Metabolism of Carbonyl-C^{14}–Labeled Imidan, *N*-(Mercaptomethyl)phthalimide-*S*-(*O*,*O*-dimethylphosphorodithioate), in Rats and Cockroaches

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The metabolism of a carbonyl-C^{14}-labeled preparation of Imidan [*N*-(mercaptomethyl)phthalimide-carbonyl-C^{14}-*S*-(*O*,*O*-dimethylphosphorodithioate)] was investigated in rats and German cockroaches. On oral administration to rats, metabolites are excreted in urine in amounts, relative to the administered dose, as follows: 40.7% as phthalamic acid, 21.0% as phthalic acid, 10.9% made up of five minor metabolites containing the phthaloyl moiety, 5.5% as undefined radioactivity, and no more than 0.04% as Imidan and its thiol analog (Imidoxon). Phthalamic and phthalic acids are excreted without chemical modification after oral administration to rats. The expected conversion of Imidan to Imidoxon occurs in the rat liver microsome–$NADPH_2$ system. Imidoxon is tentatively identified in cockroaches, but the major insect metabolite appears to be phthalamic acid. These studies support other evidence that Imidan is rapidly metabolized in mammals, mainly to innocuous, water-soluble metabolites.

The insecticide chemical Imidan, *N*-(mercaptomethyl)-phthalimide-*S*-(*O*,*O*-dimethylphosphorodithioate), is rapidly metabolized in mammals and plants to water-soluble products. Following a single oral dose of carbonyl-labeled Imidan to rats, 98% of the radioactivity is recovered; of this amount, 78% is in urine, 19% in feces, and 2.6% remains, uniformly distributed, in tissues either 72 or 120 hours after treatment (Ford *et al.*, 1966). Phthalic (PA) and phthalamic acids (PAA) are the major urinary metabolites in the steer resulting from a single dermal treatment (Chamberlain, 1965). Cotton plants convert Imidan mainly to PA and/or PAA and possibly benzoic acid or a derivative of the latter (Menn and McBain, 1964).

Further studies considered in this report concern the identity of Imidan-C^{14} metabolites formed in rats and German cockroaches and in the rat liver microsome–reduced nicotinamide adenine dinucleotide phosphate ($NADPH_2$) enzyme system. The fate of phthalamic and phthalic acids after oral administration to rats and the possibility of ring-hydroxylated metabolites of Imidan are also considered.

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MATERIALS AND METHODS

Potential Metabolites and Radiochemicals. The compounds used are listed in Table I. The Imidan-C^{14} and PAA-C^{14} samples had a specific activity of 1.53 mc. per mmole; PA-C^{14}, 0.8 mc. per mmole. The radiochemical purity of PAA-C^{14} was greater than 99%, while the Imidan-C^{14} used consisted of 92% Imidan-C^{14}, 4.5% PAA-C^{14}, and 3.5% of other radioactive impurities; these determinations were made with the EtOH paper chromatographic system (Table II).

Chromatographic Systems and Detection of Resolved Products. The paper and thin-layer chromatography (TLC) systems used to resolve Imidan, its potential metabolites, and the radioactive products recovered from rats and cockroaches treated with Imidan-C^{14} are given in Table II. Radioactive products and standard radiolabeled samples were detected by autoradiography using Kodak No-screen x-ray film. Imidan and Imidoxon were detected using a spray solution of 0.5% (w./v.) 2,6-dibromo-*N*-chloro-*p*-quinoneimine in cyclohexane (Menn *et al.*, 1957). The hydroxy derivatives of PA, 3OH-PA and 4OH-PA, appeared as blue and white fluorescent spots, respectively, under ultraviolet light (2537 A.). The remaining potential metabolites of Imidan were detected by spraying with a 3% (w./v.) $AgNO_3$ aqueous solution to give white or dark spots against a light brown background upon standing for several hours under ordinary fluorescent room light.

Table I. Name, Chemical Name, and Source of Compounds Used

Compound	Chemical Name	Source
Imidan	*N*-(Mercaptomethyl)-phthalimide-*S*-(*O*,*O*-dimethylphosphorodithioate)	Stauffer Chemical Co., Richmond, Calif.
Imidan-C^{14}	*N*-(Mercaptomethyl)-phthalimide-carbonyl-C^{14}-*S*-(*O*,*O*-dimethylphosphorodithioate)	Stanford Research Institute, Menlo Park, Calif. (Leaffer *et al.*, 1967)
Imidoxon	*N*-(Mercaptomethyl)-phthalimide-*S*-(*O*,*O*-dimethylphosphorothiolate)	Stauffer Chemical Co.
HMPI	*N*-Hydroxymethyl-phthalimide	Stauffer Chemical Co.
PI	Phthalimide	Matheson, Coleman, & Bell, Los Angeles, Calif.
PAA	Phthalamic acid	Matheson, Coleman & Bell
PAA-C^{14}	Phthalamic acid–carbonyl-C^{14}	From hydrolysis of Imidan-C^{14} in 0.1*M* K_2HPO_4 (Menn and Miskus, 1967)
PA	Phthalic acid	Eastman Organic Chemicals, Rochester, N. Y.
PA-C^{14}	Phthalic acid–carbonyl-C^{14}	New England Nuclear Corp., Boston, Mass.
3OH-PA	3-Hydroxyphthalic acid	M. E. Brokke, Stauffer Chemical Co.
4OH-PA	4-Hydroxyphthalic acid	From acid hydrolysis of 4-hydroxyphthalimide
4OH-PI	4-Hydroxyphthalimide	R. L. Smith, St. Mary's Hospital Medical School, London, England

