carbamates and trimethylsilylcarbamates investigated.

The *N*-trimethylsilylcarbamates were toxic only when the corresponding carbamate showed activity, suggesting that in vivo hydrolysis of the former to the latter occurs in the fly. The initial hydrolysis step might protect the remainder of the molecule from the action of other detoxication mechanisms before reaching the site of action. Indeed, as shown in Table II, 3-isopropylphenyl *N*-trimethylsilylcarbamate (XIX) is several times as toxic as 3-isopropylphenylcarbamate (XII). If this hypothesis is valid, it would be expected that the degree of synergism of the *N*-trimethylsilylcarbamate by piperonyl butoxide would be less than that for the corresponding carbamates and this is true for all the synergizable compounds of this nature in Table II.

It is interesting to compare the activities of the *N*-trimethylsilylcarbamates with the *N*-acylcarbamates of 3-isopropylphenol (*19*) (XXV–XXVII, Table II). These compounds have affinities for fly ChE of about 0.001 to 0.002 that of 3-isopropylphenyl *N*-methylcarbamate (I) yet retain much of the insecticidal activity of the parent carbamate. This suggests that, like the trimethylsilylcarbamates, they must undergo in vivo hydrolysis, forming, in the case of acyl compounds, *N*-methylcarbamates and the corresponding carboxylic acid.

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METABOLIC STUDIES

Metabolism of *N*-(Mercaptomethyl)-phthalimide-carbonyl-C^{14}-*S*-(*O*,*O*-dimethyl-phosphorodithioate) (Imidan-C^{14}): Balance Study in the Rat

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The fate of Imidan-C^{14} labeled in the carbonyl carbon was determined following administration of a single oral dose to rats. Ninety-eight per cent of the radioactive material was accounted for in studies with three male and two female rats. Of that recovered, 79% was excreted in the urine and 19% in the feces at the time of sacrifice, either 72 or 120 hours after treatment. Less than 1% of the administered compound appeared in the urine as Imidan or its phosphorothiolate analog, *N*-(mercaptomethyl)phthalimide-*S*-(O,O-dimethylphosphorothiolate)(Imidoxon). Tissue residues accounted for 2.6% of the administered radioactivity with no selective storage in any tissue. Little, if any (<0.04%), radioactivity was detected in the expired CO_2.

THE FATE of the insecticide, *N*-(mercaptomethyl) phthalimide-*S*-(*O*, *O*-dimethylphosphorodithioate) (Imidan, Stauffer Chemical Co.), has been determined in the cotton plant (*15*) and in a steer (*10*). Imidan-C^{14} is absorbed and metabolized primarily to phthalamic and/or phthalic acids and possibly to benzoic acid or its derivatives following surface application to cotton leaves (*15*). Approximately 10% of a dermally applied dose of Imidan-C^{14} appeared in the urine and feces of a hereford steer within seven days after treatment. The primary degradation products in the urine of the steer were considered to be phthalic and phthalamic acids (*10*).

Metabolism of several other phosphorodithioate insecticides in mammals has also been studied using phosphorus-32–labeled materials (*1, 8, 9, 17*). Rapid degradation, extensive excretion in the urine and feces, and incorporation of trace amounts of P^{32} into normal biological constituents has usually been noted. With only a few exceptions (*5, 8, 10, 14, 16*), the fate of nonphosphorus-

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containing metabolites has not been critically evaluated. Imidan-C^{14} radiolabeled in the carbonyl groups of the phthalimide portion of the molecule was considered to be most appropriate to ascertain certain peculiarities, if any, that might occur in metabolism of this insecticide.

The present study was designed to determine the extent of gastrointestinal absorption, the rates and routes of excretion, and the deposition sites, if any, of metabolites following a single oral dose of Imidan-C^{14} to three male and two female rats. In addition, characterization of urinary metabolites was initiated.

Materials and Methods

Chemicals. The chemicals used in this study, including the synthesis, specific activity, and radiochemical purity of the Imidan-C^{14}, have previously been reported (*15*).

