B.Sc., M.Sc.

* * * * *

The Ohio State University
1964

Approved by

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INTRODUCTION

Two of the principal factors affecting the toxicity of an insecticide to a particular insect species are the rate of penetration of the toxicant into the insect and its subsequent metabolism within the insect tissues. The investigation reported herein was conducted to determine the rate of absorption and the in vivo metabolism of endosulfan by the housefly, Musca domestica (L.).

Endosulfan, 6, 7, 8, 9, 10, 10-hexachloro-1, 5, 5a, 6, 9, 9a-hexahydro-6, 9-methano-2, 4, 3-benzodioxathiopen-3-oxide is a sulfur-containing insecticide developed by Farbwerke Hoechst AG in Germany. It is known chemically in the United Kingdom (B.S.I.) as 1, 2, 3, 4, 7, 7-hexachlorobicyclo [2, 2, 1] -2-heptene 5, 6-bis (hydroxymethylene) - sulfite (I)¹. The technical material contains up to 90 per cent endosulfan as a mixture of two stereoisomers melting between 80 and 90°C. The isomeric mixture contains a high-melting (mp 208-210°C.), and a low-melting (mp 108-110°C.) isomer. The remaining 10 per cent is made up of endosulfan alcohol (mp 198-200°C.) (II) (1, 4, 5, 6, 7, 7-hexachloro-2, 3-bis (hydroxymethyl)-bicyclo [2, 2, 1] heptene-5) and endosulfan ether.

Endosulfan is manufactured by a three-stage synthesis (Farbwerke Hoechst, U.S. Pat. 1955) illustrated in Figure 1. The product (III)

¹Roman numerals refer to structures in Figure 1.

Fig. 1.—Structure of endosulfan and related derivatives.

Fig. 2.—Calotte models of the preferred constellations of the isolated isomers of endosulfan. (After Riemschneider, 1963.)

formed from hexachlorocyclopentadiene and cis - 1, 4 diacetoxymethylene-2 by the Diels-Alder reaction is saponified to the diol (II). This, in turn, reacts with thionyl chloride to yield endosulfan and HCl. In glass apparatus, the diol can be obtained directly from C_5Cl_6 and cis-butene-2-diol-1, 4, if the components in tetrahydrofuran and dioxane are heated in a tube for 15 hours at $140^{\circ}C$. (Riemschneider, 1963). Stereoisomers of endosulfan are discussed by Riemschneider and Hilscher (1960), the Calotte models of the two toxic isomers are shown in Figure 2.

Endosulfan is manufactured in the United States by the Niagara Chemical Division, Food Machinery and Chemical Corporation, and is used to control a variety of arthropod pests. Of the four components of

technical endosulfan, only the high- and low-melting isomers appear to have appreciable insecticidal activity (Finkenbrink, 1956).

Lindquist and Dahm (1957) investigated some of the chemical properties of endosulfan by using column and paper chromatography, melting point determinations, ultraviolet and infrared spectroscopy as well as LD₅₀ determinations on houseflies and male white rats. They found a 6.7 ug/g LD₅₀ to susceptible houseflies, while the oral LD₅₀ to male white rats was between 40 and 50 mg/kg of body weight.

Various colorimetric methods of determining endosulfan residues have been described (Cassil, 1958, Graham, 1959) which entail the evolution of sulfur dioxide into an absorbing solution and addition of a Fuchsin dye reagent to develop a color. An improved colorimetric method for determining endosulfan residues was described by Maitlen, et al. (1963) wherein a pyridine-methanolic sodium hydroxide reagent was mixed with endosulfan extracts to develop colored samples. The colored samples were analyzed photometrically. The sensitivity of these tests was in the microgram range, making them more suited to the determination of endosulfan residues on plants and in soil rather than on insects.

