Metabolism of Furadan (NIA-10242) in Rats and Houseflies

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Furadan [(NIA-10242) 2,3-dihydro-2,2-dimethyl-7-benzofuranyl *N*-methylcarbamate] was metabolized by rats and houseflies by hydrolytic and oxidative mechanisms. Only a small amount of the hydrolytic product, 2,3-dihydro-2,2-dimethyl-7-hydroxybenzofuran, was detected in the free form, although it occurred in rather large quantities in the conjugated form. The major step in the oxidative metabolism of Furadan was the formation of 2,3-dihydro-2,2-dimethyl - 3 - hydroxybenzofuranyl - 7 - *N*-methylcarbamate. This material was either conjugated directly or metabolized to the 3-keto-carbamate derivative. The latter metabolite was hydrolyzed and the resulting hydrolytic product rapidly conjugated. Only that portion of the conjugated metabolite derived from the Furadan molecule was identified. However, four groups of intact conjugates were resolved by thin-layer chromatography.

Furadan [(NIA-10242) 2,3-dihydro-2,2-dimethyl-7-benzofuranyl *N*-methylcarbamate] has shown excellent insecticidal action when tested under field conditions. Not only is it effective against insects attacking a wide range of crops, but it is active as a contact insecticide when applied as a foliar spray or as a plant systemic when applied to the soil (Harris and Mazurek, 1966; Pass, 1966; Arthur and Guyer, 1967; Turnipseed, 1967). As with all new products of potential commercial importance, the fate of Furadan in various organisms must be evaluated. Such information aids in establishing the manner in which the product may be used safely and is essential to the development of analytical methods. In addition, a more thorough knowledge of the metabolic fate of this carbamate insecticide might be of value in predicting the occurrence of cross-resistance among populations of insects that are resistant to other insecticides.

PROCEDURES AND RESULTS

Chemicals. Furadan-carbonyl-C^{14} was synthesized with acetyl bromine-C^{14} (specific activity 2 mc. per mmole) as the labeled intermediate (Krishna *et al.*, 1962). The carbamate was isolated from the crude reaction mixture by thin-layer chromatography (TLC) on glass plates coated with silica gel G (1 mm. thick). A 2 to 1 ether-hexane mixture was used to develop the chromatograms, and radioautography was used to detect the radioactive materials. A 35% yield was obtained. Radio-assay of the final product revealed that the specific activity was 16,000 c.p.m. per μg. as determined by liquid scintillation counting. Furadan-ring-C^{14} (2,3-dihydro-2,2-dimethyl-7-benzofuranyl *N*-methylcarbamate-7a-C^{14}) was supplied by Niagara Chemical Division, FMC Corp., Middleport, N. Y. The specific activity was 0.07 mc. per mmole, yielding 200 c.p.m. per μg. by scintillation counting. A Packard Tri-Carb counter (Model 3365), adjusted for 70% counting efficiency, was used for all radioassays.

Several theoretical metabolites of Furadan were furnished by FMC Corp. and used in the identification of metabolites. Those compounds tentatively identified as metabolic products of Furadan are shown in Table I.

Metabolism by Rat Liver Enzymes. A 20% rat liver homogenate (w./v.) in 0.05*M* sodium phosphate buffer (pH 7.3) was prepared and separated into three fractions that were evaluated for their efficiency in metabolizing Furadan. One fraction was prepared by centrifuging the homogenate at 15,000 G for 30 minutes; the supernatant was used as the enzyme source. The other two fractions were prepared by centrifuging the 15,000 G supernatant at 105,000 G for 1 hour. The supernatant obtained after centrifugation at 105,000 G was used as an enzyme source, as was the particulate fraction (microsomes) after dispersal in a volume of phosphate buffer that made the suspension equivalent to the original 20% homogenate.

For incubation, 1.5 ml. of the enzyme source and 1.0 ml. of the pH 7.3 phosphate buffer containing 2 μmoles of $NADPH_2$ were added to a 25-ml. Erlenmeyer flask containing the labeled carbamate. After incubation at 37° C. for 2 hours, the contents of each flask were transferred to a 15-ml. centrifuge tube, and the flask was rinsed with 1 ml. of distilled water and 4 ml. of diethyl ether. The aqueous layer was extracted four times with 4-ml. portions of ether, and the ether extracts were combined and dried with anhydrous sodium sulfate. Aliquots from both the water and organic solvent phases were radioassayed. Ether extracts were evaporated to about 0.1 ml. and spotted on TLC plates (0.3 mm. thick). The plates were developed in a 3 to 1 ether-hexane mixture, exposed to x-ray film for 3 days, and the areas of the gel corresponding to darkened areas on the radioautogram were scraped into scintillation vials for counting.