Whenever a urine or fecal extract was examined for metabolites, reference standards were co-chromatographed or were added to an appropriate blank extract for chromatographic comparison.

Analysis of Urine and Feces from Rats Treated with Imidan-C^{14}. The solvent-partitioning characteristics of Imidan and its potential metabolite standards were studied by extracting fortified rat urine samples (pH 6.2 to 6.6) several times with equal volumes of benzene. The compound distribution was determined either by paper chromatography or by measurement of radioactivity in cases where the standard was available in radioactive form. Imidan, Imidoxon, phthalimide (PI), and *N*-hydroxymethyl phthalimide (HMPI) partitioned entirely into benzene, while PA, PAA, 3OH-PA, and 4OH-PA remained in the aqueous phase.

The urine and feces samples analyzed were from rats treated at an average oral dose of 27 mg. per kg. (Ford *et al.*, 1966). The feces had been homogenized with 50% aqueous methanol and the liquid phase recovered by centrifugation for analysis. Urinary and fecal samples were extracted three times with equal volumes of benzene and the two phases were separated by centrifugation. The benzene extracts generally were prepared soon after the samples were collected, to minimize loss of Imidan and any benzene-soluble metabolites through hydrolysis. Because of the low level of benzene-soluble radioactivity, the benzene extracts of the samples from the male and female rats were composited at each analysis interval and then reduced to small volumes for subsequent chromatography. Aliquots of the remaining aqueous phases from extraction of urine were analyzed separately by sex and were spotted for chromatography without altering the original volumes. The aqueous fecal extracts were handled in the same fashion as the urinary samples, except that they were reduced in volume by lyophilization.

The radioactive urinary and fecal metabolites of Imidan-C^{14} were hydrolyzed in acid to determine whether they could be reduced to a common degradation product. Aliquots of composited male and female rat urine collected after 12 and 24 hours and the benzene-soluble fractions from these samples were hydrolyzed in 2*N* HCl for 4 hours under reflux. No radioactivity was lost in this hydrolysis procedure, as determined by comparative radioassays of pre- and posthydrolyzed samples. The hydrolyzed samples were chromatographically analyzed along with their nonhydrolyzed counterparts and appropriate standards.

In order to quantify the radioactive Imidan-C^{14} metabolites, paper chromatograms were cut into radioactive and nonradioactive areas, using as a guide the autoradiograms and nonradioactive standards incorporated for co-chromatography. With TLC plates, radioactive and nonradioactive gel regions were scraped free of the glass support. The sectioned areas or powdered gel were then placed in vials for radioassay by liquid scintillation counting. The counting solution used was that described by Ford *et al.* (1966).

Radioactive samples of PA and PAA were given orally to rats, at a dose approximating by weight that used in the experiment with Imidan, and the animals were housed in metabolism chambers as described by Ford *et al.* (1966). About 2.5 mg. of PA-C^{14} or PAA-C^{14} were administered separately in 0.5 ml. of water by oral intubation to each of two female rats of the Long-Evans strain. A 2-ml. aliquot of the 0- to 24-hour urine was extracted twice with 5 ml. of benzene. No radioactivity was recovered in the benzene, as expected because of the partitioning properties of PA and PAA. Only aliquots from the aqueous phase were chromatographed, using the EtOH paper chromatographic system. Comparison was made with an aliquot of the 6- to 12-hour urine collection from an Imidan-C^{14}–treated rat. Appropriate controls were prepared by fortifying and chromatographing urine samples from an untreated rat with PA-C^{14} and PAA-C^{14}. In addition, PA-C^{14} and PAA-C^{14} standards were chromato-