Paper and gas chromatography have been used previously to determine endosulfan residues in bean plants (Terranova and Ware, 1963). Eldefrawi and Hoskins (1961) used paper chromatograms spotted with C¹⁴-labeled Sevin to determine its rate of penetration and metabolism upon three species of insects. The mechanisms of resistance of the housefly to cyclodiene insecticides have been investigated (Brooks, 1960) and the relations between structure metabolism on toxicity of

cyclodiene insecticides were studied by Brooks and Harrison (1963). Perry (1960) gives an excellent general discussion of metabolism of insecticides by various insect species, while an earlier investigation (Perry, Matson and Buckner, 1958) covered the metabolism of heptachlor, a cyclodiene insecticide closely related to endosulfan, by susceptible and resistant houseflies.

MATERIALS AND METHODS

Rearing.--- Housefly larvae were reared in one-gallon plastic jars with screened tops on CSMA standard dry larval medium² following a modification of the Peet-Grady method (1961). The rearing room was artificially illuminated by fluorescent lamps which operated continuously. Temperature was controlled to $80^{\circ}\text{F} \pm 5^{\circ}$, while the relative humidity ranged from 40 to 60%. Freshly oviposited eggs were collected and washed in tap water before seeding 0.3 ml (approximately 2100) eggs in each container. Contents of the jars were moistened as necessary to prevent mold growth. The media within the jars was covered with sand on the fourth day following the seeding of eggs to afford a dry pupation site for the larvae and also to facilitate separation of the pupae from the rearing medium. Pupae were separated from the medium six days after seeding by pouring the top sandy layer into a 12-inch enamel basin half filled with lukewarm water. The pupae floated readily, while the sand and any loose chaff sank, thus efficiently separating the pupae and cleaning them in one operation.

The separated pupae were air dried and placed in a cylindrical stainless steel cage with a screen cone top (Figure 3). These cages accommodated up to 5000 flies without crowding. Males were selectively removed by placing a screen cylinder over the top of the cone for the

²Obtained from Ralston Purina Company, Checkerboard Square, St. Louis, Missouri 63102.

Fig. 3--Stainless steel rearing cage.

first twenty-four hours of adult emergence. The males emerged first, crawled into the cylinder and were trapped by inserting two index cards between the cylinder and the cage. The top index card was used to close the cylinder containing a high percentage of male flies when the cylinder was removed, while the bottom card closed the top of the cage. The flies thus collected were discarded. The remaining flies were collected similarly when two days old, anesthetized lightly with CO₂, sexed (males rejected), counted, and placed into pint glass jars with screened screw tops for testing on the following day. Flies were fed 15% sugar water on cellucotton placed on the screened lids.

The flies used in these tests were a normally susceptible (CSMA) strain which had been laboratory-reared at O.S.U. for several years and a cyclodiene-resistant strain obtained from the United States Department of Agriculture Laboratory, Orlando, Florida. Both strains were reared under identical conditions for five generations prior to LD₅₀ determinations. Toxicity tests were conducted on each generation for five successive generations.

Description of test compounds.--- The C¹⁴-labeled test material furnished by the Niagara Chemical Division, FMC Corporation had a specific activity of 0.577 microcurie per milligram of technical endosulfan. Highly purified samples of non-radioactive endosulfan ether (EE), endosulfan alcohol (EA), low-melting point endosulfan (E₁), high-melting point endosulfan (E₂), and an oxidized endosulfan identified as endosulfan sulfate (E₃) were also supplied by the Niagara Chemical Division. The C¹⁴ label was located on the

hexachlorocyclodiene ring at carbons 5 and 6 as marked in Figure 1.

Toxicity tests.-- Dosage mortality determinations for technical endosulfan, its four components, and endosulfan sulfate were made by topical application of a one microliter droplet of insecticide in acetone to the mesothoracic sternum. An ISCO Model M³ foot-actuated microapplicator (Figure 4) fitted with a No. 26 gauge needle was used for topical application. Flies were anesthetized with carbon dioxide to facilitate handling. Treated flies were held in screen-topped pint jars for twenty-four hours before mortality counts were made. The criterion for knockdown was the ability of the affected flies to walk around the jar without losing their balance. Moribund flies were considered dead. Results were plotted on log-probit paper after correction using Abbott's (1925) formula where applicable. Five replicates of twenty female flies were treated with each of five concentrations of the insecticide to obtain the LD₅₀ determinations; controls were treated with acetone alone. The concentrations used on the susceptible strain were 1:9 dilutions (insecticide:acetone) of those used on the resistant strain.