Apparatus and Design. Three male rats (M-1, M-2, M-3) and two female rats (F-1, F-2) of the Long Evans strain, weighing between 73 and 111 grams, were housed in all-glass and stainless steel metabolism cages similar to that described by Roth (*18*) and modified for this laboratory by the Stanford Glassblowing Laboratories, Inc., 970 San Antonio Road, Palo Alto, Calif. Air was drawn through the system at a rate of 0.4 to 0.7 liter per minute. In the case of rat M-1, the incoming air was freed of moisture and CO_2. Water and feed were available *ad libitum*. Exhaled CO_2 was trapped in a scrubber containing either a solution of sodium hydroxide (rats M-1, M-2, F-1) or a solution of ethanolamine in 2-ethoxyethanol (rats M-3, F-2).

Radioanalysis. A Packard Tri-Carb liquid scintillation spectrometer was used for all radioanalyses. Two counting formulations were used: solution A for counting toluene-soluble samples, consisting of 5 grams PPO (2,5-diphenyloxazole), 100 mg. POPOP (1,4-bis-5-phenyloxazoly)-benzene, and enough reagent grade toluene to bring the volume to 1 liter; and solution B for analyzing powders and other toluene-insoluble materials, previously described as diotol counting solution (*13*).

Duplicate aliquots of each sample (except the 11-hour blood, rat M-1), were prepared in 20-ml., low-potassium-glass vials and counted to determine reproducibility. One of each replicate was then fortified with an internal standard (toluene-C^{14}) and recounted to determine efficiency of counting.

Sample Preparation. Preliminary work was conducted with rat M-1. Lyophilization of urine and homogenized feces and tissues was performed routinely to retard the rate of decomposition of any water-sensitive metabolites, and also to improve conditions for radioanalysis. Prompt extraction with benzene obviated the necessity to remove the water from the remaining samples and was, therefore, not continued. Except where specified, the other techniques of sample preparation were essentially the same for all rats.

Urine. After the water was removed from the urine of rat M-1, the oily residue was reconstituted in 2 ml. of a 50% aqueous methanol solution. The urine samples from rats M-2, M-3, F-1, and F-2 were diluted directly with water to 20 ml. Aliquots representing a suitable fraction were suspended in counting solution B. At the end of the study, each cage was washed with 100 ml. of 50% methanol to ensure removal of residual radioactive urine adhering to the cage. One-milliliter aliquots were prepared, as above, for counting.

Feces. The wet feces from rat M-1 were homogenized in pH 6.75 phosphate buffer and lyophilized. A portion of dry feces was further reduced to a fine powder in a ground glass tissue grinder using dioxane as solvent. The wet feces from the other four rats were ground with the phosphate buffer directly in the tissue grinder and diluted with water to a known volume. Aliquots of known amounts were suspended in counting solution B and counted.

Carbon Dioxide. The NaOH scrubbing solutions were concentrated by lyophilization to 60 ml. and then diluted to 100 ml. with 5 ml. of dioxane and 35 ml. of methanol. One-milliliter aliquots of this suspension were prepared for radioassay by first mixing thoroughly with 1 ml. of polyglycol E-200 (Dow Chemical Co.), and then shaking vigorously with 15 ml. of counting solution B for 30 minutes on a mechanical shaker. Two-milliliter aliquots ($1/50$ of each collection) of the ethanolamine solution, containing the trapped CO_2, were suspended directly in counting solution B.

Tissues. Tissues and organs assayed for total carbon-14 content are listed in Table III. Samples of tissues from rat M-1, except the hide (the carcass was preblended with pH 6.75 phosphate buffer in a Waring Blendor), were finely pulverized with a small amount of the phosphate buffer in a ground glass tissue grinder and then freeze dried. Fifty-milligram aliquots of the dry tissues were suspended in counting solution B. The blood samples were bleached with a few drops of 30% H_2O_2 to reduce color quenching.

Tissue samples from the other rats (in this instance the carcasses were preblended with 50% methanol and dried under vacuum) were finely homogenized with 50% methanol and diluted to a known volume. Aliquots representing $1/25$ or $1/50$ of each sample were suspended in counting solution B. Highly colored tissues were bleached with H_2O_2.

Portions of the hide weighing from 100 to 500 mg. were digested by the method of Braun-Cantilo (*4*). Hydroxide of Hyamine 10-X (trade mark of Rohm and Haas), *p*-(di-isobutylcresoxyethoxyethyl)dimethylbenzylammonium hydroxide, containing the trapped CO_2 was dissolved either in counting solution A or suspended in B.

