Absorption and Excretion, Distribution, and Metabolism of Carbon-14–Labeled DDT by the American Cockroach

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Radioactive DDT and DDE, topically applied to American cockroaches, are rapidly absorbed and widely distributed internally. As much as 75% of the DDT applied is excreted as metabolite(s) in the feces over a 24-day period. About 80% of the radioactivity in the feces is due to metabolites containing the diphenyl-2-carbon moiety of DDT; less than 10% is due to DDT, DDE, or DDA. Less than 1% of DDT applied or injected is excreted as $C^{14}O_2$. The synergist "piperonyl cyclonene," used with DDT, inhibits absorption of DDT and excretion of metabolites.

THE SYNTHETIC ORGANIC INSECTICIDE DDT, 2,2-bis(*p*-chlorophenyl)-1,1,1-trichloroethane, has probably been the object of more mode-of-action studies than any other insecticidal chemical. Interest in it has been heightened by the development of resistance to DDT by the housefly and other insects.

The results of these studies have been summarized in a number of publications (*1*, *2*, *4*, *7*, *21*, *22*). Sternburg and Kearns (*31*), Sternburg and others (*34*), Perry and Hoskins (*25*, *26*), March and Metcalf (*20*), and Tahori and Hoskins (*36*) have reported that DDT-resistant strains of houseflies are capable of dehydrohalogenating a greater percentage of absorbed DDT to the relatively nontoxic ethylene analog DDE, or 2,2-bis(*p*-chlorophenyl)-1,1-dichloroethylene, than nonresistant strains. Other insects used to study the metabolic fate of DDT have included the large milkweed bug (*9*), the American cockroach (*37*), a series of insect species that possessed varying degrees of natural resistance to DDT (*32*), and susceptible and resistant German cockroaches (*3*). Sternburg and Kearns (*33*) reported the presence of toxins, other than DDT, in the blood of DDT-poisoned American cockroaches. These findings emphasized the importance and necessity of further metabolism studies, and indicated the possibility that a hypertoxic metabolic mechanism [such as that enunciated by Williams (*39*, *40*)] is involved with this compound. Sternburg and others (*35*) demonstrated the presence of an enzyme system capable of rapidly dehydrochlorinating DDT to DDE in a strain of DDT-resistant flies. This same enzyme system either was absent in a susceptible strain or had such low activity that it could not be detected.

Several studies relating to this problem have been made with radioactive molecules. A comparatively low specific activity *p,p'*-C^{14}–ring-labeled DDT was used by Lindquist and others (*18*, *19*), Hoffman and others (*13*, *14*), and Roth and others (*28*) to study the absorption, distribution, and metabolism of DDT in resistant and nonresistant houseflies. Winteringham and others (*43*) used $DBr^{82}DT$, the bromine-82 analog of DDT or 2,2-bis(*p*-bromo-82-phenyl)-1,1,1-trichloroethane, and a reversed-phase paper partition chromatographic analytical method (*42*) to study the problem of resistance of houseflies to DDT. The DDT-resistant flies were resistant also to the $DBr^{82}DT$ analog. The dehydrohalogenated compound, $DBr^{82}DE$, was the only metabolite found. Dahm and others (*8*) reported the use of *p,p'*-ring–labeled DDT and DDE to study the distribution and metabolism of these compounds by the American cockroach. The major portion of sublethal, topically applied radioactive DDT was eliminated in the feces of the cockroach, within a few days, as one or more metabolites which did not have spectral transmittance characteristics (*29*) in the visible region of DDT, DDE, or DDA. Butts and others (*5*) have used 2-C^{14}–ethane-labeled DDT to study the detoxication of DDT in the American cockroach. As much as 55% of the radioactive DDT injected into the body of the insect was converted to an unknown, "conjugated" metabolite. None of the radioactive DDT appeared to be respired as $C^{14}O_2$.

In the present study, solutions of radioactive DDT (both *p,p'*-C^{14}–ring-labeled and 2-C^{14}–ethane-labeled) were administered topically and by injection, and a solution of radioactive DDE (*p,p'*-C^{14}–ring-labeled) was administered topically to determine the absorption, metabolism, and excretion of these compounds by adult female American cockroaches.

Materials and Methods

All insects used in these experiments were laboratory-reared, female, adult American cockroaches, *Periplaneta americana* (L.). All experiments were carried out at a temperature of about 28° C. because of the reported negative-temperature coefficient of action of DDT for both topical and injected applications of the compound (*37*).

Insecticidal Compounds The four C^{14}–labeled compounds used in these studies are recorded in Table I. Compound 1 was used only in preliminary investigations because of the later availability of compound 3, which had the same labeling but a much higher specific activity. To correct for the concentration of insecticides by solvent evaporation, the observable specific activities were determined by radiometric assay of 10 to 15 samples of standard solutions immediately following preparation.

Nonradioactive DDA [2,2-bis(*p*-chlorophenyl) acetic acid] was used in the paper-chromatographic studies and was prepared from the *p,p'* isomer of DDT (*38*). The recrystallized DDA had a melting point of 166.0–166.5° C. The "piperonyl cyclonene" used in the synergism studies was technical grade material (specific gravity 1.08 at 25° C.) [consisting of 80% of 3-*iso*amyl-5-(3:4-methylenedioxyphenyl)-2-cyclohexene-1-one and its 6-carbethoxy derivative and 20% of related compounds.]

The four radioactive compounds listed in Table I were tested for both chemical and radiochemical purity. The radioactive DDT was found to be as pure as the nonradioactive, *p,p'*-DDT standard using the colorimetric method of an-

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alysis of Schechter and others (*29*). The radioactivity of the compounds provided a convenient method to check possible losses of DDT or DDE throughout the analytical procedure. By radiometric analysis, it was found that mean values of 98% of the DDT-radioactivity and 80% of the DDE-radioactivity were present at the terminal step of the colorimetric procedure.

The radiochemical purities were evaluated also by paper chromatography (*16*, *17*, *42*). This method is extremely sensitive; as these analyses indicated the absence of any detectable radioactive impurities, it was assumed the compounds were radioactively pure.

Administration of Insecticidal Compounds. Two methods of application were used; injection into the haemocoele and topical application. Injections of acetone and corn oil solutions of the radioactive ring-labeled DDT were made only in respiration and $C^{14}O_2$ excretion studies. The roaches were anesthetized with carbon dioxide, then fastened lightly by a rubber band to the top of a Büchner funnel, which was covered with $^1/_4$-inch hardware cloth, and through which flowed a stream of carbon dioxide sufficient to keep the insect continuously anesthetized during the injection. A micropipet (5 μl. in volume), fitted at the tip with a short length of a No. 27 hypodermic needle, was used for all injections. The micropipet plus the hypodermic needle tip had a total volume of 7.5 μl. as calibrated radiometrically. The insecticidal solution was injected into the haemocoele by inserting the tip of the hypodermic needle through the intersegmental membrane between the fourth and fifth abdominal sternites. The fluid in the micropipet was controlled by a manually operated, screw-type micropipettor.

Topical applications were made by first anesthetizing the roach and placing it on the Büchner funnel covered with hardware cloth as described above. The rubber band not only held the wings of the insect away from the abdomen while the insecticide was applied but also held the insect in place. The insecticides and piperonyl cyclonene were applied in acetone solutions by using micropipets (10 to 50 μl.) and a screw-type micropipettor. The site of all topical applications was the mesal abdominal tergites. After the acetone had been allowed to evaporate, the wings were returned to their normal position, thus covering the site of application and eliminating the possibility of loss of compounds through contact with the holding cage. The absence of radioactivity in acetone rinses of holding cages at the termination of several experiments indicated that very little of the insecticides thus applied was ever lost.

