Furadan-C^{14} Metabolism in a Lactating Cow

G. Wayne Ivie and H. Wyman Dorough

Furadan (2,3-dihydro-2,2-dimethyl-7-benzofuranyl-N-methylcarbamate) administered orally to a cow was altered chemically by oxidation of the number 3 carbon and of the N-methyl group, hydrolysis of the ester linkage, and by conjugation of metabolites containing a hydroxyl group. Carbamate metabolites in the milk were the 3-hydroxy, 3-keto, and 3-hydroxy-N-hydroxymethyl derivatives of Furadan. These materials were present in both the free and conjugated forms. Conjugated 2,3-dihydro-2,2-dimethyl-3-keto-7-hydroxybenzofuran was the major hydrolytic product of Furadan in the milk. The same metabolites also were components of the cow urine and/or feces. Data indicate that of a single oral dose of Furadan fed to a cow, approximately 0.2% would be eliminated in the milk, 0.7% in the feces, and 94% in the urine.

Furadan, 2,3-dihydro-2,2-dimethyl-7-benzofuranyl-N-methylcarbamate, exhibits excellent insecticidal action both as a foliar spray and as a plant systemic (Broersma, 1967; Forsythe, 1966; Rawlins and Gonzalez, 1966). One area in which it has the greatest potential is in the control of the alfalfa weevil. In field tests designed to evaluate its pesticidal qualities, foliar applications of Furadan at rates as low as 0.25 pound per acre effectively controlled this insect pest (Pass, 1966). Furadan also demonstrated good alfalfa weevil control when applied to the soil in granular form (Armbrust and Gyrisco, 1966). Whether or not the compound can ever be used for controlling the alfalfa weevil will depend largely upon its fate in animals that may feed on the treated crop. This is especially true for dairy animals since alfalfa often constitutes a major portion of the diet and any consumed Furadan might possibly be passed along in the milk to humans. This study was conducted to determine the metabolic fate of orally administered Furadan in dairy animals and to gather information on the magnitude and chemical nature of residues in the milk.

METHODS AND MATERIALS

A lactating Jersey cow was placed in a metabolism stall several days before treatment and a rubber hose was cemented to the vulva so that the urine could be collected separately from the feces. For the Furadan-carbonyl-C^{14} study, an intravenous catheter was inserted into the animal's jugular vein for the withdrawal of blood as desired. The cow was provided alfalfa hay ad libitum and was fed crushed grain at the morning and evening milkings.

For treatment, the insecticide was dissolved in approximately 1 ml. of acetone and the solution added to a 1-ounce gelatin capsule containing crushed grain. The capsule was administered to the cow with the aid of a balling gun. Initially, the animal was treated with 222 mg. of Furadan-carbonyl-C^{14} (specific activity 2.7 mc. per mmole) which was equivalent to 0.52 mg. per kg. of body weight. Thirty-eight days later, the cow was given 429 mg. of Furadan-ring-C^{14} (specific activity 0.145 mc. per mmole), the dose being 1.0 mg. per kg. The amount of radioactivity, based on scintillation counting, received by the cow was 5×10^9 and 5×10^8 c.p.m., respectively, for the two treatments. After the Furadan studies had been completed, the cow was administered 3.0 mc. of sodium bicarbonate-C^{14} in the same manner as was the insecticide.

Milk, urine, and feces were collected following the three treatments until radioassay showed that the samples were free of radiolabeled residues. In addition, approximately 5 ml. of blood were taken at frequent intervals after the Furadan-carbonyl-C^{14} treatment. To prevent coagulation of the blood in the intravenous catheter, it was flushed with a heparin solution after collection of each sample.

The samples were assayed for radioactivity using a liquid scintillation spectrometer (Packard Tri-Carb Model 3365) adjusted to count at 80% efficiency. A 2 to 1 mixture of toluene and methoxyethanol containing 5.5 grams of 2,5-diphenyloxazole per liter was used as the scintillation mixture. When quenching occurred, the necessary corrections were made by utilizing automatic external standardization. All liquid samples, except the blood, and extracts were radioassayed by the direct counting of aliquots. The radioactivity in the blood and feces was quantitated by oxygen combustion of samples weighing approximately 1 gram (wet weight) and counting aliquots of a carbon dioxide trap solution (Andrawes et al., 1967).