The 15,000 G soluble fraction was much more efficient in metabolizing Furadan than either the 105,000 G soluble fraction or the microsomes. With the 15,000 G supernatant, 36.2% of the added carbamate was metabolized. Corresponding values for the 105,000 G soluble fraction and microsomes were 5.9 and 11.8%, respectively. The more efficient 15,000 G soluble fraction was selected for use in the future studies.

To determine the maximum quantity of Furadan metabolites that could be produced, the 15,000 G soluble fraction was incubated with concentrations of the carbamate rang-

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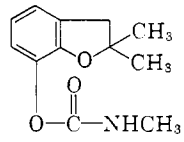
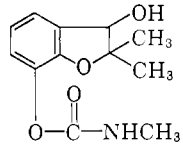
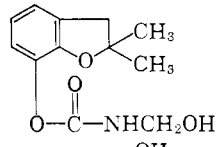
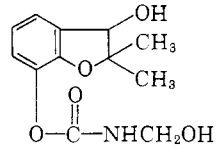
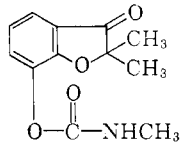
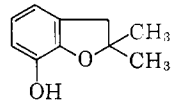
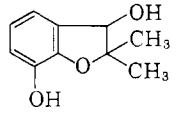
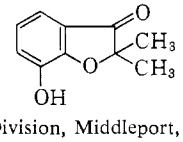
Table I. Chemical Identity of Furadan (NIA-10242) and Certain Analogs Considered as Possible Metabolites

Chemical Name	Structure	Designations Used in Text and Tables
2,3-Dihydro-2,2-dimethyl-7-benzofuranyl *N*-methylcarbamate		Furadan[a] Metabolite H
2,3-Dihydro-2,2-dimethyl-3-hydroxy-benzofuranyl-7-*N*-methylcarbamate		3-Hydroxy-Furadan[a] Metabolite E
2,3-Dihydro-2,2-dimethyl-7-benzofuranyl *N*-hydroxymethylcarbamate		*N*-Hydroxymethyl-Furadan Metabolite D
2,3-Dihydro-2,2-dimethyl-3-hydroxybenzo-furanyl-7-*N*-hydroxymethylcarbamate		3-Hydroxy-*N*-hydroxy-methyl Furadan Metabolite C
2,3-Hihydro-2,2-dimethyl-3-keto benzo-furanyl-7-*N*-methylcarbamate		3-Keto-Furadan[a] Metabolite G
2,3-Dihydro-2,2-dimethyl-7-hydroxy benzo-furan		Furadan-phenol[a] Metabolite K
2,3-Dihydro-2,2-dimethyl-3,7-dihydroxy benzofuran		3-Hydroxy-Furadan-phenol[a] Metabolite I
2,3-Dihydro-2,2-dimethyl-3-keto-7-hydroxy benzofuran		3-Keto-Furadan-phenol[a] Metabolite J

[a] Authentic samples supplied by FMC Corp., Niagara Chemical Division, Middleport, N. Y.

ing from 50 to 1000 μg. Increasing the Furadan level resulted in a corresponding decrease in total metabolism. When 50 μg. of Furadan was used, 53.3% of the carbamate was metabolized; however, at the highest level, 1000 μg., only 13.7% of the insecticide was altered by the 15,000 G soluble fraction. However, this higher concentration was used in tests designed to isolate large quantities of organo-extractable metabolites since, on a microgram basis, the metabolites were present in greater amounts.

The rate of Furadan metabolite formation was investigated by incubating the insecticide with the 15,000 G soluble fraction for various intervals up to 5 hours. For these studies, 100 μg. of Furadan-carbonyl-C^{14} were added to each flask. A incubation time of only 20 minutes resulted in approximately the same degree of metabolism as occurred during the 2-hour period used in all previous tests. In fact, increasing the incubation time to 5 hours yielded only a slightly greater concentration of metabolites. The consistency of the relative abundance of the metabolites in mixtures incubated from 20 minutes up to 5 hours indicated that, once formed, the metabolites were quite stable.