Absorption and metabolism studies.-- One hundred flies of each strain were treated topically with a one-microgram dose of C¹⁴-tagged technical endosulfan in one microliter of acetone as described earlier. Twenty treated flies were placed into each of five holding jars. After exposure periods of 2, 4, 8, 12, and 24 hours the flies

³Manufactured by Instrumentation Specialties Co., 5624 Seward, Lincoln, Nebraska 68507.

Fig. 4--ISCO Model M microapplicator.

of each strain were anesthetized with carbon dioxide, removed from the holding jar and rinsed externally with five 4-ml volumes of glass-distilled hexane to remove the unabsorbed insecticide. The washed flies were placed in a 10 ml ground-glass tissue grinder and macerated five times with 4-ml volumes of glass-distilled hexane to extract the hexane-soluble insecticide which had been absorbed through the cuticle. Each rinse was filtered through anhydrous sodium sulfate as it was poured into a 25 ml screw-top glass vial to remove moisture from the sample. The vials were appropriately labeled and the combined extracts were evaporated to dryness prior to storage in a deep freeze at 0°C.

Each empty holding jar was routinely rinsed with five 4-ml volumes of glass-distilled hexane to remove any unchanged endosulfan and its hexane-soluble metabolites excreted in the feces. In addition to the routine hexane rinse, the jars from the 24-hour holding period were also rinsed with five 4-ml volumes of acetone and finally with five 4-ml volumes of distilled water.

Fifty resistant female flies were killed by placing them in a drying oven for two hours at 300°C. After they were removed from the oven and cooled to room temperature, they were treated topically with 1 ug of technical endosulfan and processed as previously described to determine whether the detoxication mechanism was enzymatically controlled. The hexane extracts thus obtained were analyzed by gas chromatography.

Fifty resistant female flies were injected with a 1 ug dose of technical endosulfan dissolved in 1 ul of acetone, kept four hours

in holding jars, and rinsed externally to determine whether the hexane rinses, as done routinely in the absorption and metabolism studies, would remove insecticide which had penetrated the cuticle. Injections were made by the ISCO microapplicator fitted with a 30 gauge needle. The 1 ul dose was injected between the second and third abdominal sclerites into the ventral abdominal cavity of each fly as it was hand-held. The flies thus treated were held on the needle for one minute to prevent the injected dose from leaking back through the injection site. The rinses were combined as before, evaporated to dryness, and held at freezing for analysis by gas chromatography.

Paper chromatography.-- Ascending, reversed phase paper chromatography was used to analyze the fly extracts. The methods used were based upon those of Mitchell (1953, 1958) as modified by Terranova and Ware (1963). Standards were prepared by weighing 10 mg each of C^{14} -labeled technical endosulfan and its individual components and dissolving each in 10 ml of acetone. These standards were used to make the desired dilutions for topical application to flies as well as the standards used in gas and paper chromatography.

Whatman No. 1 chromatographic paper was cut into sheets measuring 8" x 10", the scraps being used to make strips 1" x 8" which were folded in half lengthwise, placed over the top of an 8" x 10" sheet and stapled in such a way that a 1/8 inch glass rod could be inserted in the fold to afford support for the sheet. A line was drawn 3/4 inch from the bottom with a graphite pencil, the resulting line marked at inch intervals for spotting. The sheets were washed successively

with distilled water, re-distilled acetone, re-distilled hexane to remove impurities, then air dried. The dried sheets were sprayed on both sides with a 4% (v/v) solution of USP medium grade mineral oil in hexane prior to spotting with 10 to 20 μ l of the test standards. The hexane-soluble external, internal, and fecal extracts were diluted to 0.1 ml with hexane prior to spotting on chromatogram sheets, while the original solvents were used to bring the acetone- and water-soluble extracts up to the same volume. Chromatograms were developed in 8" x 10" hard rubber chromatographic chambers (Figure 5) using a 65% solution of methyl cellosolve in water as the mobile solvent.