Extraction of Urine and Feces. Aliquots of diluted urine and homogenized feces from rats M-2, M-3, F-1, and F-2 were extracted three times with equal volumes of benzene. The urine samples were all extracted within 24 hours of collection while a lapse of approximately 10 days occurred in the case of the feces. Imidan and Imidoxon, as determined by chromatropic acid (*3*), partitioned into benzene from water to the extent of 99.96% and 95.5%, respectively. The benzene extracts were subjected to direct radioassay, while the procedure for counting the aqueous phase was described in the subsections Urine and Feces.

Animal Treatment

Predosage Period. Prior to the administration of the Imidan-C^{14}, the animals were acclimated to the metabolism cages and tail cups for a period of 4 to 5 days. Daily samples of urine, feces, and one CO_2 sample from each animal were collected for control purposes. The urine, immediately after excretion, was funneled into a plastic vial packed in dry ice. Feces were collected separate from urine by the use of tail cups similar to that described by Barnes (*2*), and stored under refrigeration.

Postdosage Period. Imidan-C^{14} (2.51 to 2.57 mg., 12.06 to 12.33 μc.) dissolved in 1.0 gram of ethanol–polyglycol E-200 (1 to 3) immediately prior to treatment, was administered orally by stomach tube. The dose in terms of active ingredient ranged from 23 to 35 mg. Imidan per kilogram of body weight (Table III), was well below the oral LD_{50} value of 230 mg. per kg. for males and 299 mg. per kg. for females. The syringe was weighed both before and after treatment, the difference being the amount administered. Additional weighed portions of the dosing mixture were similarly taken as standards to calibrate accurately the radioactive dose.

During the postdosage period, samples of urine, CO_2, and feces were collected, as described, at regular intervals for 72 or 120 hours. In addition, a sample of blood was taken from the retro-orbital venous plexus of rat M-1 11 hours after dosing. At termination of the 3 or 5 day postdosage period, the rats were sacrificed by cervical transection. The blood was drained into a vial and observations for gross pathology were made.

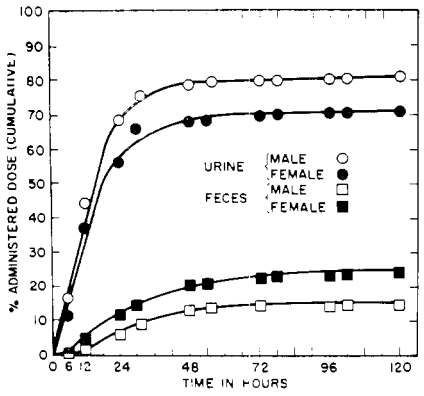

Figure 1. Average radioactivity excreted in the urine and feces of three male and two female rats after a single oral administration of Imidan-C^{14}

The appropriate tissues and organs were dissected, and the hides were removed from the carcasses. All portions of the test animals were stored in the freezer prior to preparation for radioanalysis.

Results

The rats exhibited generally normal appearance and behavior throughout the study. Weight gains were satisfactory (except for rat F-1 which gained only 6 grams; 20 to 25 grams would be considered normal), as were feed consumption and urinary and fecal excretion. Separation of urine and feces was good. There was no observable gross pathology at the time of sacrifice.

Elimination of Imidan, and/or its C^{14}-labeled metabolites, was most rapid during the initial 24 hours, gradually subsiding until only trace amounts were found after 72 hours (Figure 1). Excretion of the radioactivity in the urine of three male rats averaged approximately 10% more than that of the two female rats (81.4% and 71.5%, respectively), while elimination via the feces accounted for approximately 10% less from the males than from the females (14.7% and 23.4%, respectively). These data were calculated from Table I and are also presented graphically in Figure 1. Of the radioactivity administered to two male (M-2, M-3) and two female (F-1, F-2) rats, 75% appeared in the urine and 16% appeared in the feces 48 hours after dosing; benzene extracted 0.8% of the dose from the urine and 0.5% from the feces (Table II). An average total for all five rats of 95.7% (range: 94.2% to 97.1%) of the radioactive dose was eliminated via the urine and feces (Table I).