In the above experiments the organo-extractable metabolites were combined and quantitated so that the conditions for maximum metabolism might be determined. To evaluate the nature and quantity of individual metabolites, both Furadan-carbonyl-C^{14} and Furadan-ring-C^{14} were incubated with the 15,000 G soluble fraction from the rat liver homogenate. One hundred micrograms of the carbamate were added to each flask and incubated at 37° C. for 1 hour.

Analysis of the organic extract with TLC using a 3 to 1 ether-hexane mixture indicated that eight radioactive areas were obtained with Furadan-carbonyl-C^{14}, and 11 with Furadan-ring-C^{14} (Table II). Approximately 60% of the added Furadan was unchanged after incubation,

Table II. Comparative Metabolism of Furadan-Carbonyl-C^{14} and Furadan-Ring-C^{14} by the 15,000 G Solubles from Rat Liver

	Metabolites	R_f[a]	% of Added Radioactivity: Carbonyl-C^{14}	Ring-C^{14}
Organo-extractables				
A	Unknown I	0.00	0.07	0.10
B	Unknown II	0.07	0.11	0.09
C	3-OH-N-CH_2OH-Furadan	0.19	1.76	2.07
D	N-CH_2OH-Furadan	0.33	6.40	7.05
E	3-OH-Furadan	0.37	21.09	20.13
F	Unknown III	0.40	0.05	0.03
G	3-Keto-Furadan	0.48	0.28	0.22
H	Furadan	0.54	60.29	58.50
I	3-OH-Furadan-phenol	0.61	0	0.02
J	3-Keto-Furadan-phenol	0.86	0	0.02
K	Furadan phenol	0.90	0	3.82
	Water-solubles		5.89	7.01
	Total recovery		95.94	99.06

[a] Silica gel G chromatograms developed in 3:1 ether-hexane.

and most of the metabolites were present only in very small quantities with the exception of metabolites D and E, which accounted for 7 and 20% of the original radioactivity, respectively.

Stability of Furadan Metabolites on Silica Gel G. For experiments designed to establish the identity and biological activity of individual metabolites, it was necessary to determine the stability of the products on the silica gel plates and the efficiency of the procedure used to extract them from the gel.

To determine the stability of the metabolites on silica gel, a developed chromatogram of Furadan-ring-C^{14} metabolites was exposed to x-ray film at room temperature for 2 days, and then developed in a second dimension using the same solvent system. That the metabolites were stable on silica gel under these conditions for 2 days was indicated by the fact that only the original radioactive areas were observed on the radio-autogram after the second development. With an exposure period of more than 3 days at room temperature, Metabolite E was degraded slightly to a product having a 0.0 R_f value. Exposure of the chromatogram to film for 2 weeks in a deep freeze prevented any degradation of metabolites. Therefore, all subsequent chromatograms were placed in a deep freeze for exposure to the x-ray film.

Recovery of the major organo-extractable metabolites of Furadan (C, D, E, and H) from the silica gel was effectively accomplished by extraction with either acetone, chloroform or ether. Over 95% of these materials were extracted from the gel and could be rechromatographed without chemical change. Metabolite K was the only material that was rapidly lost during normal evaporation procedures. It was essential that the solvent was not evaporated completely to dryness when dealing with this metabolite.

Characteristics of Individual Furadan Metabolites Formed by Rat Liver Enzymes. The chemical nature of Furadan metabolites formed by the 15,000 G soluble fraction of rat liver homogenates was investigated as follows:

Chemical Degradation. The carbamate metabolites were hydrolyzed by incubation in 5 ml. of 0.1*N* sodium hydroxide for 30 minutes at room temperature. Complete hydrolysis was assured when all of the radioactivity was released as $C^{14}O_2$ when using Furadan-carbonyl-C^{14} metabolites. Furadan-ring-C^{14} metabolites were used to determine possible modification of the ring structure. To recover the hydrolyzed ring fragment from the aqueous mixture, the solution was acidified with 0.5*N* HCl and extracted with chloroform. The hydrolytic products were subjected to different tests as indicated below. Certain of the more abundant organo-extractable metabolites also were hydrolyzed in sulfuric acid and the liberation of formaldehyde was determined with a chromotropic acid reagent (Frisell *et al.*, 1954).