After the chromatograms were suspended in the chambers on glass rods, enough mobile solvent was added to cover the bottom of the tank without touching the sheets. The glass top was sealed in place with masking tape to form an air-tight compartment and the contents allowed to equilibrate for two hours before adding sufficient mobile solvent to raise the level 1/4 inch above the lower edge of the suspended chromatograms. The chromatograms were developed for five to six hours or until the front was approximately one inch from the suspending rods. The resulting chromatograms were removed and air-dried prior to treating with a chromogenic agent or exposed to Kodak No-screen Medical (8" x 10") X-ray film in Kodak cassettes to produce autoradiograms.

Autoradiograms.-- Autoradiograms were also made by neutron irradiation of paper chromatograms in The Ohio State University nuclear reactor. The chromatograms developed from unlabeled endosulfan were inserted into a water-tight plexiglass cylinder, lowered into

Fig. 5--Chromatographic chamber.

the reactor, and bombarded by a neutron flux of 10^{10} neutrons/cm²/second for two hours. After removing the chromatograms from the cylinder they were immediately stapled to X-ray film plates and placed in cassettes for a three-hour exposure. The film was developed in DK-50 developer for five minutes, fixed for five minutes, and washed in running tap water for four hours.

Metabolite synthesis.-- When preliminary experiments revealed a hexane-soluble metabolite in the internal extracts an effort was made to identify it in the laboratory. Low-melting point endosulfan was refluxed for two hours with aqueous potassium permanganate and sulfuric acid in a round bottom flask to which a water-cooled reflux condenser was attached. The flask contents were extracted with hexane in a separatory funnel, the hexane layer collected, evaporated to dryness, diluted to 10 ml with hexane and analyzed by gas chromatography.

A sample of endosulfan alcohol was refluxed with a mixture of acetic acid, hydrogen peroxide and sulfuryl chloride for two hours as described above. The flask contents were extracted with hexane and processed similarly to the permanganate oxidation. The residue remaining after extraction with hexane was extracted with acetone to determine the amount of endosulfan derivatives present.

Gas chromatography.-- A Model 15 Barber-Colman gas chromatograph⁵ using an A 4072 radium-source electron-capture detector and an 8000 series single-point recorder with 1/3 inch per minute chart

⁵Manufactured by the Barber-Colman Co., Rockford, Illinois.

speed was used for analysis of all endosulfan extracts. Analyses were done on a 3- and 4-foot, U-shaped, Pyrex glass columns 1/4 inch inside diameter which were packed with 1.5% SE-52 silicone gum rubber coated on 60/80 mesh Gaschrom⁶ solid support. Column temperature was held at 225°C, detector at 240°C, and injection block at 250°C throughout the determinations except where specifically noted. Hi-Pure⁷ nitrogen gas which had passed through a molecular seive was used as the carrier gas. Flow rate at the column effluent was maintained at 100-120 ml/min.

Samples were injected with a 10 ul Hamilton micro-syringe. Uniformity of sample size was controlled by filling the empty syringe several times to eliminate air bubbles and to wet the plunger to prevent blow-by. A column of approximately 1.5 ul of hexane was drawn into the barrel immediately below the plunger, the bottom meniscus of the column being drawn up to the 1 ul mark so that the needle and barrel, up to the 1 ul mark, were filled with air. The needle was wiped with a clean tissue and a 2 ul sample was drawn into the syringe. After removing the needle from the sample vial another column of air was drawn into the syringe below the sample. The correct sample size was thus clearly marked by a column of air on either end, while the 1.5 ul column of hexane rinsed the syringe and needle on the downstroke of the plunger and prevented blow-by. Practice

⁶Manufactured by Applied Science Laboratory, 140 N. Barnard Street, State College, Pennsylvania.