Table II. Chromatographic Systems and Their Uses

Name	Specification	Use
E/G paper chromatography system	Whatman No. 1 paper coated with stationary phase of 12% glutaronitrile in acetone; moving phase of isopropyl ether saturated with glutaronitrile	Separates Imidan, Imidoxon, PI, and HMPI
EtOH paper chromatography system	Whatman No. 1 paper and absolute ethanol-water–concentrated NH_4OH (16:3:1)	Separates PA, PAA, and 4OH-PA, but fails to resolve PA and 3OH-PA
PAW paper chromatography system	Whatman No. 1 paper and pyridine–*n*-amyl alcohol–water (7:7:6) (Schumacher *et al.*, 1965a)	Excellent resolution of both benzene- and water-soluble metabolites of Imidan-C^{14} from rats as well as of PA and PAA
BAW paper chromatography system	Whatman No. 1 paper and 1-butanol–glacial acetic acid–water (2:1:1)	Resolves PA from insect tissue hydrolyzates
BAW TLC system	Glass plates coated with 0.25-mm. layer of silica gel G slurried in 0.1*M* boric acid; developed with 1-butanol–glacial acetic acid–water (2:1:1)	Separates PA and 3OH-PA but fails to resolve PA and 4OH-PA
EB TLC system	Silica gel G precoated TLC plates (Brinkmann Instruments, Westbury, N.Y.); developed with ethyl acetate–benzene (9:1)	Separates Imidan, Imidoxon, and other metabolites from microsome-$NADPH_2$ in vitro system

graphed in the absence of urine. Radioactivity on the chromatograms was detected using a Nuclear-Chicago Actigraph II strip scanner.

Studies with Rat Liver Microsomes. Rat liver microsomes were prepared according to the procedure of Gram and Fouts (1966), using the 9000 × G supernatant fraction. One-milliliter aliquots were incubated with 150 μg. of Imidan-C^{14}, with or without the addition of 2.2 μmoles of $NADPH_2$ (Calbiochem, Los Angeles, Calif.), for 50 minutes at 37° C. in a Dubnoff metabolic shaker. A portion of the ether extract of each reaction mixture was co-chromatographed with standard Imidan and Imidoxon in the EB TLC system.

Analysis of German Cockroaches Treated with Imidan-C^{14}. Anesthetized nymphs of *Blattella germanica* L., weighing 40 to 50 mg. each, were topically treated on the dorsum with 1.5 μg. of Imidan-C^{14} in 1 μl. of acetone per cockroach. The treated cockroaches were placed in groups of 30 in 100-ml. metabolism chambers and held at room temperature without food or water. Carbon dioxide, collected by passing the air exhausted from the chamber through a 5% NaOH scrubbing solution, was precipitated with 10% $BaCl_2$ as $BaCO_3$. The precipitate was washed with acetone, followed by absolute methanol, dried, and counted, using a gas flow counter with a Mylar window.

Individual groups of cockroaches were sacrificed for analyses at 0, 2, and 72 hours after treatment. An initial rinse with acetone removed external radioactivity. Acetone-soluble absorbed materials were recovered by homogenizing three times with fresh portions of acetone, centrifugation, and clean-up on 3-mm. thickness Whatman filter paper with acetonitrile according to the procedure of Menn *et al.* (1960). Purified extracts, further concentrated to a final volume of 0.1 ml., were subjected to paper chromatography using the E/G and EtOH systems. Radioactivity on the chromatograms was detected with a strip scanner and by autoradiography.

The acetone-insoluble residue was pooled and hydrolyzed by refluxing in 6*N* HCl for about 12 hours. The hydrolyzate was filtered free from the unhydrolyzable particulate fraction (cuticle), neutralized with NaOH, and reduced to dryness under vacuum. The residue was washed with several portions of absolute ethanol followed by pyridine [these solvents selectively extract amino acids and sugars in that order (Long, 1961)]. Aliquots of these extracts were chromatographed in the BAW paper chromatographic system and PA was added as a co-chromatographic standard was detected as a yellow spot on a blue background following spraying with 0.04% bromphenol blue in ethanol.

RESULTS

Identity of Imidan-C^{14} Metabolites in Rat Urine. The aqueous fraction remaining after extraction of the urine with benzene contains 98.9% of the labeled Imidan-C^{14} metabolites excreted in urine. No Imidan, Imidoxon, PI, or HMPI appears in this fraction at any time interval after Imidan-C^{14} administration, but instead only more polar products are found (Figure 1); this is expected because partitioning of fortified urine samples with benzene results in complete extraction of each of these products from the aqueous phase into the benzene phase. The benzene extract (1.1% of Imidan-C^{14} metabolites excreted in urine) contains little, if any, Imidan or Imidoxon, but consists largely of more polar products (Figure 1), except for a trace of compound in the chromatographic position of PI in the 12- to 24-hour sample. Quantitative results on the benzene extract more clearly show the trace levels of nonpolar metabolites, moving free of the original in the E/G paper chromatographic system and ranging from a low of 0.014% to a high of 0.062% of the administered dose for Imidoxon and PI, respectively (Table III). Even these values may be misleading, because streaking occurs, counting levels of about twice background were involved, and the products were not resolved into distinct spots. Since Imidan, Imidoxon, PI, and HMPI partition quantitatively into benzene from urine, the per cent values given in Table III can be considered as maximum. Most of the metabolites in the benzene extract remain at the origin, indicating their relatively polar nature.