Cochromatography. Approximately 50 μg. of each available standard (Table I) were added to a mixture of the organo-extractable metabolites from the 15,000 G soluble fraction. The mixture was applied to TLC and developed first in 3 to 1 ether-hexane, and then in a second dimension with a 4 to 1 mixture of methylene chloride and acetonitrile. A 1% aqueous solution of potassium permanganate was sprayed on the plates to detect the nonlabeled standards, and the radioactive areas were located by radioautography. If cochromatography of one of the standards with a labeled metabolite was indicated, the metabolite was isolated in sufficient quantity, when possible, to allow chromatography with the standard in each of the following two-dimensional solvent systems: 5:1 ether-hexane, 2:1 methylene chloride–acetonitrile, 2:1 ether-benzene, 9:1 dioxane-methanol, and 5:1 ethyl acetate–methanol, 1:1 dioxane-hexane. Chromatographic behavior was considered to be identical when the metabolite and authentic compound cochromatographed in all of the solvent systems.

Infrared Analysis. Only Metabolite E was obtained in great enough quantity for IR analysis. (Beckman IR-8A, Beckman Instrument Inc., Fullerton, Calif.). Based on these criteria, the following conclusions were drawn concerning the identity of the Furadan metabolites.

Metabolite A. This metabolite appeared in only trace amounts, but was detectable following incubation of both the carbonyl-C^{14}- and ring-C^{14}-labeled Furadan. Its chemical identity was not determined because of its minute quantity.

Metabolite B. This material also was present only in trace amounts, and attempts to isolate enough for characterization were unsuccessful. Since both labeled samples of Furadan yielded Metabolite B in a radioactive form, it is likely that the product has the basic carbamate structure intact.

Metabolite C. Metabolite C retained the carbamate moiety, and after hydrolysis, its ring-labeled hydrolytic product cochromatographed with 3-hydroxy-Furadan-phenol. Sulfuric acid hydrolysis of the metabolite resulted in a positive formaldehyde test. The metabolite was tentatively identified as 2,3-dihydro-2,2-dimethyl-3-hydroxybenzofuranyl-7-*N*-hydroxymethylcarbamate.

Metabolite D. When hydrolyzed in aqueous sodium hydroxide, the ring-labeled hydrolysis product of Metabolite D cochromatographed with an authentic sample of Furadan-phenol. This indicated that the ring was not modified and that some change of the carbamate moiety probably had occurred. The most obvious modification

would be the formation of the *N*-hydroxymethyl derivative of Furadan. Its designation as *N*-hydroxymethyl Furadan was further supported by the fact that formaldehyde was detected upon acid hydrolysis of the metabolite.

Metabolite E. The major metabolite formed by the rat liver enzyme system was Metabolite E. Hydrolysis of the metabolite yielded a product that cochromatographed with 3-hydroxy-Furadan-phenol. When the hydrolytic product was reacted with methylisocyanate, three carbamate compounds were formed. The chromatographic behavior of one of these products was identical to that of Metabolite E. Although not identified, one of the other materials may have been a dicarbamate and the other a monocarbamate with carbamylation occurring at the 3-hydroxy position. In addition, Metabolite E was chromatographically the same as 3-hydroxy-Furadan and the infrared spectrum of the metabolite was consistent with a spectrum obtained with this standard. Thus the evidence indicated that Metabolite E was 2,3-dihydro-2,2 - dimethyl - 3 - hydroxybenzofuranyl - 7 - *N* - methylcarbamate.

Metabolite F. A minor metabolite that was formed with both carbonyl-C^{14}- and ring-C^{14}-labeled Furadan.

Metabolite G. Very little work was done on the identification of this product because it was formed in small concentrations. However, this metabolite cochromatographed with 3-keto-Furadan when the chromatoplates were developed in 3 to 1 ether-hexane. The two materials also were similar in that they gave a blue fluorescence when viewed under ultraviolet light.

Metabolite H. The radioactive area on the TLC designated as metabolite H was identified as Furadan by cochromatography.

Metabolites I and J. These products were identified as 3-hydroxy-Furadan-phenol and 3-keto-Furadan-phenol, respectively, by cochromatography with the standards.

Metabolite K. This metabolite was identified as Furadan-phenol because it cochromatographed with the authentic compound, and reacted with methylisocyanate to yield Furadan.