In the studies in which both DDT and piperonyl cyclonene were applied to roaches, the piperonyl cyclonene was always applied first; the acetone was allowed to evaporate, and the insecticide was then applied.

All solutions of radioactive compounds were assayed radiometrically before the roaches were treated. The counting data were used to check the concentration of radioactive compounds in the standard solutions. This technique served as a check against concentration of the standard solutions due to solvent evaporation.

In the studies of respiration and $C^{14}O_2$ excretion, roaches were allowed time for complete recovery from the carbon dioxide used as anesthetic before the experiments were begun.

Radiometric Measurements The radiometric analyses were made using conventional scalers and a windowless, gas-flow Geiger counter (Model D-46A Q-gas counter, Nuclear Instrument & Chemical Corp., Chicago 10, Ill.). All counting was done at a uniform temperature (27–28° C.) and humidity (approximately 50%). The samples were mounted on aluminum planchets designed for use in the windowless counter. Two C^{14}–labeled polystyrene standards with counting rates of approximately 1000 and 3000 counts per minute were used as reference sources.

All samples were prepared in triplicate and the mean corrected counting rate was used in computing radioactivity. The samples were counted long enough to attain a standard error of $\pm 5\%$ (*6*).

Absorption Studies In these studies, 30 female adult cockroaches were anesthetized and treated topically with 40 γ of ring-labeled DDT per roach as described above. The roaches were placed in cages which contained food and water and were held at 27–28° C. At 24-hour intervals, five roaches were chosen at random from the cages, anesthetized with carbon dioxide, and rinsed in three 100-ml. portions of acetone. These rinses were combined, concentrated, and evaluated radiometrically and chromatographically.

Distribution Studies In each of the four tissue distribution studies, seven female roaches were anesthetized with carbon dioxide and treated topically with the appropriate compound. Each group of treated roaches was placed in a metabolism cage consisting of an inverted, wide-mouthed, pint glass jar and the screw band containing a circular piece of $^1/_4$-inch hardware cloth. Legs were attached to the screw band and the cage was placed over a Petri dish to collect feces. A shell vial filled with sucrose solution and plugged with cotton provided food and water for the roaches. This vial was attached to the inside wall of the cage. The treated roaches were held 72 hours at 27–28° C.

Seventy-two hours after treatment, the roaches were anesthetized and weighed. The external, unabsorbed radioactivity was removed by rinsing the roaches in three 100-ml. portions of acetone.

The insects were dissected as follows: The tegmina and wings were cut off close to the body, the antennae and legs were removed, and all of these parts were placed in a tared aluminum-foil weighing boat. The head capsule was opened by making two incisions just below the compound eyes from the edges of the *foramen magnum*, and joining in the center of the front of the head capsule. The top of the head capsule was then cut from the previous incision to the upper edge of the *foramen magnum*. A longitudinal incision was made along the dorsum and to one side of the heart. The head capsule and body were pinned open and the following organs and

Table I. Carbon-Labeled Compounds Used

Compound	Labeling	Source	Specific Activity, γc./Mg.	Observable Specific Activity[a], C.P.M./γ	Melting Point Range, ° C.	Method of Preparation
1. DDT	*p,p'*-C^{14}–ring-labeled	U.S.D.A.[b]	0.153	145	108.5–109.5	Fields and others (*11*)
2. DDE	*p,p'*-C^{14}–ring-labeled	U.S.D.A.[b]	0.153	132	87.0	Jackson and Hopkins (*16*)
3. DDT	*p,p'*-C^{14}–ring-labeled	O.R.N.L.[c]	2.120	1936	107.5–108.2	Fields and others (*11*)
4. DDT	2-C^{14}–ethane-labeled	U.S.P.H.S.[d]	0.470	425	107.0–107.5	Pearce and Jensen (*24*)

[a] Determined with Nuclear Model D46A, windowless counter.
[b] From Division of Soil Management and Irrigation Agriculture, Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Dept. Agriculture, Beltsville, Md.
[c] From Oak Ridge National Laboratory (Carbide and Carbon Chemicals Co.), Oak Ridge, Tenn.
[d] From Technical Development Laboratories, Communicable Disease Center, U. S. Public Health Service, Savannah, Ga.

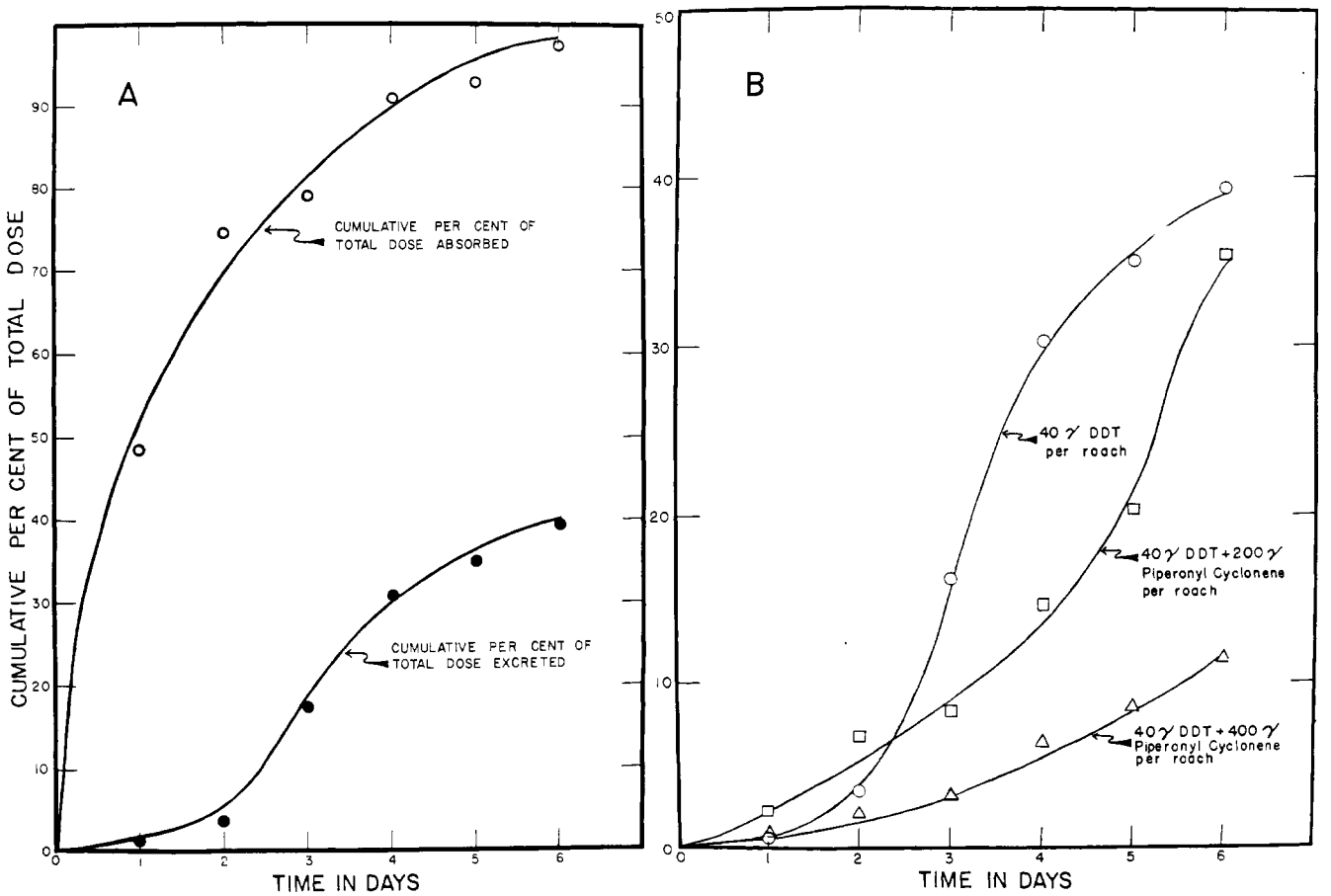


Figure 1. Absorption and excretion of radioactive DDT, alone and in combination with piperonyl cyclonene, topically applied to adult, female American cockroaches