For extraction of the radiolabeled residues from milk,

Texas A & M University, College Station, Tex. 77843

10 ml. of a 5% aqueous solution of potassium oxalate were added to 100 ml. of whole milk and the solution was thoroughly mixed (Moffitt, 1963). One hundred milliliters of ethanol, 200 ml. of diethyl ether, and 100 ml. of pentane were added to the mixture. Upon the addition of each solvent, the mixture was shaken vigorously for 1 to 2 minutes. After separation of phases, the organic solvent layer was removed and the aqueous mixture again extracted with diethyl ether and pentane.

The combined extracts were dried with anhydrous sodium sulfate and the solvents removed using a rotary evaporator. The oily residue was transferred to a separatory funnel with 5 ml. of hexane and the evaporating flask was rinsed with 5 ml. of acetonitrile, which also were added to the funnel. The mixture was shaken and the acetonitrile layer removed. The hexane was again extracted with 5 ml. of acetonitrile. The combined acetonitrile extracts were then washed with a small amount of hexane to remove any traces of oils. This extraction procedure yielded three milk fractions—acetonitrile-solubles, hexane-solubles, and water-solubles. The two organic solvent fractions, or organoextractables, were concentrated and further analyzed by thin layer chromatography (TLC).

The water-soluble radioactivity suspected of being conjugated metabolites was subjected to acid treatment in an attempt to free the carbamate moiety. For each milliliter of the water-soluble fraction analyzed, 0.1 ml. of $5N$ hydrochloric acid was added. The acidified water-solubles were placed in a 95° C. water bath for 10 minutes, cooled, and the mixture was extracted three times with ethyl acetate. The ethyl acetate extractable materials were analyzed on TLC.

Certain milk samples also were analyzed utilizing the acetonitrile extraction procedure described by Timmerman et al. (1961). Extraction efficiencies obtained were comparable to those achieved using the above procedure. However, the acetonitrile coagulated the milk solids and

prevented analysis of the radioactivity in the solids by acid treatment and extraction with ethyl acetate. By using the potassium oxalate procedure, the milk solids were suspended in the aqueous milk fraction and were included in the water-soluble fraction of the milk.

Cow urine was extracted with equal volumes of ethyl acetate until the last solvent extract was free of radioactivity. The urine was then subjected to acid conditions in the manner described for the water-soluble fraction of milk and was re-extracted with ethyl acetate.

Twenty-five grams of feces containing sufficient radioactivity for evaluation were extracted with 100 ml. of acetone in a Waring Blendor. The homogenate was filtered and the residue washed several times with small volumes of acetone. The residue was dried and portions burned to determine the quantity of unextractable radioactivity. The volume of the filtrate was reduced until only an aqueous solution remained. This was extracted four times with ethyl acetate; the extracts were combined and then concentrated to near dryness. Approximately 2 ml. of acetone, 20 ml. of coagulating solution, 0.5 gram of ammonium chloride, and 1 ml. of phosphoric acid in 400 ml. of water (Johnson, 1964) were added. The mixture was allowed to stand at room temperature for 30 minutes with occasional swirling and then was filtered under slight vacuum through a fritted glass funnel coated with Celite 545. The filtrate was extracted four times with 20-ml. volumes of ethyl acetate and the combined extracts were dried with sodium sulfate.

Silica gel G thin layer plates were used to separate Furadan and its metabolites extracted from the milk, urine, and feces with organic solvents. The chromatograms were developed in a 3 to 1 ether-hexane mixture, the radioactivity located by radioautography and then quantitated by counting the gel containing the radiolabeled materials. Nonradioactive Furadan and metabolite standards (Table I) used for cochromatographic purposes were detected colorimetrically (Dorough, 1968).