Incubation of Metabolites with Rat Liver Enzymes. Tests were conducted to determine the sequence of metabolite formation. Ring-labeled metabolites C, D, E, and K, as well as the hydrolytic product of Metabolite E, were purified on TLC and incubated individually with the rat liver 15,000 G soluble fraction. An 80-μg. quantity (Furadan-C^{14} equivalents) of each material was added to the standard incubation mixture (Table II). Metabolite C was converted largely to water-soluble materials and to a small amount of a single organo-extractable product which remained at the origin on TLC. The manner in which Metabolite C and the hydrolysis product of Metabolite E (3-OH-Furadan-phenol or Metabolite I) were metabolized was almost identical. Based on the tentative identification of Metabolite C as 3-hydroxy-*N*-hydroxymethyl Furadan, this would be expected since upon hydrolysis of Metabolite C the two compounds would be the same. A small amount of a product that cochromatographed with a 3-keto-Furadan-phenol standard was formed from the hydrolysis product of Metabolite E.

Both Metabolites D and E were converted into Metabolites, A, C, hydrolysis product of E, and into water-soluble material. Metabolite K, identified as Furadan-phenol, was metabolized primarily to water-soluble materials by the rat liver enzyme system.

In Vivo Metabolism of Furadan in Rats. The in vivo fate of carbonyl-C^{14}- and ring-C^{14}-Furadan in laboratory rats was investigated. Animals weighing approximately 200 grams each were treated orally with the carbamate dissolved in 0.5 ml. of Tween 20. Rats treated with Furadan-carbonyl-C^{14} received a 0.4 mg. per kg. dose, and those treated with Furadan-ring-C^{14} received a 4.0 mg. per kg. dose. Symptoms of poisoning were evident in the rats treated at the higher level.

The treated rats were held in glass metabolism cages so that urine and feces could be collected separately. Carbon dioxide-C^{14} expired by rats treated with the carbonyl-C^{14} carbamate also was measured. Urine was analyzed in the same manner as described for the rat liver incubation studies, and the radioactive content of the feces was determined by oxygen combustion (Andrawes *et al.*, 1967).

The amount of $C^{14}O_2$ collected from the Furadan-carbonyl-C^{14} treated rats indicated that approximately 45% of the administered dose was hydrolyzed within 32 hours after treatment (Table IV). Hydrolysis of the carbamate was further indicated in that 92% of the ring-C^{14} dose was excreted in the urine while only 38% of the carbonyl-C^{14} dose was excreted by this route. Feces from both groups of rats contained approximately 4% of the radioactivity administered.

Table III. Metabolism of Certain Furadan-Ring-C^{14} Metabolites by Rat Liver 15,000 G Solubles

	Products Resulting from Metabolism	Furadan Metabolites Isolated and Re-incubated, % Conversion to Indicated Metabolite				
		C	D	E	E–H[a]	K
A	Unknown I	9.2	2.2	0.6	2.1	0.9
C	3-OH-NCH_2OH-Furadan	60.5	6.9	8.9	0	0
D	N-CH_2OH-Furadan	0	62.9	0	0	0
E	3-OH-Furadan	0	0	63.2	0	0
I	3-OH-Furadan-phenol	0	4.1	3.9	56.1	0
J	3-Keto-Furadan-phenol	0	0	0	6.0	0
K	Furadan-phenol	0	0	0	0	32.4
	Water-solubles	30.3	23.9	23.4	35.8	66.7

[a] E–H, the ring-labeled hydrolytic product of metabolite E.

Table IV. Elimination of Radioactivity from Rats Treated Orally with Carbonyl-C^{14}- and Ring-C^{14}-Furadan

Hours after Treatment	% of Administered Dose-Accumulative				
	Carbonyl-C^{14}			Ring-C^{14}	
	$C^{14}O_2$	Urine	Feces	Urine	Feces
2	5.6	2.7	0.0	5.9	0.3
4	20.2	15.3	0.4	15.0	0.5
6	30.8	24.6	0.8	21.4	0.5
12	40.0	29.9	1.0	37.8	0.9
24	43.4	36.8	1.9	72.2	2.3
32	44.6[a]	38.4[a]	2.6	87.7	2.4
48	44.6	38.4	3.8	89.0	2.5
56	44.6	38.4	4.4[a]	89.7	3.0
72	44.6	38.4	4.4	90.8	3.3[a]
96	44.6	38.4	4.4	91.4	3.3
120	44.6	38.4	4.4	91.6[a]	3.3
	Total	87.4%		94.9%	

[a] Last sample containing radioactivity.