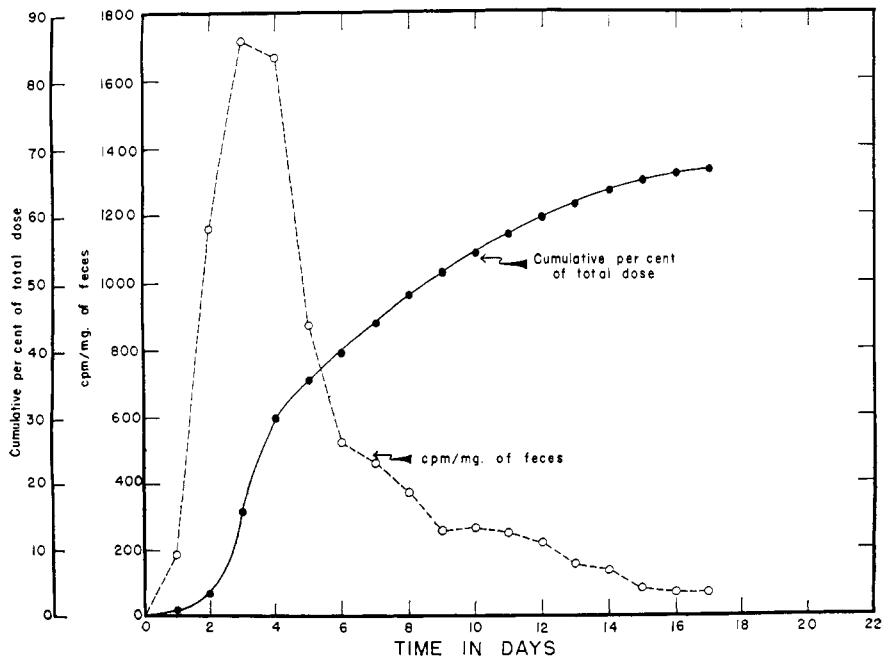
40 γ of *p,p'*-C^{14}-labeled DDT per roach

A. Cumulative per cent of total DDT absorbed obtained from pooled, external rinses of groups of five roaches and of total DDT and metabolites excreted in feces.

B. Cumulative per cent of total DDT and metabolites excreted in feces of three groups of roaches treated with DDT alone and in combination with piperonyl cyclonene in ratios of 1 to 5 and 1 to 10.

Figure 2. Excretion of radioactive DDT, DDE, and metabolites in feces following topical application of radioactive DDT and DDE to cockroaches

40 γ *p,p'*-C^{14}-labeled DDT per roach. Mean weight of roaches 1.294 grams (range 1.078–1.690)



tissues removed: fore-, mid-, and hindgut plus Malpighian tubules, brain and thoracic ganglia, thoracic muscle samples, and abdominal fat samples. These tissues were transferred rapidly to aluminum-foil weighing boats and placed in a Petri dish containing a water-saturated piece of cotton to reduce dehydration before weighing. The portion of insect body remaining after dissection and removal of tissues was added to wings, tegmina, legs, and antennae; these were collectively designated as the "remainder." The tissues were weighed rapidly on a torsion balance and frozen pending extraction. The accumulated feces for the 72-hour period were weighed also and stored in a refrigerator.

The tissues and feces were extracted by the following procedure: Samples were transferred quantitatively to a glass mortar, acidified with dilute sulfuric acid, thoroughly ground with sand, and extracted with five aliquots of an acetone-water solution. The extracts were filtered, and concentrated, and the final volume was recorded. Measured aliquots were pipetted in triplicate onto aluminum planchets and the liquid was evaporated. Sufficiently small aliquots were used to obviate

self-absorption due to lipoid materials in the extracts. These lipoid substances tended, however, to give a more even distribution of the material on the planchet and to reduce loss of activity through volatility. The tissue residues remaining after extraction were assayed radio-metrically to determine completeness of extraction.

In the haemolymph distribution studies, 20 roaches were treated topically as described above. The roaches were placed in a metabolism cage and held at 27–28° C. At 24-hour intervals, groups of five roaches were chosen at random, anesthetized, and weighed. The haemolymph was collected by clipping the antennae and freezing the blood droplets with carbon dioxide on a tared cover slip as described by Sternburg and Kearns (*33*). The haemolymph was weighed on a torsion balance and extracted in the same manner as the other tissues.

$C^{14}O_2$ Measurements. Following application of the insecticide, the roaches were placed in a respiration apparatus where the expired carbon dioxide was collected as barium carbonate and assayed radiometrically for the presence of $BaC^{14}O_3$. The respiration apparatus was a modification of systems described for mammalian studies (*12*, *30*). The methods of conducting the experiments, sample preparation, radiometric assay, calculation and correction for self-absorption, and statistical evaluation of the results have been described (*45*).

Fecal Metabolites Groups of 12 roaches were treated topically with insecticidal solutions as described above. The roaches were placed in large metabolism cages similar to those described under absorption studies. The roaches were fed dog-food pellets, and water was available in cotton-stoppered glass vials. Both the pellets and water vial were attached to the wall of the metabolism cage to prevent loss of feces and contamination of the food and water with excreta. Feces were collected at 24-hour intervals, weighed, and stored in a refrigerator until extracted. The extracting solution, method of extraction, and radiometric evaluation of the extracts were the same as those described under tissue distribution studies. The fecal residues remaining after extraction were checked radiometrically for completeness of extraction.