Table I. Identification and Thin Layer Chromatographic Characteristics of Furadan and Its Metabolites Detected in Milk, Urine, and Feces of a Cow Fed Furadan

Metabolites[a]	R_f[b]	Chemical Name	Abbreviation Used in Text
A	0.00	Unknown	Unknown I
B	0.07	Unknown	Unknown II
C	0.19	2,3-Dihydro-2,2-dimethyl-3-hydroxy-benzofuranyl-7-N-hydroxymethyl-carbamate	3-OH-N-CH₂OH-Furadan
E	0.37	2,3-Dihydro-2,2-dimethyl-3-hydroxy-benzofuranyl-7-N-methylcarbamate	3-OH-Furadan
F	0.40	Unknown	Unknown III
G	0.48	2,3-Dihydro-2,2-dimethyl-3-keto benzofuranyl-7-N-methylcarbamate	3-Keto Furadan
H	0.54	2,3-Dihydro-2,2-dimethyl-7-benzo-furanyl-N-methylcarbamate	Furadan
I	0.61	2,3-Dihydro-2,2-dimethyl-3,7-dihydroxybenzofuran	3-OH-Furadan phenol
J	0.86	2,3-Dihydro-2,2-dimethyl-3-keto-7-hydroxybenzofuran	3-Keto Furadan phenol
K	0.90	2,3-Dihydro-2,2-dimethyl-7-hydroxybenzofuran	Furadan phenol

[a] Metabolite designations from Dorough (1968).
[b] Silica gel G chromatograms developed in 3 to 1 ether-hexane.

RESULTS AND DISCUSSION

Following treatment with Furadan-carbonyl-C^{14} at 0.52 mg. per kg., the cow was quite nervous for about 3 hours, but there were no definite signs of carbamate poisoning. However, the ring-labeled Furadan dose, 1.0 mg. per kg., caused definite symptoms of poisoning. They were first noticed approximately 50 minutes after treatment, with salivation, tearing, hyperactivity, and diarrhea being readily apparent. The symptoms were most severe 2 hours after treatment and then began to subside gradually. By 4 hours, the cow had almost completely recovered, the only sign of poisoning being a slight loss of balance which persisted for an additional 2 to 3 hours.

Blood. Radioassay of blood taken from the cow after the Furadan-carbonyl-C^{14} treatment showed that radioactivity was present in the blood very quickly. The labeled residues were first detected in the sample withdrawn after 40 minutes (Figure 1). There was a rapid increase in the level of radioactive materials with the maximum concentration (0.24 p.p.m. Furadan equivalents) occurring 2 hours after treatment. Based on the total blood volume in a cow, 7.7% of the body weight, this level of radioactivity was equivalent to 3.6% of the administered dose. The radioactivity in the blood declined steadily but was detectable in all samples withdrawn within 144 hours. The pattern in which the residues appeared in the blood coincided with the onset of symptoms, the time they were most severe, and with their disappearance.

Milk. Table II shows the magnitude of radiolabeled residues in the milk following treatment of the cow with carbonyl-C^{14} and ring-C^{14} labeled Furadan. The peak concentration of the carbonyl-C^{14} residues, 0.569 p.p.m. at 12 hours, was approximately twice the peak concentration of the ring-C^{14} residues, 0.26 p.p.m. at 8 hours. That this excretion pattern was maintained at each sampling interval was readily evident by the data in Figure 2. Two per cent of the administered Furadan-carbonyl-C^{14} dose was eliminated in the milk, whereas only 0.16% of the Furadan-ring-C^{14} dose was detected. This was unexpected since the latter treatment was twice the Furadan-carbonyl-C^{14} dose.
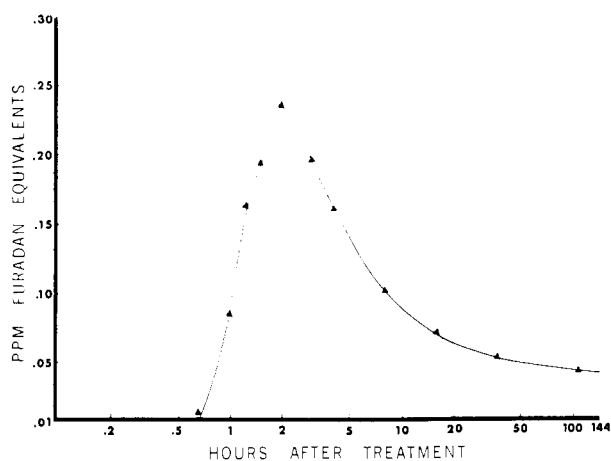
.30