Analysis of the organo-extractable metabolites from the rat urine (Table V) indicated that the in vivo metabolism of Furadan followed the same general pattern as observed in the in vitro rat liver enzyme studies. Unknown III (Table II) and 3-Hydroxy-Furadan-Phenol were the only metabolites that were detected in the rat urine.

Although adequate quantities of organo-extractable metabolites were formed for studies of their chemical nature, greater than 90% of the radioactivity in the urine of rats treated with Furadan-C^{14} could not be extracted with organic solvent (Table V). Since a metabolic pathway for Furadan in rats could not be established if these water-soluble materials were not characterized, attempts were made to cleave and identify the Furadan moiety of the conjugated metabolite.

Pooled rat urine, collected during a 72-hour period after oral administration of Furadan-ring-C^{14}, was acidified, boiled for 10 minutes, and then extracted with chloroform. The maximum conversion of water-soluble radioactivity to chloroform-extractable products (90 to 95%) was achieved when hydrochloric acid was added to the urine in a quantity sufficient to make a 0.5*N*-equivalent solution. Control tests demonstrated that Furadan and its carbamate metabolites were not hydrolyzed or changed in any manner when added to rat urine and treated similarly with acid. In addition, Furadan-phenol, 3-hydroxy-Furadan-phenol, and 3-keto-Furadan-phenol were not altered by the same treatment. Thus, if the water-soluble conjugates were cleaved by acid hydrolysis to yield the above Furadan-related products, they could be recovered, measured, and characterized without being altered chemically by the techniques employed.

Six metabolites of Furadan were cleaved from the water-soluble rat urine conjugates by acid hydrolysis (Table VI). All of these products had previously been identified as organo-soluble metabolites that were formed in vitro by rat liver enzymes or extracted from the urine. Two products, Unknown III and 3-keto-Furadan-phenol, were not detected per se in the rat urine but were present as water-soluble conjugates. Unknown III was detected only in trace amounts, but 3-keto-Furadan-phenol accounted for over 50% of the total water-soluble-C^{14} in the rat urine. Less than 10% of the original water-soluble metabolites in the urine remained in the aqueous layer after the acid treatment and chloroform extraction.

When samples of the urine from Furadan-C^{14} treated rats were concentrated and applied directly to TLC, the radioactivity was resolved into four components (Table VII). The relative quantity of each component in urine from rats given Furadan-ring-C^{14} and those that received Furadan-carbonyl-C^{14} was different although each component was detected in both samples of urine. This indicated that each group of conjugates was comprised of both hydrolytic and nonhydrolytic metabolites of Furadan. Most of the carbonyl-C^{14} metabolites were conjugated with a product(s) that moved as a single band (R_f 0.77, Table VII) on TLC. The Furadan-ring-C^{14} metabolites, representing both hydrolytic and nonhydrolytic products, were more evenly distributed among the four groups of conjugates.

Although a dose of Furadan-ring-C^{14} to rats could be recovered almost quantitatively within 3 to 4 days, it was of interest to determine the distribution of the labeled material during the first few hours after treatment. Rats weighing between 200 and 250 grams were given oral doses of Furadan-ring-C^{14} and then certain animals were sacrificed at 1, 2, 4, and 8 hours following treatment. Analysis of several body tissues by combustion (Andrawes *et al.*, 1967) showed that the liver contained more Furadan-equivalents (1.43 p.p.m. maximum on dry weight basis) than any of the other tissues tested (Table VIII). None of the other tissues had detectable residues in excess of 0.9 p.p.m.