Tissue residues accounted for an additional 2.6% (Table I). These residues, in Imidan-C^{14} equivalents, averaged 0.71 p.p.m. for all tissues from those animals sacrificed at 72 hours and 0.41 p.p.m. for all tissues from those sacrificed at 120 hours (Table III).

Table I. Summary of Radioactivity Recovered from Three Male and Two Female Rats after a Single Oral Administration of Imidan-C^{14}

	Administered C^{14} Dose, %					
	M-1	M-2	M-3	F-1	F-2	Average
Urine	75.8	83.1	85.3	64.6	78.6	77.5
Feces	18.5	13.9	11.8	31.2	15.6	18.2
CO_2	<0.04	⩽0.01	⩽0.01	⩽0.01	⩽0.01	
Tissues	3.4	2.9	2.3	1.8	2.6	2.6
Totals	97.7	99.9	99.4	97.6	96.8	98.3

Table II. Radioactivity Recovered from the Urine and Feces of Two Male (M-2, M-3) and Two Female (F-1, F-2) Rats after a Single Oral Administration of Imidan-C^{14}

	Administered C^{14} Dose, %					
	Urine			Feces		
	Aqueous Phase		Benzene phase, composite	Aqueous Phase		Benzene phase, composite
Collection Interval, Hours	Male	Female		Male	Female	
6	17.0	11.7	0.212	0.1	0.2	0.034
12	27.8	25.4	0.253	1.2	3.9	0.066
24	30.5	22.7	0.336	3.6	7.8	0.353
30	3.1	6.0	0.029	3.2	2.8	0.025
48	3.3	1.9	0.012	3.9	5.6	0.030
Totals	81.7	67.7	0.842	12.0	20.3	0.508

Table III. Radioactive Residues Detected in the Tissues of Three Male and Two Female Rats after a Single Oral Administration of Imidan-C^{14} [a]

	Tissue Residues, P.P.M. of Imidan-C^{14} Equivalents						
	72 Hours[b]				120 Hours[b]		
Tissues and Organs	M-1	M-2	F-2	Average	M-3	F-1	Average
Fat	0.33	0.17	0.20	0.23	0.09	0.10	0.10
Gonads	0.18	0.21	0.18	0.19	0.13	0.09	0.11
Intestines	0.68	0.32	0.70	0.57	0.05	0.21	0.13
Brain	0.49	0.36	0.43	0.43	0.26	0.24	0.25
Spleen	0.47	0.42	0.37	0.42	0.28	0.23	0.26
Heart	0.58	0.45	0.41	0.48	0.35	0.26	0.30
Liver	0.91	0.41	0.62	0.65	0.32	0.32	0.32
Carcass[c]	0.94	0.69	0.63	0.75	0.43	0.40	0.42
Hide[c]	0.98	0.62	0.47	0.69	0.35	0.53	0.44
Lungs	0.60	0.41	0.59	0.53	0.23	0.69	0.46
Muscle	1.04	0.80	0.75	0.86	0.63	0.50	0.56
Kidney	1.55	1.12	1.09	1.25	0.65	0.56	0.60
Blood	1.93	0.63	0.63	1.07	1.21	1.25	1.23
Total animal	0.93	0.62	0.61	0.71	0.39	0.43	0.41

[a] Dose (mg./kg.) 35.2 (M-1), 25.5 (M-2), 27.1 (F-2), 23.0 (M-3), 26.0 (F-1).
[b] Time of sacrifice.
[c] The hides were removed from the carcasses and analyzed individually.

The over-all range was 0.05 p.p.m. in intestines of rat M-3 to 1.93 p.p.m. in the blood of rat M-1. The 11-hour blood sample from rat M-1 contained 2.62 p.p.m. Except for the relatively high levels of radioactivity remaining in the blood and kidneys, the tissue residues (Table III) were fairly evenly distributed throughout the body, the greatest amount being found in the somatic tissues (approximately 0.75 p.p.m. at 72 hours and 0.45 p.p.m. at 120 hours) and lesser amounts being found in the viscera (0.4 to 0.6 p.p.m. at 72 hours and 0.25 to 0.45 p.p.m. at 120 hours). The gonads and fat contained exceptionally low residues (0.2 p.p.m. at 72 hours and 0.1 p.p.m. at 120 hours). The total amount of Imidan-C^{14} equivalents remaining in the tissues at 120 hours was only 58% of that found at 72 hours, indicating progressive and continued elimination of C^{14} residues from the animal. The apparent increase in C^{14} residues found in the blood (compare rats M-2, F-2, with rats M-3, F-1, Table III) was probably a result of inadvertently diluting, with an unknown amount of water, the blood taken from rats M-1 and F-2 at time of sacrifice.