⁷Manufactured by Liquid Carbonics Division of General Dynamics.

using this technique resulted in remarkable duplication of peak size with repeated samples of similar volumes.

Samples of external, internal, and fecal extracts in hexane were injected, in that sequence. A minimum of two peaks identical in height served as the criterion for reproducibility, and in no case was it necessary to make more than two injections. Acetone-soluble fecal extracts were dried and redissolved in hexane prior to injection to obtain comparable results, while water-soluble fecal extracts would not partition into hexane and were not analyzed by gas chromatography.

Samples of metabolite synthesis extracts were injected after standards and fly extracts had been analyzed to circumvent sensitivity loss in the detector due to excess amounts of unknown deposits accumulating on the element.

RESULTS AND DISCUSSION

Dosage-mortality determinations of technical endosulfan and its individual components are given in Table 1. The LD₅₀ of technical endosulfan to susceptible female (CSMA) houseflies (60 ug/g) compares favorably with that published by Lindquist and Dahm (1957) (6.7 ug/g) as does the slope of the dosage mortality curve. The dosage-mortality curves for resistant and susceptible flies are shown in Figure 6.

Table 1.-- LD₅₀ values of technical endosulfan and related derivatives to susceptible and resistant three day old female houseflies.

Component	LD ₅₀ (ug/g)	
	Susceptible	Resistant
Technical endosulfan	6.0	140.0
endosulfan ether	>500	>500
endosulfan alcohol	>500	>500
endosulfan IMP	5.5	110.0
endosulfan HMP	9.0	200.0
endosulfan sulfate	9.5	235.0

Flies treated with a toxic dose of technical endosulfan began showing symptoms of intoxication ten minutes after treatment. Affected flies lacked coordination and displayed increased activity beginning shortly after they recovered from anesthesia and lasting approximately twenty minutes when actual knockdown began. Leg and mouthpart movement continued in moribund flies for as long as six hours after initial knockdown.

Fig. 6.--Dose-mortality curves comparing technical endosulfan, high- and low-melting point isomers and endosulfan sulfate on resistant and susceptible female houseflies.

The purified low-melting point isomer was slightly more toxic than technical endosulfan to resistant and susceptible houseflies (see Table 1), the resistant flies showed approximately a 20-fold resistance to both toxicants. The high-melting point isomer and the endosulfan sulfate were less toxic than technical endosulfan and the low-melting point isomer. The sulfate of endosulfan, which appears rapidly as a metabolite in internal extracts, is of the same order of toxicity as the high-melting point isomer when applied topically. Flies treated with 10 ug of either endosulfan alcohol or endosulfan ether showed no symptoms of poisoning.

Paper chromatography of the individual components of technical endosulfan and the sulfate of endosulfan gave R_f values shown in Table 2. Figure 7 is a representative chromatogram of the standards.

Table 2.-- R_f values of technical endosulfan and related derivatives.

Standard	R_f *
endosulfan ether	0.375
endosulfan alcohol	0.95
endosulfan HMP isomer	0.575
endosulfan LMP isomer	0.15
endosulfan sulfate	0.885

*Mobile phase = 65% methyl cellosolve in water.
Stationary phase = 4% mineral oil.

A comparison of the chromatogram map with the chromatograms of resistant and susceptible fly extracts (Figures 8 and 9) show that the hexane-soluble metabolite extracted in all the internal rinses has the same R_f as does the endosulfan sulfate. The metabolite (E_3) was never

Fig. 7.—Chromatograph trace of endosulfan standards. Six-hour development at 25°C. Mobile phase, 65% methyl cellosolve with water. Stationary phase, 4% mineral oil in hexane.