Table V. Nature and Magnitude of Metabolites in Urine of Rats Treated Orally with Furadan-ring-C^{14}

	Metabolites	% of Total C^{14} in Urine/Hours Sample 2	4	6	24
A	Unknown I	1.01	1.73	1.35	1.33
B	Unknown II	0.08	0.09	0.16	0.17
C	3-OH-N-CH_2OH-Furadan	0.73	1.41	1.05	1.13
D	N-CH_2OH-Furadan	0.32	0.27	0.08	0.05
E	3-OH-Furadan	1.64	0.91	0.73	0.25
G	3-Keto-Furadan	0.03	0.02	0.02	0.01
H	Furadan	0.92	0.08	0.04	0.02
J	3-Keto-Furadan-phenol	0.91	0.44	0.23	0.04
K	Furadan-phenol	0.20	0.33	0.21	0.20
	Water-solubles	94.16	94.72	96.13	96.80

Table VI. Metabolites Recovered by Acid Hydrolysis of Water-Soluble-C^{14} Products in Urine of Rats Treated Orally with Furadan-ring-C^{14}

	Metabolites	% of Total C^{14}-Water-Solubles in Urine[a]
C	3-OH-N-CH_2OH-Furadan	4.02
E	3-OH-Furadan	14.78
F	Unknown III	0.04
I	3-OH-Furadan-phenol	1.43
J	3-Keto-Furadan-phenol	50.54
K	Furadan-phenol	21.12
	Water-solubles[b]	8.07

[a] Urine from rats collected during 72-hour period following oral dose of 4 mg./kg. Furadan-C^{14}.
[b] C^{14}-materials remaining in the aqueous layer after acid treatment and extraction with chloroform.

Table VII. Separation by TLC of Water-Soluble-C^{14} Metabolites in the Urine of Rats Treated Orally with Furadan

R_f on Silica Gel G TLC[a]	Distribution of Radioactivity, % Total C^{14}-Water-Solubles: Furadan-ring-C^{14}	Furadan-Carbonyl-C^{14}
0.43	11.3	2.6
0.55	15.5	8.2
0.68	27.6	8.8
0.77	45.5	80.3

[a] Silica gel G TLC developed in one dimension with a 5:3:1 mixture of ethylacetate–*n*-propanol–water.
[b] Urine from rats collected during 72-hour period following oral doses of 2.5 mg./kg. Furadan-C^{14}. In Furadan-ring-C^{14} treatment, water-soluble-C^{14} residues in urine equaled 91% of dose and in Furadan-carbonyl-C^{14} treatment 25% of dose was a water-soluble metabolite in the urine.

Table VIII. Distribution of Radioactivity in Rats Following Oral Treatment of Furadan-Carbonyl-C^{14} at 1.0 Mg./Kg. (Body Weight)

Tissue	P.P.M. (Dry Weight) Furadan-Carbonyl C^{14} Equivalents at Indicated Hours			
	1	2	4	8
Blood	0.47	0.84	0.23	0.30
Brain	0.30	0.15	0.11	0.09
Bone	0.08	0.09	0.06	0.06
Kidney	0.38	0.52	0.17	0.14
Liver	1.43	1.25	0.68	0.78
Leg muscle	0.19	0.22	0.07	0.06

Furadan Metabolism in Houseflies. Limited studies were conducted on the metabolism of Furadan-carbonyl-C^{14} in houseflies so that the metabolic pathway of this carbamate in mammals and insects could be compared. Since the approximate LD_{50} of Furadan to the susceptible flies used in these tests was 0.1 μg. per fly, it was impossible to use the ring-labeled material because of its low specific activity.

Adult houseflies, 6 days old, were treated topically with Furadan-carbonyl-C^{14} at a rate of 0.05 μg. per fly. This amounted to about 800 c.p.m. per insect and was adequate for metabolism studies since 100 flies were processed as a single replicate. A detailed examination of Furadan metabolism was conducted 1 hour after treatment of the flies.

Radioactivity remaining on the surface of the flies was removed by rinsing the insects with acetone. Internal radioactivity was extracted by homogenizing the treated flies with a 1 to 1 acetone-water mixture. The filtered extract was partitioned with chloroform, and the radioactivity separated into organo-extractable and water-soluble products depending on partitioning characteristics. Vials that held the treated flies were rinsed with acetone and water and the wash partitioned with chloroform. Water-soluble Furadan-C^{14} metabolites were hydrolyzed with acid and extracted with chloroform in the same manner as described for the rat urine water-soluble-C^{14} materials.

A uniform distribution of the topically applied Furadan-carbonyl-C^{14} was observed 1 hour after treatment of the flies (Table IX). Sufficient radioactive products were recovered from the fly surfaces, internal extracts, and from the holding vials to permit identification of the products. After 1 hour, approximately 83% of the applied dose was recovered in these three fractions, and 3% could not be extracted from insect solids. The remainder of the dose probably was lost as $C^{14}O_2$ as hydrolysis of the carbamate occurred.