Little, if any, radioactivity was eliminated as CO_2 (Table I). Less than 0.04% of that given to rat M-1 and not more than 0.01% of that given to rats M-2, M-3, F-1, and F-2 was associated with the expired air. The total radioactivity recovered from each of the five rats averaged 98.3% (range: 96.8% to 99.9%) of the calculated administered dose (Table I).

Discussion

Imidan-C^{14} is rapidly absorbed from the gastrointestinal tract and eliminated

by rats, primarily in the urine and, to a lesser extent, through the feces. Preliminary data indicate phthalamic acid or a closely related water-soluble derivative to be the major metabolite of excretion, thus suggesting that in the rat, as in the cotton plant (*15*) and the steer (*10*), Imidan detoxication involves hydrolysis to water-soluble metabolites. In this respect, it follows a pattern similar to that established for other phosphate esters (*1, 14*).

Radioactivity remaining in tissues accounted for only 2.6% of the administered dose and no tissue selectively stored radioactive residues. The low fat residues is in contrast to findings with chlorinated hydrocarbon insecticides which accumulated in fat and is in accord with metabolic studies with several organophosphate insecticides (*6, 7*). Since there was a general reduction in radioactive residues in all tissues between the 72- and 120-hour intervals, it was not possible to assess fully whether the radioactivity found in the various tissues represented C^{14} metabolites which were incorporated into normal body constituents or whether the radioactive residues were only transiently stored and weakly bound to tissues.

Essentially no cleavage of the carbonyl carbon in the phthalimide moiety occurred in vivo, since no more than a trace of $C^{14}O_2$ was liberated. Faigle and coworkers (*12*) also found essentially no $C^{14}O_2$ in the exhaled air from rats and dogs which were orally fed with Thalidomide-C^{14} (α-phthalimidoglutarimide) uniformly labeled in the carbonyl groups. Although the trace amount of $C^{14}O_2$ found in this study could possibly have been liberated from an impurity, it is suspected that it may represent bacterial disruption of the phthaloyl moiety either in the gastrointestinal tract or in the feces. Dagley and coworkers (*11*) reported that many bacteria were capable of cleaving benzoates and polycyclic aromatic compounds to yield $C^{14}O_2$.

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HERBICIDE ADSORPTION STUDIES

Adsorption and Leaching of Herbicides in Hawaiian Sugarcane Soils

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With 16 increments of water, each equivalent to 1 acre-inch, leaching was negligible below a depth of 2 inches in a sugarcane topsoil for diuron applied at experimental rates up to 20 pounds per acre. Monuron at rates up to 10 pounds per acre did not leach appreciably below 4 inches. The soil had a saturation adsorption limit for diuron of about 47 pounds per acre-inch when the diuron was applied as a nearly saturated aqueous solution. Leaching of these herbicides at usual field rates in normal sugarcane topsoils is a minor consideration; it is a major contributory factor to sugarcane injury in exposed subsoils. The equilibrium availability of the herbicide in a given soil solution depends on adsorption, herbicide concentration, and the soil-water ratio.

Most Hawaiian sugarcane topsoils show an unusually high degree of physical adsorption of herbicides from aqueous solutions compared to most U. S. mainland soils (*5, 11*). Subsoils do not exhibit the same degree of adsorption. Two major factors are believed to account for the ability of the topsoils to fix most of the applied herbicide: a relatively high "organic matter" content and a considerable quantity of carbon from the combustion of dry leaf trash and cane tops prior to crop harvest. The "organic matter" represents accumulated organic residues and the decomposition products of 12 to 15 tons of sugarcane roots and variable quantities of leaf trash incorporated in the soil after each two-year crop cycle. In dry, irrigated areas (about 50% of the total acreage in sugarcane) analysis of "or-