Fig. 8.—A 16-day exposure autoradiogram of extracts from resistant flies treated with ^{35}S -labeled technical endosulfan.

recovered from hexane extracts of feces in sufficient quantities to be detectable via paper chromatography either using chromogenic agents or autoradiograms made from the chromatograms. Autoradiograms made from the chromatograms which were spotted with either the C^{14} -labeled standard or C^{14} -treated fly extracts were considerably more sensitive in detecting spots than were any of the chromogenic agents tried during the preliminary tests. Papers spotted with as little as 1 ug/spot were easily detected on an autoradiogram after X-ray films were exposed to developed chromatograms for sixteen days.

Figure 10 shows a water-soluble metabolite (R_f 0.78) and an acetone-soluble metabolite (R_f 0.87) extracted from feces of 25 flies treated with endosulfan ether at the rate of 5 ug/fly. The mobile solvent was 55% methyl cellosolve in water. Corresponding R_f s of other components developed on this system are shown in Table 3.

Table 3.-- R_f values of endosulfan standards and metabolites developed in 55% methyl cellosolve in water.

Component	R_f
endosulfan ether (EE)	0.117
endosulfan alcohol (EA)	0.538
endosulfan sulfate (E_3)	0.415
endosulfan IMP (E_1)	0.06
endosulfan HMP (E_2)	0.189
water-soluble metabolite (E_4)	0.78
acetone-soluble metabolite (E_5)	0.87

The high polarity of these two metabolites, and the fact that they are relatively insoluble in hexane, indicates they may be conjugates of the endosulfan molecule with either a glucoside, a glucuronide, or an ethereal sulfate as described by Smith (1955).

Fig. 10.—Chromatogram trace of fecal extracts and standards. Mobile phase, 55% methyl cellosolve in water. Stationary phase, 4% mineral oil in hexane.

Analysis of fly extracts by gas chromatography showed that the basic difference between the resistant and susceptible strains was the rate of detoxication of the absorbed endosulfan. Figure 11 shows little difference in the amount of unabsorbed insecticide between the two strains, while Figure 12 shows a great disparity between the strains when the amount of absorbed insecticide is considered.

Considerably more of all absorbed components of technical endosulfan, as well as the hexane-soluble sulfate, is apparent earlier (two hours after treatment) in the susceptible strain. While all components can be detected unchanged in measurable quantities in fecal extracts of both strains, the amount excreted by the susceptible strain is greater than twice that excreted by the resistant strain (Figure 13). Considering that the rate of absorption is essentially the same in both strains, and the 20-fold resistance of the resistant strain, a mechanism other than differential rate of excretion of unchanged endosulfan is indicated. The amount of water-soluble and acetone-soluble metabolites (E_1 and E_5 respectively), while not measured quantitatively, may account for most of the metabolized endosulfan which was insoluble in hexane. No quantitative or qualitative determinations were made on these two metabolites during this study.

A comparison of the relative amount of endosulfan sulfate in internal extracts of the two strains shows a rapid accumulation of the oxidized endosulfan in the susceptible strain; whereas extracts from the resistant strain show little accumulation throughout the twenty four hour test period. Two possible explanations are that

Fig. 11.--Gas chromatograms of external rinses from resistant and susceptible flies treated with 1 µg technical endosulfan per fly. Extractions made at indicated hours after treatment.

Fig. 12.--Gas chromatograms of internal extracts from resistant and susceptible flies treated with 1 µg technical endosulfan per fly. Extractions made at indicated hours after treatment.

Resistant

Susceptible

Fig. 13.--Gas chromatograms of fecal extracts from resistant and susceptible flies treated with 1 ug technical endosulfan per fly. Extractions made at indicated hours after treatment.

(1) the susceptible strain may be unable to further metabolize the oxidized endosulfan which is itself nearly as toxic as high-melting point endosulfan to the flies; and (2) the resistant strain may not oxidize endosulfan as rapidly as does the susceptible strain.

Further studies using a synergist such as sesamex are contemplated to test these explanations. A probable route of detoxication would be a conjugation of the oxidized endosulfan as previously mentioned. This may account for the two metabolites, found in the feces, which were insoluble in hexane. Endosulfan sulfate (E_3) may be an intermediate through which the two toxic isomers (E_1 and E_2) must pass enroute to further detoxication.