Furadan metabolites in houseflies, identified and measured by the techniques mentioned earlier, are listed in Table IX. The parent compound was the major radioactive component of the organo-extractable fraction. The major metabolite in this fraction (6% of the applied dose) was 3-hydroxy-Furadan. This product was located primarily in the internal extracts of the flies. Other metabolites occurring in progressively lesser quantities were 3-keto-Furadan, 3-hydroxy-*N*-hydroxymethyl-Furadan, and Unknown I.

Carbamate metabolites recovered from the water-soluble-C^{14} conjugates were the same as the organo-soluble products except that 3-keto-Furadan was not detected as a conjugate. Again, 3-hydroxy-Furadan was the major metabolite recovered. Only 8% of the total water-soluble-C^{14} material formed in insects could not be converted into organo-extractable products by acid treatment.

Biological Activity of Furadan Metabolites. Three metabolites, 3-hydroxy-Furadan, 3-keto-Furadan, and 3-hydroxy-*N*-hydroxymethyl Furadan, were isolated from the in vitro rat liver incubation medium, and their anticholinesterase activity was estimated colorimetrically (Simpson *et al.*, 1964) with housefly heads as the enzyme source. The molar I_{50} values obtained were 3-hydroxy-Furadan, 2.1×10^{-1}; 3-keto-Furadan, $3.3. \times 10^{-7}$; and 3-hydroxy-*N*-hydroxymethyl-Furadan, 1.4×10^{-6}.

Table IX. Distribution and Nature of Radioactive Material in Houseflies One Hour after Topical Application of Furadan-Carbonyl-C^{14} (0.05 μg. per Fly)

Radioactive Materials as:	% of Applied Dose			
	Surface residues	Internal residues	Excreted residues	Total recovered
Total	23.9	42.4	17.0	83.3
Organo-extractables				
Furadan	22.9	12.3	7.1	42.3
3-OH-Furadan	0.3	5.7	0.4	6.4
3-OH-N-CH_2OH-Furadan	0.2	0.7	0.3	1.2
3-Keto-Furadan	0.2	1.2	2.0	3.4
Unknown I	0.3	0.6	0.4	1.2
Total	23.9	20.5	10.2	54.5
Carbamate water soluble conjugates				
3-OH-Furadan	0	11.4	4.3	15.7
3-OH-N-CH_2OH-Furadan	0	2.1	0.1	2.2
N-CH_2OH-Furadan	0	1.8	0.2	2.0
Unknown I	0	0.2	0.4	0.7
Water-solubles[a]	0	6.4	1.8	8.2
Total	0	21.9	6.8	28.8

[a] Those C^{14} materials which remained in the aqueous layer following acid hydrolysis of the conjugates and extraction with chloroform.

All of these materials were less active than Furadan which had an I_{50} of 3.3×10^{-8}.

DISCUSSION

Studies of the metabolism of carbamate insecticides have become increasingly complex as work on these compounds has progressed. In the early studies, investigators were satisfied with results that indicated products such as carbaryl underwent hydrolytic degradation yielding nontoxic materials. More detailed studies, made possible primarily by improved techniques, have recently shown that oxidation, hydroxylation, and conjugation are just as important as hydrolysis in the metabolism of the carbamate compounds (Dorough and Casida, 1964; Oonnithan and Casida, 1966; Metcalf *et al.*, 1966; Andrawes and Dorough, 1967). All of these types of reactions may play a part in the metabolism of a single carbamate. Although progress has been made, evidently a great deal more study, especially in definining mechanisms of formation of water-soluble carbamate metabolites, will be required before complete metabolic pathways may be presented.

The metabolism of Furadan in rats and houseflies was found in the current study to involve biochemical reactions similar to those reported for other aromatic carbamates. Hydroxylation of the molecule produced carbamate metabolites that still retained a certain degree of biological activity. These metabolites, as well as the parent compound, were altered by hydrolysis and by conjugation to form metabolites of a water-soluble nature. The carbamate metabolites, whether present in the free or conjugated form, must be considered carefully in the development of practical analytical methods of residue detection. Fortunately, their number is limited, their potential toxicity less than Furadan, and when present as conjugates, may be converted easily to the free form for detection and identification.

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