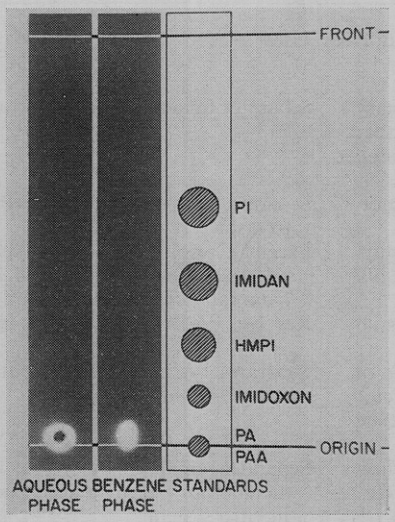

Figure 1. Chromatographic behavior of 0- to 48-hour composite samples of water- and benzene-soluble rat urinary metabolites of Imidan-C^{14} and standard Imidan analogs and degradation products

Separated in E/G paper chromatographic system and detected by autoradiography or chromogenic reagents

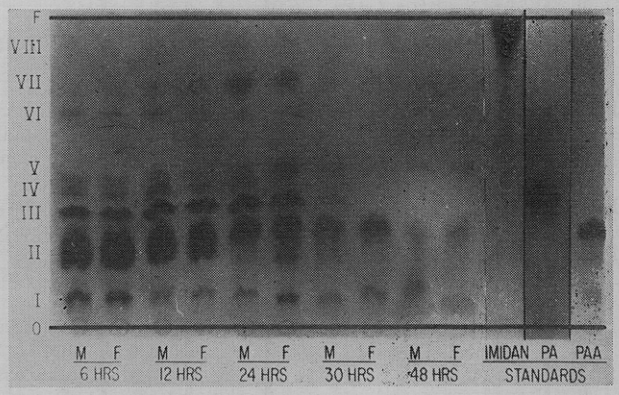

Figure 2. Chromatographic behavior of water-soluble rat urinary metabolites of Imidan-C^{14}

Separated in PAW paper chromatographic system and detected by autoradiography

Chromatographic systems other than the E/G paper chromatographic system considered above were used to obtain better resolution of the metabolites in the aqueous fraction of urine from Imidan-C^{14}–treated rats. The EtOH paper chromatographic system revealed one major product in the chromatographic position of PAA (R_f = 0.5) and this product in the 12- and 30-hour interval urine co-chromatographed with PAA. This metabolite is present in equal amounts in urine of both male and female rats. The PAW paper chromatographic system provided the best resolution of metabolites in the aqueous fraction; eight metabolites (I through VIII) were resolved and other regions lacking resolved metabolites were designated as A, B, and C (Figure 2 and Table IV). Fewer metabolites are detected in the autoradiograms for the 30- and 48-hour intervals because of the low total radiocarbon levels. The major product (II) is PAA, because this product in the 12- and 30-hour urine samples from males and females co-chromatographs with authentic PAA-C^{14}. [Occasionally the latter is not resolved as a discrete spot (24-hour interval, Figure 2), possibly because of formation of salts with pyridine. Chamberlain (1965) encountered similar problems with aromatic acid metabolites of Imidan in steer urine.] The second metabolite of importance (III) is PA because it similarly co-chromatographs with authentic PA-C^{14}. The third product of importance (I) appears to be a PA derivative; it possibly is a salt form of PA, because a labeled material with the R_f of compound I appears as a minor product even when authentic PA-C^{14} alone is chromatographed in the PAW system. The five remaining metabolites (IV to VIII) are not identified.

The Imidan-C^{14} metabolites from urine that are recovered on benzene extraction are chromatographically the same as metabolites VI, VII, and VIII which are present in the aqueous fraction (Figure 3). Because of limitations in radiocarbon levels, this comparison was made only for the 24-hour urine sample. The partitioning ratio of the mixture of metabolites VI to VIII is about 4 to 1 between urine (pH 6.5) and benzene, indicating their relative polar nature in confirmation of the finding that these metabolites do not migrate in the E/G paper chromatographic system.

Acid hydrolysis of the Imidan-C^{14} metabolites in both the benzene extract and aqueous fraction of urine converts a significant portion of the total products to PA (III, Figure 3). On re-examination of the hydrolysis products in paper and TLC systems adequate for separation of PA,3OH-PA, and 4OH-PA, it is evident that only trace amounts, if any, of the hydroxylated materials are present, because radioactive regions of appropriate R_f were not detected on autoradiography. Quantitative consideration of these results (Table V) showed that 3OH-PA in the hydrolyzed aqueous fraction accounts for not more than 1.2% of the administered radiocarbon and in the hydrolyzed benzene extract for not more than 0.02% of the administered radiocarbon, while 4OH-PA accounts for no more than 2.5 and 0.05% in the aqueous fraction and benzene ex-

Table III. Nature and Amounts of Imidan-C^{14} Metabolites in the Benzene Extract of Urine from Male and Female Rats as Separated by the E/G Paper Chromatographic System

Area of Chromatogram	Description	% of Administered Radiocarbon at Indicated Interval after Treatment, Hr.[a]				
		0–6	6–12	12–24	24–30	Total
1	Origin	0.189	0.237	0.251	0.015	0.692
2	Imidoxon[b]	0.004	0.004	0.005	0.001	0.014
3	HMPI	0.013	0.014	0.014	0.002	0.043
4	Imidan[b]	0.003	0.004	0.017	0.002	0.026
5	PI	0.001	0.001	0.051	0.009	0.062
6	Front	...	...	0.002	0.001	0.003
	Total	0.21	0.26	0.34	0.03	0.840

[a] An additional 0.01% of administered radiocarbon appears in this fraction for 30–48-hour interval. Level too low for satisfactory chromatographic analysis of components.
[b] Identification based on co-chromatography.