Experiments conducted to synthesize the hexane-soluble metabolite were successful in both cases. Results are shown in Figure 14. The endosulfan sulfate peak of the fly extracts and those obtained by oxidizing endosulfan with potassium permanganate-sulfuric acid and the peracetic acid-sulfuryl chloride oxidation had identical retention times. The sample of purified endosulfan sulfate supplied by the Niagara Division, FMC Corporation, produced a peak with a retention time identical with those produced by fly extracts and my locally synthesized samples. These results were confirmed by gas chromatography at Niagara Chemical Division, FMC Corporation, using a disc integrator-equipped Dohrman gas chromatograph. A trace of results obtained by this analysis is shown in Figure 15.

External rinses of flies injected with technical endosulfan showed no traces of the toxicant when flies were rinsed as described. These extracts showed traces of endosulfan if the flies were soaked

Fig. 14.—Gas chromatograms of hexane extracts from laboratory synthesis of endosulfan sulfate.

Fig. 15.--A chromatogram trace of 250 ul of 4 hr internal extract from fifty resistant flies tested with 1 ug technical endosulfan per fly.

in the initial rinse for 30 minutes, while approximately one-third of the injected dose was recovered in the initial rinse if the flies were soaked for one hour.

Heat-killed flies showed no difference in the rate of absorption than that displayed by live flies; however, a slight trace of E_3 was recovered in the internal hexane extracts at the 12-hour interval after treatment.

Activation analysis or irradiation of developed chromatograms which have been spotted with insecticides containing chlorine and other neutron absorbing elements appears to be a technique warranting further investigation. Faint, but readily detectable, spots were produced on an autoradiogram made from a chromatogram which had been spotted with 20 μ l each of acetone-soluble and water-soluble fecal extracts from twenty-five flies treated with 5 μ g endosulfan ether per fly. The total volume of each extract was 0.1 ml; thus the maximum amount of the metabolites which could be shared by the two spots obtained is 12.5 μ g. This is presuming there was no loss due to volatilization, and none of the unchanged ether remained undetected in the external and internal rinses.

CONCLUSIONS

Of the methods of detoxication of insecticides discussed by Smith (1962), those most likely to play a major role in detoxifying technical endosulfan appear to be oxidation and conjugation. The hexane-soluble metabolite (endosulfan sulfate) was detectable rapidly (as little as thirty minutes) in internal extracts from resistant and susceptible houseflies, yet it was never found in fecal extracts when flies were treated with either technical endosulfan or its individual components. The fact that the sulfate was recovered in fecal extracts of flies treated with the purified sulfate indicates that the rate of metabolism of the sulfate may be the most important aspect of the entire detoxication process. When technical endosulfan was applied topically its components did not penetrate at the same rate. The alcohol and ether were almost completely absorbed in less than fifteen minutes whereas the HMP and LMP isomers were still detectable in external rinses twenty-four hours after treatment. Thus the two toxic isomers were absorbed comparatively slowly and over a twenty-four hour period. These isomers, in turn, must be oxidized to the sulfate at a given rate. If this rate is greater than whatever the sulfate degradation process, the accumulation of the sulfate may overwhelm the fly and cause death. When the sulfate is applied as a pure compound the absorbed material is in an oxidized form and may be present in such large amounts that it cannot be metabolized to the water- and

acetone-soluble metabolites at a sufficient rate to avoid a toxic accumulation within the fly. Further studies into the chemical composition of the two metabolites which were insoluble in hexane will be necessary before the metabolism of endosulfan is fully elucidated.

The relative polarity of the water- and acetone-soluble fecal extracts suggests a probable conjugation as a glucoside or glucuronide, while their presence in feces from flies treated with endosulfan ether eliminates the possibility that the conjugation could occur at the terminal sulfur of either of the two toxic endosulfan isomers.

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