Figure 1. Levels of radioactive residues in the blood of a cow following oral administration of 0.52 mg. per kg. of Furadan-carbonyl-C^{14}

Table II. Radiolabeled Residues in the Milk of a Cow Fed Furadan-Carbonyl-C^{14} and Furadan-Ring-C^{14}

	P.P.M. Furadan-C^{14} Equivalents[a]		
Hours after Treatment	Furadan-Carbonyl-C^{14}		Furadan-ring-C^{14}
	I[b]	II[c]	
2	0.124	0.097	0.24
4	0.325	0.073	0.19
8	0.553	0.100	0.26
12	0.569	0.074	0.10
24	0.236	0.027	0.03
48	0.041	0.005	<0.02
72	0.018	<0.005	<0.02
96	0.012	<0.005	<0.02
120	0.007	<0.005	<0.02
132	0.005	<0.005	<0.02

[a] Limit of sensitivity using Furadan-carbonyl-C^{14} was 0.005 p.p.m. and 0.02 p.p.m. with Furadan-ring-C^{14}.
[b] I = Furadan-C^{14} equivalents based on total radioactive content of milk samples.
[c] II = Furadan-C^{14} equivalents based on actual Furadan-type materials as estimated by substracting the radioactivity incorporated into natural milk components from the total Furadan-C^{14} equivalents.

The higher specific activity of the Furadan-carbonyl-C^{14} allowed a 10 times greater sensitivity in the detection of residues in the milk. This made it possible to quantitate carbonyl-C^{14} residues in the milk for 132 hours after treatment. Although the magnitude of the ring-C^{14} milk residues may have been equivalent to the carbonyl-C^{14} materials after 24 hours, the amount of radioactivity present was too low for detection. This, in part, is the reason for an apparent increase in the percentage of the Furadan-carbonyl-C^{14} dose eliminated in the milk. However, other factors were likely involved since over 90% of the residues in the milk were eliminated within 24 hours. a period when radioactive materials were detectable in both groups of samples. Evidence will subsequently be presented to show that the increased carbonyl-C^{14} residues resulted from the incorporation of carbon-14 dioxide into naturally occurring constituents of the milk.

The efficiency of the extraction method used to analyze Furadan-C^{14} residues in the milk was determined by fortifying milk from untreated cows with radioactive Furadan, 3-OH-Furadan, 3-keto-Furadan, Furadan-phenol, and 3-OH-Furadan phenol. Recovery of the parent carbamate and its metabolites, isolated in the radioactive form from rats (Dorough, 1968), ranged from 92 to 98%. Analysis of the products on TLC showed that they were not altered chemically during the process.

The extraction characteristics and chemical nature of the Furadan-C^{14} residues in milk are given in Table III. Over 75% of the radioactivity in both the 2-hour carbonyl-C^{14} and ring-C^{14} milk were extractable into organic solvents. In samples taken later, the organoextractables constituted a progressively lesser quantity of the total radiolabeled materials in the milk. The rate at which their relative magnitude decreased was much faster in milk containing carbonyl-C^{14} residues. This difference in the solubility properties of carbonyl-C^{14} and ring-C^{14} milk residues suggested that the carbonyl carbon could be removed from the benzofuranyl ring and be independently eliminated in the milk. These nonbenzofuranyl-contain-