Table IV. Nature and Amounts of Imidan-C^{14} Metabolites in the Aqueous Fraction of Urine from Male and Female Rats as Separated by the PAW Paper Chromatographic System[a]

Metabolite Code	Description	% of Administered Radiocarbon at Indicated Interval after Treatment, Hr. 0–6	6–12	12–24	24–30	30–48	Total
A	Origin	0.20	0.25	0.33	0.12	0.10	1.0
I	PA derivative	1.62	1.50	1.95	0.72	0.62	6.41
II	PAA	9.32	15.51	12.23	2.75	0.93	40.74
III	PA	1.73	5.81	6.78	0.17	0.06	14.55
IV and V	Unknown	1.06	2.00	2.00	0.23	0.20	5.49
B	Area between V and VI	0.12	0.30	0.17	0.02	0.01	0.62
VI	Unknown	0.32	0.56	0.33	0.02	0.02	1.25
VII	Unknown	0.20	0.72	1.72	0.20	0.16	3.00
VIII	Unknown	0.08	0.22	0.72	0.06	0.04	1.12
C	Area between VIII and front	0.02	0.11	0.22	0.10	0.04	0.49
Remainder of chromatogram	...	0.36	0.78	1.33	0.41	0.52	3.40
Total		15.03	27.76	27.78	4.80	2.70	78.07

[a] Chromatogram illustrated in Figure 2. Results of male and female rats were consistently similar and so are averaged for tabulation.

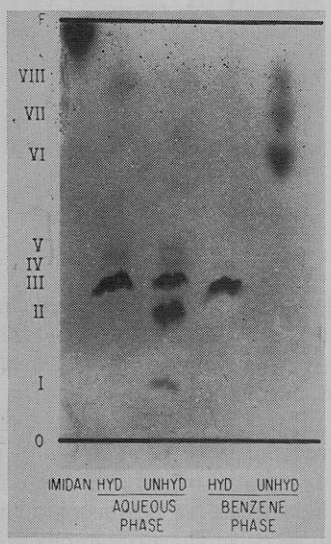

Figure 3. Chromatographic behavior of water- and benzene-soluble rat urinary metabolites of Imidan-C^{14} for 12- to 24-hour interval, pre- and postacid hydrolysis

Separated in PAW paper chromatographic system and detected by autoradiography. Area at left (Imidan) result of fortifying urine with authentic labeled compound and chromatographing benzene phase resulting from extraction of this urine

tract, respectively. These are maximal values for the hydroxylated PA derivatives, because some or all of the measured radiocarbon may possibly represent other metabolites that were not hydrolyzed and appear in these regions. Some streaking of radioactivity was also evident on the autoradiogram, thus contributing to the values assigned to these metabolites.

Table V. Nature and Amounts of Imidan-C^{14} Derivatives in Hydrolyzates of Aqueous Fraction and Benzene Extract of Urine from Male and Female Rats for the 0- to 30-Hour Interval after Treatment

Compound	R_f	% of Administered Radiocarbon: Aqueous fraction	Benzene extract
EtOH Paper Chromatographic System			
PA + 3OH-PA	0.17	73.87	0.73
4OH-PA	0.5	2.50	0.05
Unknown	Remainder of chromatogram	1.56	0.06
Total		77.93	0.84
BAW TLC System			
PA + 4OH-PA	0.83	72.3	0.80
3OH-PA	0.40	1.2	0.02
Unknown	Remainder of chromatogram	4.5	0.02
Total		78.0	0.84

Identity of PA-C^{14} and PAA-C^{14} Metabolites in Rat Urine. PAA-C^{14} and PA-C^{14} are largely excreted in the urine without chemical modification (Figure 4). A minor peak near the origin is evident following PAA-C^{14} administration, probably PA-C^{14} derived from either in vivo metabolism or hydrolysis of PAA-C^{14} in the urine. The major Imidan-C^{14} metabolite co-chromatographs with the PAA-C^{14} product and not with the PA-C^{14} product. Further evidence that PA-C^{14} is excreted without modification is provided by acid hydrolysis of the PA-C^{14} product, in which case the hydrolyzate co-chromatographs with PA-C^{14} in both the EtOH and PAW paper chromatographic systems.