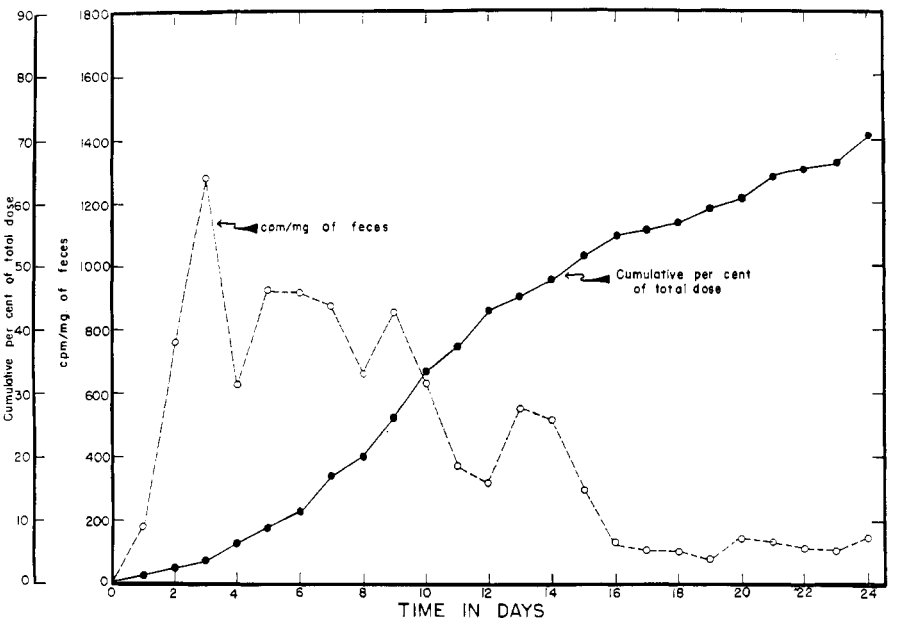
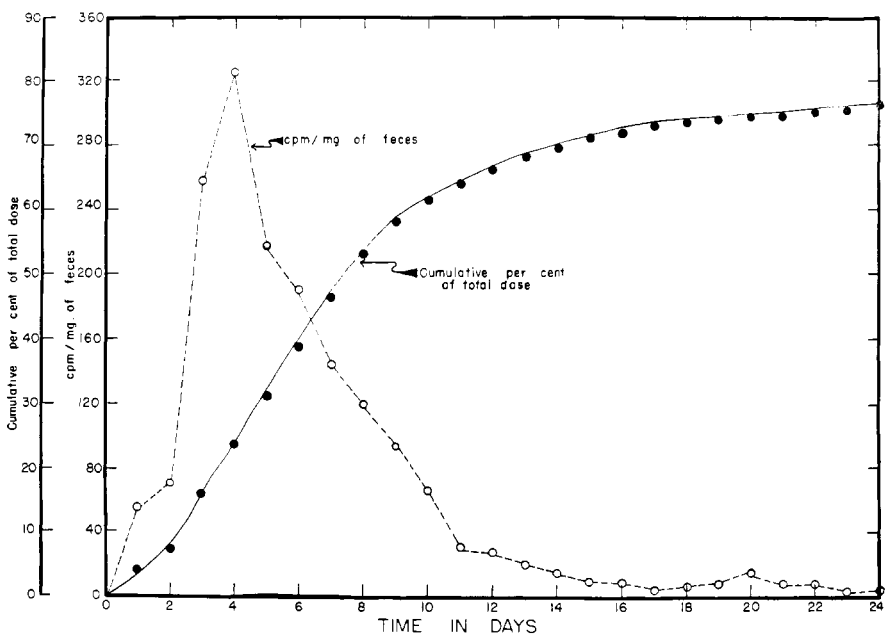


Figure 4. Excretion of radioactive DDT, DDE, and metabolites in feces following topical application of radioactive DDT and DDE to cockroaches

40 γ *p,p'*-C^{14}-labeled DDT and 400 γ piperonyl cyclonene per roach. Mean weight of roaches 1.321 grams (range 1.168–1.638)

Figure 3. Excretion of radioactive DDT, DDE, and metabolites in feces following topical application of radioactive DDT and DDE to cockroaches

40 γ 2-C^{14}-ethane-labeled DDT per roach. Mean weight of roaches 1.468 grams (range 1.263–1.844)



Evaluation of Fecal Metabolites. The radioactivity in the feces of treated roaches was evaluated by chemical and biological methods in an attempt to identify the compounds present. Chemical evaluation was undertaken using the colorimetric method of Schechter and others (*29*), and a modification of the paper chromatographic method of Winteringham and others (*42*) nearly identical to the method reported by Jackson and Hopkins (*16*, *17*) was used.

Spectrophotometric analyses were performed with a Coleman, Model 14, Universal spectrophotometer. Standard transmittance curves for both DDT and DDE were established using radioactive compounds. Radioactive DDT and DDE were used also to establish R_f values (ratio of the distance traveled by the material to that of the solvent front, measured from the point of application) for these compounds by paper chromatographic analyses. All chromatograms were evaluated radiometrically by cutting the strips into sections and counting these in a windowless, gas-flow counter. R_f values were assigned to the radioactive peaks on the strips. The coating solution, the process of coating the strips, temperature control during the run, and saturation of the atmosphere of the chromatography chamber with the mobile phase of the

Table II. Radioactivity in Pooled Tissue Samples from Groups of Seven Adult, Female American Cockroaches Individually Treated on Abdominal Tergites with Insecticides

Dissections and fecal collections made 72 hours after application of insecticides

Samples Assayed Radiometrically	Tissue Weights[a]		Radiometric Data				
	Weight, mg.	% of body wt.	Total activity, c.p.m.	Activity per unit of weight, c.p.m./mg.	Total γ equivalent of DDT	γ equivalent of DDT/roach	Distribution of applied dose, %
		Group A[b]. 42.4γ *p,p'*-C^{14}-labeled DDT					
Tissues							
Fore-gut	64.2	5.8	53,657	119.4	27.7	4.0	9.4
Mid-gut	27.2	2.4	5,534	29.1	2.9	0.4	1.0
Hind-gut	39.6	3.4	41,632	150.1	21.5	3.1	7.3
Brain and thoracic ganglia	6.5	0.6	1,150	25.3	0.6	0.08	0.2[c]
Fat sample	36.6	3.3	19,207	74.9	9.9	1.4	3.4[c]
Muscle sample	13.9	1.2	4,654	47.8	2.4	0.3	0.8[c]
Remainder	852	73.7	211,100	35.4	109.0	15.6	36.9
Feces	14.1		21,362	216.7	11.0	1.6	3.7
Rinse			141,700		72.2	10.3	24.8
Total		90.4[d]					87.5[e]
		Group B[b]. 40 γ 2-C^{14}-ethane-labeled DDT					
Tissues							
Fore-gut	68.7	4.8	4,247	8.8	10.0	1.4	3.6
Mid-gut	32.7	2.3	1,007	4.4	2.4	0.3	0.9
Hind-gut	48.9	3.4	5,729	16.8	13.5	1.9	4.8
Brain and thoracic ganglia	8.2	0.6	230	4.0	0.5	0.07	0.2[c]
Fat sample	64.5	4.6	4,819	10.7	11.3	1.6	4.0[c]
Muscle sample	19.1	1.3	728	5.4	1.8	0.3	0.6[c]
Remainder	1032	72.2	37,000	5.1	87.1	12.4	31.1
Feces	26.9		8,451	44.9	19.9	2.8	7.1
Rinse			33,405		78.6	11.2	28.1
Total		89.2[d]					80.4[e]
		Group C[b]. 42.2 γ *p,p'*-C^{14}-labeled DDT and 400 γ piperonyl cyclonene					
Tissues							
Fore-gut	40.0	3.3	62,922	224.8	32.5	4.6	11.0
Mid-gut	22.7	1.9	5,497	34.6	2.8	0.4	1.0
Hind-gut	37.9	3.1	35,573	134.0	18.4	2.6	6.2
Brain and thoracic ganglia	6.1	0.5	773	18.1	0.39	0.06	0.1[c]
Fat sample	52.1	4.1	9,503	26.0	4.9	0.7	1.7[c]
Muscle sample	11.9	1.0	1,629	19.6	0.84	0.12	0.3[c]
Remainder	922	74.7	162,355	25.2	83.9	12.0	28.4
Feces	15.9		15,862	142.6	8.2	1.2	2.8
Rinse			223,025		115.2	16.5	39.0
Total		88.6[d]					90.5[e]
		Group D[b]. 220 γ *p,p'*-C^{14}-labeled DDE					
Tissues							
Fore-gut	33.5	2.7	12,261	52.3	92.9	13.3	6.0
Mid-gut	23.8	1.8	4,519	27.1	34.2	4.9	2.2
Hind-gut	35.3	2.9	12,790	51.8	96.9	13.8	6.3
Brain and thoracic ganglia	6.8	0.6	385	8.1	2.9	0.4	0.2[c]
Fat sample	38.2	2.9	9,030	33.8	68.4	9.8	4.4[c]
Muscle sample	22.3	1.7	1,057	6.8	8.0	1.1	0.5[c]
Remainder	905	72.7	75,285	11.9	570.3	81.5	37.0
Feces	6.5		5,562	122.0	42.1	6.0	2.7
Rinse			68,551		519.3	74.2	33.7
Total		85.3[d]					93.0[e]

[a] All tissue weights are mean values and "wet weights" as described in test.
[b] Mean weights of seven cockroaches. Group A, 1.150 grams (range 1.012–1.375); B, 1.429 grams (range 1.198–1.622); C, 1.232 grams (range 1.088–1.510); D, 1.250 grams (range 0.982–1.700).
[c] For these samples a better comparison can be made by referring to data in Activity per Unit of Weight column.
[d] Total in this column does not equal 100% because of weight losses in dissection and weighing. See text for description of methods.
[e] Total in this column does not equal 100% largely because of extraction losses. See text for description of methods.

solvent were critical factors in the method.