| | Per Cent of Total Radiolabeled Residues in Sample (Hours) | | | | | | | |
| | 2 | | 4 | | 8 | | 12 | |
Milk Fraction	Carbonyl-C^{14}	Ring-C^{14}	Carbonyl-C^{14}	Ring-C^{14}	Carbonyl-C^{14}	Ring-C^{14}	Carbonyl-C^{14}	Ring-C^{14}
Organoextractables	75.00	83.33	17.85	63.16	8.86	26.92	14.76	20.00
Acetonitrile-solubles								
Furadan	5.80	3.75	0.54	1.83	0.16	0.61	0.01	0
3-OH-Furadan	66.05	63.50	14.96	53.32	6.55	22.11	0.49	16.84
3-OH-N-CH$_2$OH-Furadan	0.73	9.35	0.64	4.45	0.35	1.80	0.08	2.12
3-Keto-Furadan	2.32	2.60	0.44	1.83	0.30	1.05	0.02	0
3-OH-Furadan phenol	0	0.75	0	0.69	0	0.43	0	0.58
Unknown 1	0.10	3.38	0.04	1.04	0.05	0.92	0.05	0.46
Hexane-solubles	0	0	1.23	0	1.45	0	14.11	0
Water-solubles	25.00	16.67	82.15	36.84	91.14	73.08	85.24	80.00
3-OH-Furadan	0.78	7.17	1.89	1.67	1.60	2.88	0.70	4.94
3-OH-N-CH$_2$OH-Furadan	0.14	0	0.34	0	0.35	0	0.12	0
3-Keto-Furadan phenol	0	6.06	0	25.30	0	49.27	0	50.40
Furadan phenol	0	0	0	5.47	0	11.48	0	12.46
Unknown 1	0.05	1.17	0.13	0.86	0.27	1.12	0.12	0.83
Water-solubles[a]	24.03	2.27	79.79	3.54	88.92	8.33	84.30	11.37

[a] Those C^{14} materials remaining in the aqueous layer following acid hydrolysis and extraction with ethyl acetate.

ing products made up the total radioactivity of the organo-extractables that partitioned from acetonitrile into hexane since hexane-soluble radioactivity was found only in milk containing carbonyl-C^{14} residues (Table III).

Most of the residues in the milk that were radiolabeled as a result of the Furadan-carbonyl-C^{14} treatment, but nonlabeled following the Furadan-ring-C^{14} dose, were in the form of water-soluble metabolites. Acid treatment of the carbonyl-C^{14} milk water-solubles converted only 3 to 5% of these products into chemicals that could be extracted with ethyl acetate. Identical treatment of the ring-C^{14} milk water-solubles resulted in 85 to 90% conversion of the radioactive materials into ethyl acetate extractables. Identification of the radiolabeled materials in the ethyl acetate extract after acid treatment of the carbonyl-C^{14} and ring-C^{14} milk water-solubles showed them to be typical Furadan-type metabolites. Apparently the carbonyl-C^{14} materials remaining in the water fraction were a function of only the carbamate moiety of Furadan.

The above differences in the chemical nature of Furadan-carbonyl-C^{14} and ring-C^{14} milk residues directed our attention to the possibility that feeding a carbonyl-C^{14} labeled carbamate to a cow might result in the radio-labeling of natural milk components. Supporting this possibility was the fact that the extraction characteristics of the carbonyl-C^{14} Furadan residues in the milk were unlike those of radiolabeled products appearing in the milk of cows treated with either carbaryl-1-naphthyl-C^{14} (Dorough, 1967) or Temik-S^{35} (Dorough and Ivie, 1968a). Furadan-carbonyl-C^{14} was readily hydrolyzed in mammalian systems and the resulting carbon-14 dioxide could be collected from the respiratory gases (Dorough, 1968). Additionally, 73% of the Furadan dose given to the cow in this study was hydrolyzed since the recoveries of the administered radioactivity in the urine for Furadan-carbonyl-C^{14} and Furadan-ring-C^{14} were 94 and 21%, respectively (Figure 2). Most of the hydrolysis occurred within 4 hours after treatment; therefore, there were large

quantities of carbon-14 dioxide produced in the cow's body in a rather short period of time.

Carbon from carbon-14 dioxide can be incorporated into glucose (Wood et al., 1945). Glycolytic reactions could then result in the formation of other C^{14}-carbohydrates as well as in the biosynthesis of radioactive glycerides. Possibly, then, the hexane-solubles from the carbonyl-C^{14} milk residues were glycerides and the non-Furadan type materials in the water fraction were carbohydrates.