Identity of Imidan-C^{14} Metabolites in Rat Feces. Imidan-C^{14} metabolites in the aqueous phase derived from feces 30 hours after dosing account for 11.4% of the

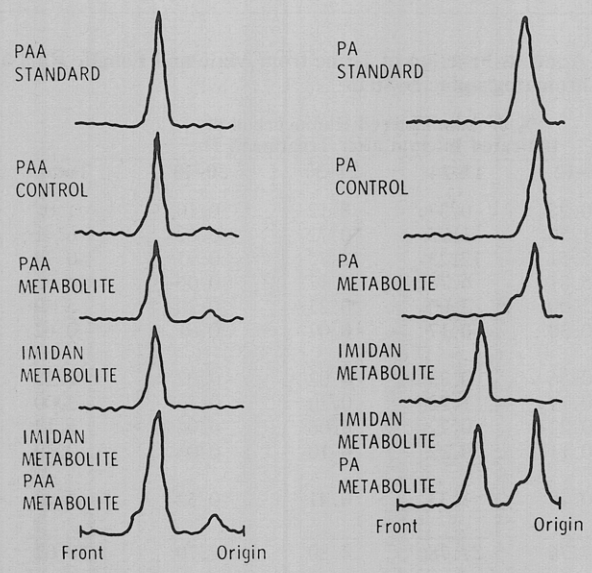

Figure 4. Chromatographic behavior of rat urinary metabolites of PA-C^{14} and PAA-C^{14}

Separated in EtOH paper chromatographic system and detected by radioscanning. Lowest scan in each case is co-chromatogram of major Imidan-C^{14} metabolites and product from PAA-C^{14} or PA-C^{14} administration

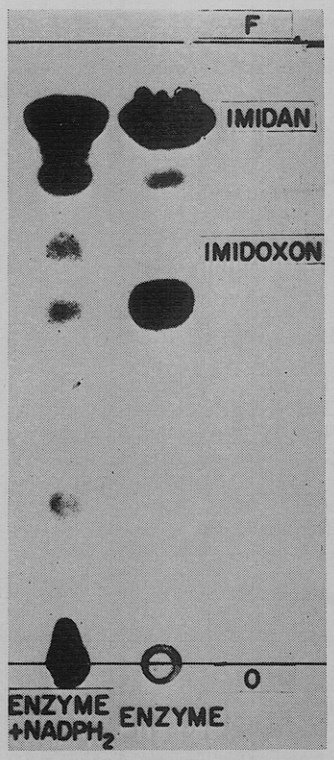

Figure 5. Autoradiogram showing Imidoxon-C^{14} formed on incubation of Imidan-C^{14} with rat liver microsome–$NADPH_2$ enzyme preparation

Separated by EB TLC system

Imidan-C^{14} administered to male and female rats and are entirely retained at the origin in the E/G paper chromatographic system, suggesting a similarity to the metabolites in the aqueous phase of urine. Using the EtOH and PAW paper chromatography systems, the major feces metabolite in the aqueous phase chromatographs, with streaking, in the position of PAA-C^{14} and, on hydrolysis, essentially all labeled metabolites are converted to PA-C^{14}.

Benzene extraction of feces through 30 hours after dosing recovers 0.48% of the radiocarbon administered as Imidan-C^{14} to male and female rats. Chromatography of this extract in the E/G paper chromatography system separates the radioactivity into the following metabolites: origin (polar metabolites) 0.22%; Imidoxon and Imidan 0.02 and 0.17%, respectively (identification based on co-chromatography); plus unidentified compounds making up the remainder of the chromatogram 0.07%.

In Vitro Conversion of Imidan-C^{14} to Imidoxon-C^{14}. A product which co-chromatographs with Imidoxon is formed from Imidan on incubation with the rat liver microsome system in the presence but not in the absence of $NADPH_2$ fortification (Figure 5). Cofactor fortification also greatly increases the amount of four other metabolites and of products remaining at the origin on chromatography. The four products moving free of the origin are not PAA, PA, 3OH-PA, or 4OH-PA, because these compounds remain at the origin in the EB TLC system.

Identity of Imidan-C^{14} Metabolites in German Cockroach. Only Imidan-C^{14} is recovered by acetone extraction of German cockroaches immediately after topical application with Imidan-C^{14}. At 2 hours, an additional product with the chromatographic behavior of Imidoxon (R_f — 0.20) in the E/G paper chromatography system is apparent (Figure 6) and this product co-chromatographs with unlabeled Imidoxon. The appearance of Imidoxon was associated with an increase in the proportion of intoxicated and moribund cockroaches. At 72 hours, the amount of Imidan-C^{14} decreases, Imidoxon is no longer detected, and more polar products appear at the origin. This material at the origin in the E/G paper chromatographic system has the chromatographic behavior of PAA in the EtOH paper chromatography system. Only a trace (less than 0.01%) of CO_2 is recovered as $BaC^{14}O_3$. The proportion of radiocarbon not removed from the tissues with repeated acetone extraction increases with time, to a level of 61% of the total internal radiocarbon at 72 hours. This labeled material is partially soluble in pyridine and ethanol and, on hydrolysis, is converted to a single radioactive material which co-chromatographs with authentic PA.