The presence of compounds in the feces toxic to houseflies was checked biologically by using a modification of the microbioassay method of Hoskins and others (*15*). The bioassays were performed using 4-day-old houseflies (1948 CSMA strain). The flies were placed inside shell vials previously coated with known amounts of either DDT or radioactivity equivalents of fecal extracts. After 0.5-hour exposure in the vials, the flies were transferred to 1-quart, wide-mouthed glass jars containing a sucrose nutrient solution. At the end of 24 hours, the mortality was assessed. The flies were rinsed externally with acetone and ground and extracted as described under tissue distribution studies, to determine the comparative absorption by the flies of DDT and the fecal metabolites used in the assays.

Control samples were used in both the chemical and biological methods of evaluation. These were prepared by extracting feces from nontreated roaches in the manner used for the feces of treated roaches. Care was taken to approximate the concentration of feces in the control solution with that obtained in the extracts of feces from treated roaches. This procedure helped eliminate the possibility of differences in absorption spectra, R_f values, and mortalities due to chemical substances other than the fecal metabolites of the labeled compounds.

Results

Absorption and Excretion Studies

The results of several absorption experiments are presented in Figure 1. It is apparent (Figure 1, *A*) that the initial absorption of DDT is rapid; about one half of the applied dose is absorbed in 24 hours and nearly three quarters after 48 hours. About 91% of the applied DDT is absorbed by the roaches in the first 96 hours, after which absorption continues at a much slower rate. The excretion of radioactivity in feces was highest for radioactive DDT alone, and progressively lower when 5 to 1 and 10 to 1 ratios of piperonyl cyclonene and DDT were used (Figure 1, *B*). These experiments cover the first 6 days, or critical period, in which more than 90% mortality occurred.

The excretion of radioactivity in the feces of roaches treated with the *p,p'*-C^{14}–labeled DDT is presented in Figure 2. Initially, the excretion was very rapid, as shown by both curves from the second through fifth days. At the end of 17 days, nearly 67% of the radioactivity of the total applied doses was accounted for in the fecal extracts. This value is a little low, as it was impossible to extract all of the radioactivity from the feces. A similar excretion pattern is apparent for the 2-C^{14}–labeled DDT (Figure 3). At the end of 24 days, 75% of the total applied doses had been excreted in the feces. The mean mortality for these two experiments was 21%.

When piperonyl cyclonene plus *p,p'*-C^{14}–labeled DDT (10 to 1 ratio) was applied to roaches the rate and amount of radioactivity excreted were much lower (Figure 4) and more irregular, and the mortality of the roaches was increased about 2.5 times. These factors indicated that the insecticide or its metabolitis were retained by the roaches for a longer period. Reference to Table II (groups A and C) will show that the combination treatment resulted in more radioactivity in the external rinses than in treatments with DDT alone. This helps explain why less radioactivity was found in the feces following the combination treatment.

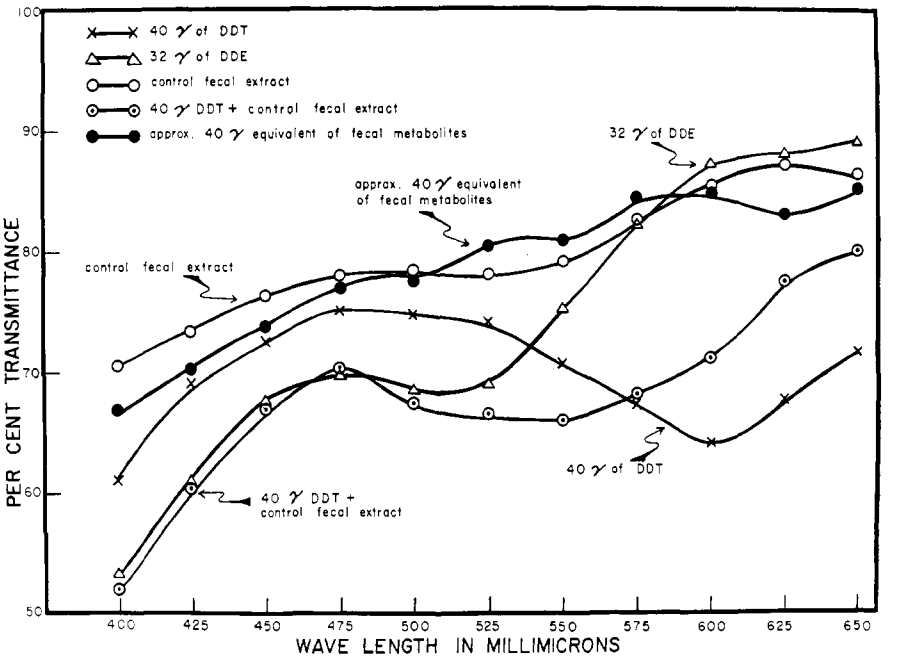


Figure 6. Absorption spectra of fecal metabolites from *p,p'*-C^{14}–labeled DDT-treated roaches, nonradioactive DDT and DDE standards, and control fecal extracts, treated for color development by Schechter method (29)

Results of the *p,p'*-C^{14}–labeled DDE excretion experiment are presented in Figure 5. Because of the low specific activity and lack of toxicity of this compound, it could be applied at a higher rate of 100 γ per roach. The curve of the cumulative radioactivity approached linearity. At the end of 24 days, about 58% of the total applied doses had been excreted in the feces.

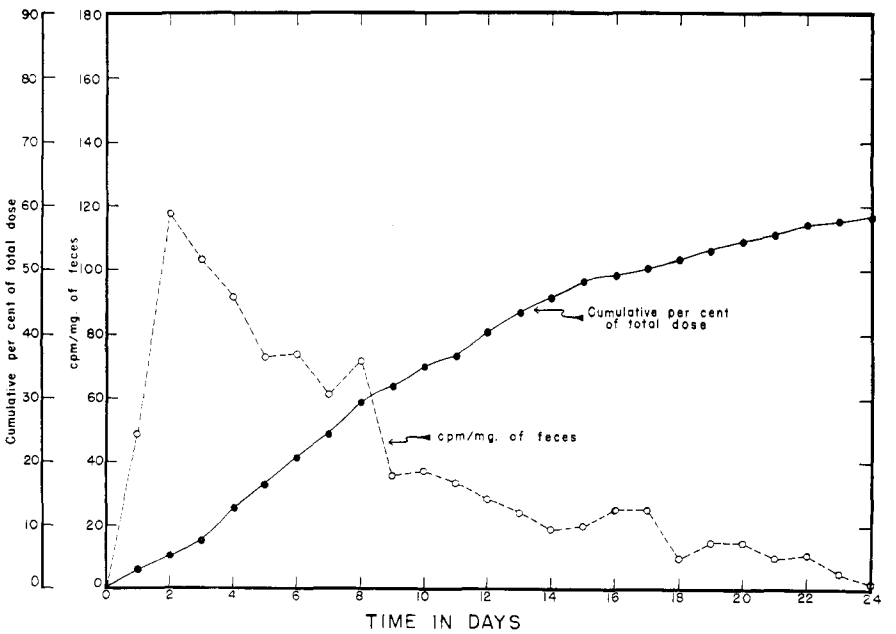
Distribution Studies

The results of four distribution studies are summarized in Table II. The activity in the tissue samples from the brain and thoracic ganglia, fat, and muscle can best be compared with other tissues by utilizing the "activity per unit of weight" column, as no attempt was made to remove all of these tissues quantitatively from the treated roaches. The radioactivity of whole organs—i.e., fore-gut, hind-gut plus Malphigian tubules, and mid-gut—and feces should be compared on the basis of the percentage "distribution of applied dose." This is true because the latter tissue samples closely approximate their total weights, and the weights of the gut portions and of the feces excreted vary with the amount of food eaten before and excreted after treatment.