DISCUSSION

Imidan is metabolized in vivo primarily via hydrolytic pathways. In this respect it resembles the metabolism of many other organophosphorus insecticides (O'Brien, 1967).

The Imidan-C^{14} metabolites excreted in urine of male and female rats are in the following amounts as per cent of the administered dose: PAA 40.7, PA 21.0, five unidentified compounds (IV to VIII, Figure 2 and Table IV) 10.9,

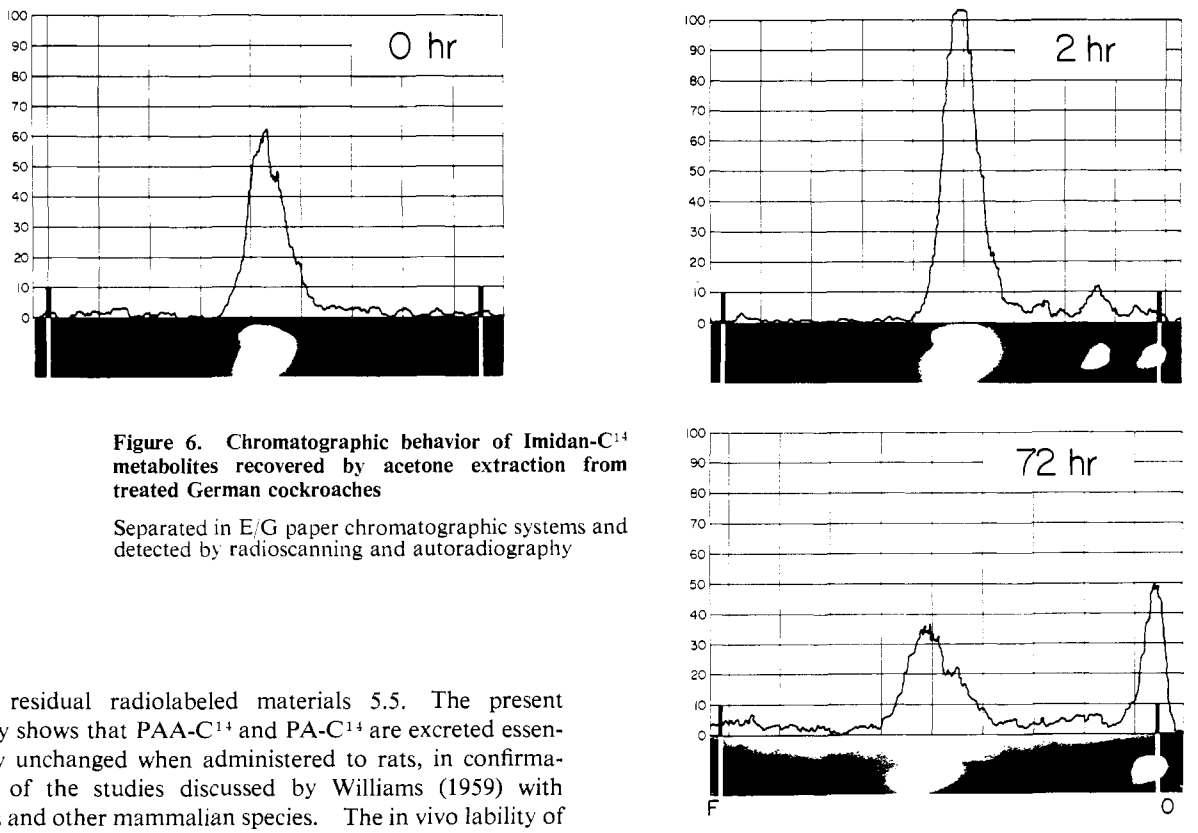

Figure 6. Chromatographic behavior of Imidan-C^{14} metabolites recovered by acetone extraction from treated German cockroaches

Separated in E/G paper chromatographic systems and detected by radioscanning and autoradiography

and residual radiolabeled materials 5.5. The present study shows that PAA-C^{14} and PA-C^{14} are excreted essentially unchanged when administered to rats, in confirmation of the studies discussed by Williams (1959) with dogs and other mammalian species. The in vivo lability of Imidan to spontaneous or enzymatic hydrolysis is further demonstrated by the finding that the major, radioactive metabolite in aqueous rat fecal extracts is PAA, suggesting that Imidan is, at least partially, decomposed before it is absorbed from the gastrointestinal tract. Although the identity of the Imidan-C^{14} metabolites (IV to VIII) is not known, they are converted to PA by acid hydrolysis, suggesting that the phthaloyl moiety is not modified in their formation. Thalidomide (α-phthalimidoglutarimide) is metabolized and excreted in human, rabbit, and rat urine in the form of two major metabolites, one of which is believed to be a derivative of 3-hydroxyphthalic acid (Smith *et al.*, 1962). In Imidan, ring hydroxylation is either not involved or is of minor importance because the maximal value for 3OH-PA plus 4OH-PA in hydrolyzates of rat urine is 3.8% of the administered radiocarbon and no discrete radioactive spots corresponding to 3OH-PA and/or 4OH-PA are detected on chromatograms. The large amounts of PAA-C^{14} and PA-C^{14} in the urine of Imidan-C^{14}–treated rats and their appearance soon after treatment also suggest that detoxication in the rat is achieved primarily via hydrolysis with minor involvement.

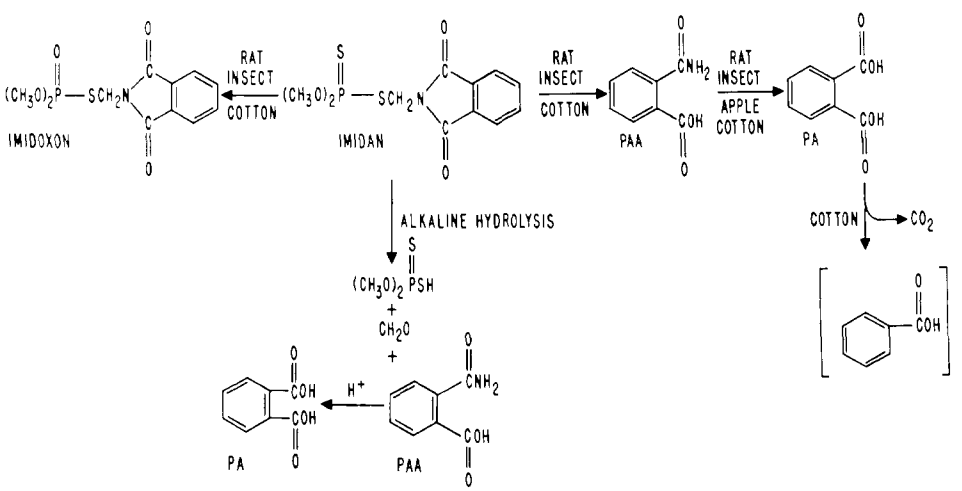

Figure 7. Proposed pathways of Imidan degradation in mammals (rat), insects (cockroach), plants [apple (Batchelder and Wise, 1963) and cotton], and in alkaline solution

if any, of ring hydroxylation prior to hydrolysis. Schumacher *et al.* (1965b) find small amounts of radioactive 3OH-PA and 4OH-PA in the urine of rabbits dosed with thalidomide-C^{14}, but not in the urine of rabbits dosed with hydrolysis products of thalidomide containing the phthaloyl moiety. This suggests that ring hydroxylation of thalidomide occurs in the parent drug itself.

In plants, CO_2 evolved from the carbonyl carbon of the phthaloyl moiety is recovered as a metabolite (Menn and McBain, 1964). The trace level of $C^{14}O_2$ recovered from German cockroaches possibly arises from microbial disruption of the phthaloyl moiety (Ford *et al.*, 1966). In all other respects the metabolic pathway of Imidan appears to be similar in cotton, the steer (Chamberlain, 1965), and German cockroach. In the latter, Imidoxon is demonstrated as a metabolite, while its occurrence in vivo in the rat, steer, and cotton is not established by direct analysis. The fly abdomen microsome–$NADPH_2$ system converts Imidan to Imidoxon (Tsukamoto and Casida, 1967) and this conversion also occurs in the rat liver microsome–$NADPH_2$ system.

Nonenzymatic hydrolysis of Imidan yields mainly PAA, PA, and *O,O*-dimethylphosphorodithioic acid (Menn *et al.*, 1965; Menn and Miskus, 1967).

The proposed pathway of Imidan metabolism and hydrolysis is given in Figure 7. Although the fate of the phosphorus-containing moiety of Imidan was not traced in vivo, it is likely that it is excreted to a large extent in the form of *O,O*-dimethylphosphorodithioic acid. This contention is based on the results of earlier studies with *O,O*-dimethyl *O*-(2,4,5-trichlorophenyl) phosphorothioate and *O,O*-dimethyl *S*-(*N*-methylcarbamoylmethyl) phosphorodithioate in which dimethylphosphorothioic and dimethylphosphorodithioic acids are identified as metabolites (Dauterman *et al.*, 1959; Plapp and Casida, 1958; Uchida *et al.*, 1964).

CONCLUSIONS

Imidan is readily metabolized in vivo to yield primarily PAA and PA. The latter is a naturally occurring substance (Cross *et al.*, 1963), is relatively nontoxic [acute oral LD_{50} in rats is 7500 to 8400 mg. per kg. (Spector, 1956)], and is readily metabolized and utilized by microorganisms (Ribbons and Evans, 1960). Consequently Imidan represents, by virtue of its rapid metabolism and elimination, a biodegradable pesticide which is not likely to leave toxic residues in the environment.

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