Of the tissues examined, the fore-gut, hind-gut, fat, and feces contained the greatest amount of radioactivity per unit of weight. The fore-gut contained the greatest amount of radioactivity in the *p,p'*-C^{14}–labeled DDT, *p,p'*-C^{14}–labeled DDT plus piperonyl cyclonene,

Figure 5. Excretion of radioactive DDT, DDE, and metabolites in feces following topical application of radioactive DDT and DDE to cockroaches

100 γ *p,p'*-C^{14}–labeled DDE per roach. Mean weight of roaches 1.177 grams (range 1.001–1.597)



and p,p'-C^{14}–labeled DDE distribution studies. In the 2-C^{14}–ethane-labeled DDT study, the fore-gut contained less radioactivity than the hind-gut and the fat, but the amount of radioactivity excreted via the feces was less than in any of the other experiments.

In the p,p'-C^{14}–labeled DDT plus piperonyl cyclonene experiment, absorption of DDT seemingly was retarded by pretreatment of the roaches with the synergist.

Blood samples were collected and extracted, at 24-hour intervals, over a 5-day period from groups of roaches that had been treated topically with p,p'-C^{14}–labeled DDT at the rate of 40 γ per roach. The counting rates of extracts of these samples were so low they could not be reported.

$C^{14}O_2$ Measurements Very low counting rates were obtained from the barium carbonate samples containing the expired carbon dioxide from treated roaches. Less than 1%—e.g., 0.5 to 0.6%—of topically applied p,p'-C^{14}–labeled DDT, ranging from 40 to 200 γ per roach, was excreted as $C^{14}O_2$ over periods of 26 to 44 hours. Doses of p,p'-C^{14}–labeled DDT, ranging from 40 to 100 γ, were injected into roaches using either acetone or corn oil as carriers. The carbon dioxide expired was collected from 4 to 56 hours. Injection of the labeled compound produced an initial increase in $C^{14}O_2$ expired about 4 hours after injection, the amount of $C^{14}O_2$ expired decreased to the same low level found in the topical application experiments and remained at this level until the roaches died or the experiment was discontinued.

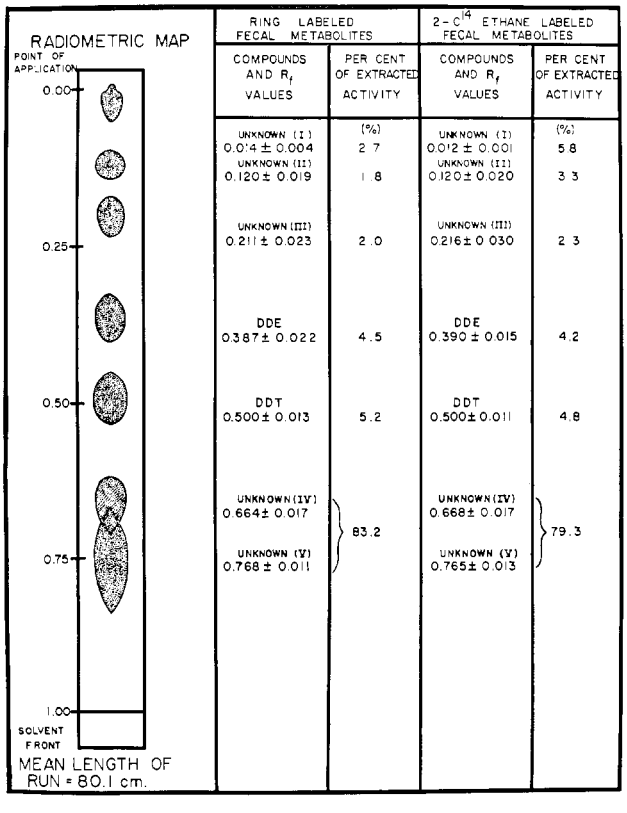
Evaluation of Fecal Metabolites Absorption spectra of fecal metabolites, DDT and DDE standards, and normal feces, which were analyzed by the method of Schechter and others (*29*), are shown in Figure 6. The presence of interfering substances after nitration of the fecal extracts made it difficult to determine the true absorption of the metabolic products. Radiometric assays were conducted at several stages of the colorimetric analysis. Only one third to one half of the radioactivity in the fecal extracts was carried completely through the procedure (compared with 98% for DDT and 80% for DDE under insecticidal compounds above), indicating that the metabolic products were very different from either DDT or DDE.

The results of the paper chromatography experiments are summarized in Figure 7. R_f values are shown for the two radioactive DDT compounds, radioactive DDE, and the radioactive compounds extracted from the feces. It is apparent that less than 10% of the radioactivity in the feces is due to DDT and DDE. About 80% of the radioactivity is associated with a region running from 0.66 to 0.77 (unknowns IV and V, Figure 7). Treatment of the strips with potassium permanganate (*41*) brought out the two distinct regions designated as unknowns IV and V. DDA has a higher R_f value (*16*, *17*) and does not give this color reaction. Efforts to resolve the two unknown substances were not successful, but enough work was done to estimate that about 80% of the radioactivity was associated with unknown V. It is strikingly apparent that application of the two radioactive DDT compounds to roaches resulted in almost identical fecal metabolites. Considering the position of the radioactive carbon atoms in the two compounds, these chromatographic resolutions suggest that the principal metabolic products in the feces must include the diphenyl-2-carbon moiety of the original molecules.

Results of a typical bioassay of fecal metabolites for toxicity are presented in Table III. Although results were generally erratic, differences were noticed in the inability of houseflies to pick up as much radioactivity from mixtures of DDT and fecal metabolites (70 to 74%) as from DDT alone (85 to 91%), indicating again that the fecal metabolites did not possess the same physiological properties as DDT.

Figure 7. Paper chromatographic resolution of radioactivity extracted from feces of female, adult, American cockroaches treated topically with p,p'-C^{14}–ring-labeled DDT or 2-C^{14}-ethane–labeled DDT

40 γ per roach. R_f values are mean values, standard deviations calculated from 10 or more chromatograms. Percentages of extracted materials are also mean values.



RING LABELED FECAL METABOLITES		2-C^{14} ETHANE LABELED FECAL METABOLITES	
COMPOUNDS AND R_f VALUES	PER CENT OF EXTRACTED ACTIVITY	COMPOUNDS AND R_f VALUES	PER CENT OF EXTRACTED ACTIVITY
	(%)		(%)
UNKNOWN (I) 0.014 ± 0.004	2.7	UNKNOWN (I) 0.012 ± 0.001	5.8
UNKNOWN (II) 0.120 ± 0.019	1.8	UNKNOWN (II) 0.120 ± 0.020	3.3
UNKNOWN (III) 0.211 ± 0.023	2.0	UNKNOWN (III) 0.216 ± 0.030	2.3
DDE 0.387 ± 0.022	4.5	DDE 0.390 ± 0.015	4.2
DDT 0.500 ± 0.013	5.2	DDT 0.500 ± 0.011	4.8
UNKNOWN (IV) 0.664 ± 0.017	83.2	UNKNOWN (IV) 0.668 ± 0.017	79.3
UNKNOWN (V) 0.768 ± 0.011		UNKNOWN (V) 0.765 ± 0.013	

Discussion

It may prove significant that in all insecticide distribution studies reported to date, in which radioactive compounds and the American cockroach were used, the fore-gut selectively accumulated the radioactivity representing the toxicant and/or its metabolites to a greater extent than any other organ or tissue. This phenomenon has been reported for a number of organic phosphates (*10*, *27*), for pyrethrins (*45*), and for DDT in this paper. The large amount of radioactivity associated with the hind-gut plus Malpighian tubules and the fat bodies may be accounted for by excretion of the labeled compounds and/or their metabolites. Woke (*44*), working with the southern armyworm, *Prodenia eridania* (Cram.), reported that of all the tissues tested for in vitro degradation of pyrethrins, the fat body was the most efficient. In a more recent study dealing with the metabolic activation of octamethyl pyrophosphoramide, O'Brien and Spencer (*23*) reported that the gut in susceptible and the fat body in nonsusceptible insects were the most effective organs in producing a "hypertoxic" oxidation. The possibility of a similar mechanism occurring with DDT in these tissues of the American cockroach appears to be a problem worthy of further exploration.

Perry and Hoskins (*25*) have given three physiological defense mechanisms of the housefly against the toxic effects of DDT: detoxication of DDT to DDE, degradation of DDT to metabolic products, and storage of DDT and/or metabolic products. All of these mechanisms seem to be available to the American cockroach. The detoxication of DDT to DDE, which is seemingly of foremost importance in resistant houseflies, appears to be of secondary importance in the American cockroach; this agrees with the findings of Vinson and Kearns (*37*). The degradation of DDT to

Table III. Typical Bioassay Experiment

p,p'-C^{14}–labeled DDT and/or roach fecal metabolites comparatively evaluated as to toxicity and absorption using 3-day-old male and female houseflies of a 1948 CSMA strain as test organisms

Vial No.	Flies Used ♀	Flies Used ♂	Flies Used Total	Residual Dose of DDT and/or Roach Fecal Metabolites per Vial	Moribund at 24 Hours Flies	Moribund at 24 Hours %	Estimated Pickup γ equivalent	Estimated Pickup % of residual dose	External Radioactivity γ equivalent	External Radioactivity % of residual dose	External Radioactivity % of pickup	Internal Radioactivity γ equivalent	Internal Radioactivity % of residual dose	Internal Radioactivity % of pickup
1 and 2	51	49	100	11.0 γ DDT	21	21.0	3.01	13.68	0.25	1.14	8.3	2.76	12.54	91.7
3 and 4	58	44	102	14.7 γ DDT	33	32.3	3.95	13.46	0.40	1.36	10.1	3.55	12.10	89.9
5 and 6	52	50	102	18.3 γ DDT	51	50.0	6.53	17.84	0.68	1.86	13.4	5.85	15.98	86.6
7 and 8	48	53	101	22.0 γ DDT	82	81.2	8.95	20.34	1.10	2.50	12.3	7.85	17.84	87.7
9 and 10	57	47	104	25.7 γ DDT	65	62.5	7.14	13.90	0.80	1.56	11.1	6.34	12.34	88.9
11 and 12	52	50	102	29.3 γ DDT	87	85.3	10.30	17.14	1.52	2.59	14.8	8.78	15.00	85.2
13 and 14	61	42	103	Cfe[a]	12	11.6	...	...	...	...	...	...	...	...
15 and 16	64	38	102	Cfe plus 7.3 γ DDT	1	1.0	1.68	11.51	0.15	1.03	9.0	1.53	10.48	91.0
17 and 18	55	47	102	12.6 γ equivalent metabolites plus 7.3 γ DDT	49	48.2	5.41	13.59	1.59	3.99	29.4	3.82	9.60	70.6
19 and 20	55	46	101	Cfe[a] plus 14.6 γ DDT	38	37.2	4.56	15.51	0.68	2.31	14.7	3.88	13.20	85.3
21 and 22	61	40	101	12.6 γ equivalent metabolites plus 7.3 γ DDT	67	66.3	7.97	14.66	2.09	3.83	26.0	5.88	10.83	74.0

[a] Control fecal extract.

metabolic products seems to be most important in the roach. There is evidence also that storage of DDT and/or its metabolites is of importance in the roach.

Summary

The absorption and excretion, tissue distribution, and metabolism of *p,p'*-C^{14}– and 2-C^{14}–ethane-labeled DDT and the tissue excretion and distribution of *p,p'*-C^{14}–labeled DDE in female American cockroaches, *Periplaneta americana* (L.), have been studied. The absorption studies showed that DDT applied topically at the rate of 40 γ per roach is very rapidly absorbed (nearly 50% in 24 hours) and after 6 days more than 95% is absorbed (see Figure 1,*A*). The internal distribution of *p,p'*-C^{14}– and 2-C^{14}–ethane-labeled DDT, *p,p'*-C^{14}–labeled DDT plus piperonyl cyclonene, and *p,p'*-C^{14}–labeled DDE in female American cockroaches has been determined 72 hours following topical application of sublethal doses of the compounds (see Table II). Of the tissues dissected and assayed radiometrically, the fore-gut, hind-gut plus Malpighian tubules, and fat contained the greatest amount of radioactivity.

The carbon dioxide expired by roaches treated topically and injected with *p,p'*-C^{14}–labeled DDT was assayed radiometrically as barium carbonate to determine if the insecticide might be degraded to $C^{14}O_2$. Less than 1% of the DDT applied or injected was excreted as $C^{14}O_2$.

The excretion of radioactivity in the feces of roaches treated topically with the same compounds used in the distribution studies summarized above has been determined (see Figures 2, 3, 4, and 5). As much as 75% of the DDT applied can be accounted for over a 24-day period. When the synergist piperonyl cyclonene is used with DDT it inhibits both the absorption and excretion of the DDT and/or its metabolic products.

Colorimetric analyses (*29*) of the fecal metabolites of roaches treated topically with *p,p'*-C^{14}–labeled DDT indicated that the major portion of the metabolites was not due to either DDE or DDA (see Figure 4). Paper chromatographic analyses (*42*) of fecal metabolites of roaches treated topically with *p,p'*-C^{14}– and 2-C^{14}–ethane-labeled DDT showed that less than 10% of the radioactivity in the feces was due to DDT, DDE, or DDA. In the case of both radioactive DDT compounds, about 80% of the radioactivity in the feces is associated with one or more metabolites which contain the diphenyl-2-carbon moiety of the original molecules (see Figure 6).

Biological evaluation of the fecal metabolites, using houseflies, demonstrated a lower toxicity and decreased ability to penetrate insect cuticle as compared with DDT.

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Literature Cited

(1) Babers, F. H., U. S. Dept. Agr., Bur. Entomol. Plant Quarantine, **E-776** (1949).
(2) Babers, F. H., and Pratt, J. J., Jr., *Ibid.*, **E-818** (1951).
(3) Babers, F. H., and Roan, C. C., *J. Econ. Entomol.*, **46,** 1105 (1953).
(4) Brown, A. W. A., "Insect Control by Chemicals," Wiley, New York, 1951.
(5) Butts, J. S., Chang, S. C., Christensen, B. E., and Wang, C. H., *Science*, **117,** 699 (1953).
(6) Calvin, M., Heidelberger, C., Reid. J. C., Tolbert, B. M., and Yankwich, P. F., "Isotopic Carbon. Techniques for Its Measurement and Chemical Manipulation," Wiley, New York, 1949.
(7) Chadwick, L. E., *Am. J. Trop. Med. Hyg.*, **1,** 404 (1952).
(8) Dahm, P. A., Robbins, W. E., Hein, R. E., and McFarland, R. H., "Distribution and metabolism of C^{14}–Labeled DDT in the American Cockroach," 64th Annual Meeting, American Association Economic Entomologists, Philadelphia, Pa., Paper **127** (Dec. 18, 1952).
(9) Ferguson, W. C., and Kearns, C. W., *J. Econ. Entomol.*, **42,** 810 (1949).
(10) Fernando, H. E., Roan, C. C., and Kearns, C. W., *Ann. Entomol. Soc. Amer.*, **44,** 551 (1951).
(11) Fields, M., Gibbs, J., and Waltz, D. E., *Science*, **112,** 591 (1950).
(12) Gould, R. G., Sinex, F. M., Rosenberg, I. N., Solomon, A. K., and Hastings, A. B., *J. Biol. Chem.*, **177,** 295 (1949).
(13) Hoffman, R. A., Roth, A. R., and Lindquist, A. W., *J. Econ. Entomol.*, **44,** 734 (1951).
(14) Hoffman, R. A., Roth, A. R., Lindquist, A. W., and Butts, J. S., *Science*, **115,** 312 (1952).
(15) Hoskins, W. M., Witt, J. M., and Erwin, W. R., *Anal. Chem.*, **24,** 555 (1952).
(16) Jackson, P. C., personal communication on preparation of C^{14}–labeled DDE, Aug. 26, 1952.
(17) Jackson, P. C., and Hopkins, H. T., "Study of the Persistance of C^{14}–Labeled *p,p'*-DDT in Various Soils," U. S. Dept. Agr., Bur.

Plant Industry, Div. Soil Management and Irrigation, Research Rept. **237** (1952).
(18) Lindquist, A. W., Roth, A. R., Hoffman, R. A., and Butts, J. S., *J. Econ. Entomol.*, **44,** 931 (1951).
(19) Lindquist, A. W., Roth, A. R., Yates, W. W., Hoffman, R. A., and Butts, J. S., *Ibid.*, **44,** 167 (1951).
(20) March, R. B., and Metcalf, R. L., *Soap Sanit. Chemicals*, **26,** 121, 123, 125, 139 (1950).
(21) Metcalf, R. L., "Mode of Action of Organic Insecticides," Natl. Research Council, Natl. Acad. Sci., Chem. Biol. Coordination Center, Rev. No. I., 1948.
(22) Natl. Research Council, Natl. Academy Sci., "Conference on Insecticide Resistance and Insect Physiology," Pub. **219** (1952).
(23) O'Brien, R. D., and Spencer, E. Y., J. AGR. FOOD CHEM., **1,** 946 (1953).
(24) Pearce, G. W., and Jensen, J. A., *Ibid.*, **1,** 776 (1953).
(25) Perry, A. S., and Hoskins, W. M., *J. Econ. Entomol.*, **44,** 850 (1951).
(26) Perry, A. S., and Hoskins, W. M., *Science*, **111,** 600 (1950).
(27) Roan, C. C., Fernando, H. E., and Kearns, C. W., *J. Econ. Entomol.*, **43,** 319 (1950).
(28) Roth, A. R., Lindquist, A. W., and Terriere, L. C., *Ibid.*, **46,** 127 (1953).
(29) Schechter, M. S., Soloway, S. B., Hayes, R. A., and Haller, H. L., *Ind. Eng. Chem., Anal. Ed.*, **17,** 704 (1945).
(30) Skipper, H. E., Bryan, C. E., White, L., Jr., and Hutchison, O. S., *J. Biol. Chem.*, **173,** 371 (1948).
(31) Sternburg, J., and Kearns, C. W., *Ann. Entomol. Soc. Amer.*, **43,** 444 (1950).
(32) Sternburg, J., and Kearns, C. W., *J. Econ. Entomol.*, **45,** 497 (1952).
(33) Sternburg, J., and Kearns, C. W., *Science*, **116,** 144 (1952).
(34) Sternburg, J., Kearns, C. W., and Bruce, W. N., *J. Econ. Entomol.*, **43,** 214 (1950).
(35) Sternburg, J., Vinson, E. B., and Kearns, C. W., *Ibid.*, **46,** 513 (1953).
(36) Tahori, A. S., and Hoskins, W. M., *Ibid.*, **46,** 302 (1953).
(37) Vinson, E. B., and Kearns, C. W., *Ibid.*, **45,** 484 (1952).
(38) White, W. C., and Sweeney, T. R., *U. S. Public Health Repts.*, **60,** 66 (1945).
(39) Williams, R. T., *Ann. Rev. Biochem.*, **20,** 441 (1951).
(40) Williams, R. T., "Detoxication Mechanisms," Wiley, New York, 1949.
(41) Winteringham, F. P. W., *Science*, **116,** 452 (1952).
(42) Winteringham, F. P. W., Harrison, A., and Bridges, R. C., *Nature*, **166,** 999 (1950).
(43) Winteringham, F. P. W., Loveday, P. M., and Harrison, A., *Ibid.*, **167,** 106 (1951).
(44) Woke, P. A., *J. Agr. Research*, **58,** 289 (1939).
(45) Zeid, M. M. I., Dahm, P. A., Hein, R. E., and McFarland, R. H., *J. Econ. Entomol.*, **46,** 324 (1953).

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PESTICIDES IN FOODS

Determination of Malathion and Its Influence on Flavor of Milk from Cows Fed Malathion-Sprayed Alfalfa

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Malathion, applied at the rate of 0.4 pound per acre in California, caused nearly 100% mortality among mosquito larvae. But no malathion was detectable in the milk of cows fed on alfalfa sprayed with malathion, and taste tests showed that more persons found off-flavor in milk from untreated than from treated pastures.

THE FAILURE OF CHLORINATED HYDROCARBON INSECTICIDES to control mosquito larvae in several areas in California during 1950 and 1951 made it necessary to search for new effective larvicides. Malathion, one of the materials tested, was found to cause nearly 100% mortality when used at the rate of 0.4 pound per acre. However, before this insecticide could be generally used in pastures where milk cows graze, it was necessary to determine the amount that might be retained in the milk and whether it caused any change in taste.

Methods

Three cows were pastured on 1/2 acre of alfalfa that was sprayed with malathion emulsion once a week for 4 weeks. The animals were removed at the time of spraying but were turned back into the pasture as soon as the spray had dried. Another portion of the field served as a check pasture. At the end of 4 weeks, because of overgrazing in the sprayed area, it was necessary to move the cattle to a new sprayed area of the field. The animals were kept in this second pasture for 4 more weeks, during the first 3 weeks of which it was sprayed with malathion weekly. The alfalfa ranged from about 6 to 16 inches in height during the tests and was irrigated about once a week.

The first application of malathion was at the rate of 0.4 pound and the six subsequent applications were at 0.5 pound per acre. The emulsion concentrate was diluted with water to a 1.5% strength and applied either with a jeep boom spray or with a 3-gallon hand-operated sprayer.

Two of the test cows were of the Holstein breed and one was a Guernsey. Milk for comparison was obtained from the same number of cows of each breed that were pastured on adjacent nontreated alfalfa. These animals obtained from one third to one half of their food from the green alfalfa and the remainder from dry feed. This is common practice in the San Joaquin Valley, although some dairies use dry feed exclusively.

Milk samples from the cows grazing on the malathion-treated and the nontreated alfalfa were collected by the