Lactose was isolated from the carbonyl-C^{14} milk and its identity confirmed by cochromatography with a standard on TLC and by a mixed melting point of the two materials.
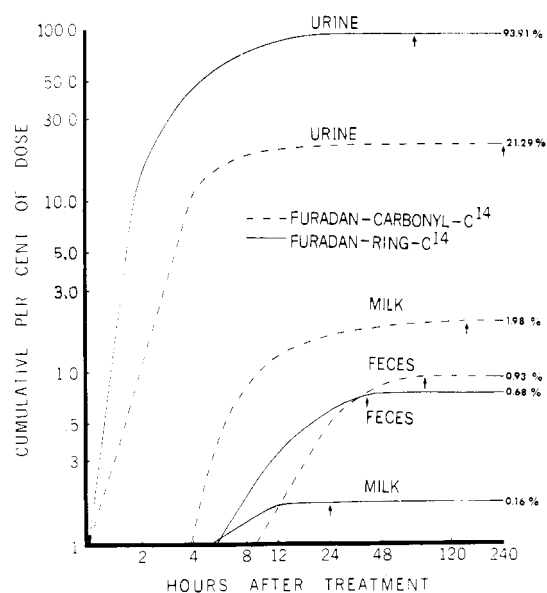

Figure 2. Elimination of radioactivity from a cow treated orally with Furadan-carbonyl-C^{14} and Furadan-ring-C^{14}. Arrows indicate the final samples containing detectable radioactive residues

The isolated lactose did contain radioactivity which could not be removed by crystallization or separated on TLC, indicating that the carbohydrate was indeed radiolabeled. Radioactivity in the hexane fraction containing the milk lipids was too low for identification of its radiolabeled components.

If incorporation of the radiolabeled carbon of Furadan-carbonyl-C^{14} had occurred in the manner described above, the same results would be obtainable should the cow be administered another compound capable of releasing large quantities of carbon-14 dioxide within the body. This was demonstrated to be the case when the cow was given sodium bicarbonate-C^{14} and the milk analyzed exactly as described for Furadan residues. Over 3% of the dose was eliminated in the milk (Figure 3). Since acidification of the whole milk did not release carbon-14 dioxide, it was concluded that none of the radioactivity was as the administered compound. Previous tests had shown that sodium bicarbonate-C^{14} added to milk could be quantitatively recovered as carbon-14 dioxide following the addition of acid.

The manner in which these radioactive residues were distributed among the various milk fractions after extraction are shown in Table IV. All of the organoextractable radioactivity was present as hexane solubles. These products accounted for as much as 27% of the radioactive content of the milk. The remainder of the radiolabeled materials was water-soluble in nature. These latter products could not be extracted into ethyl acetate following acidification and incubation.
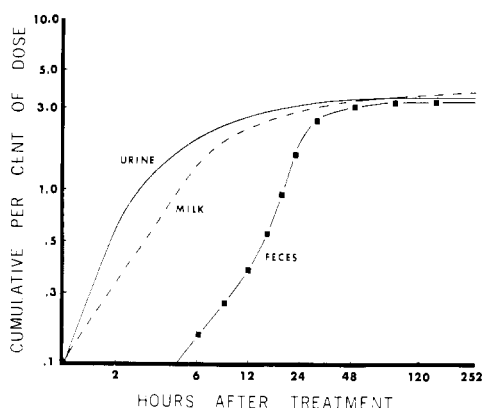

Figure 3. Elimination of radioactivity from a cow treated orally with sodium bicarbonate-C^{14}

Table IV. Extraction Characteristics of Radioactivity in Milk from a Cow Fed Sodium Bicarbonate-C^{14}

| Hours after Treatment | Per Cent of Total Carbon-14 in Sample as | | |
| | Organoextractables | | |
	Acetonitrile solubles	Hexane solubles	Water-solubles
2	0.0	2.9	97.1
8	0	25.5	74.5
12	0	26.8	73.2
24	0	20.8	79.2
72	0	25.4	74.6

Based on the series of data just presented, the authors concluded that the use of Furadan-carbonyl-C^{14} had resulted in the incorporation of the carbon-14 atom into body chemicals normally found in the milk. A communication to this effect was presented earlier (Dorough and Ivie, 1968b). These naturally occurring materials constituted an estimated 90% of the radioactive residues in some of the carbonyl-C^{14} milk samples (Table II). Taking this into account, the peak concentration of carbonyl-C^{14} Furadan and metabolites was 0.1 p.p.m. in the 8-hour milk sample. The amount of incorporated products was calculated by summing the quantity of hexane-solubles and the difference between the quantity of nonhydrolyzable water-soluble radioactivity in the carbonyl-C^{14} and ring-C^{14} milk residues. Subtracting this value from the total radioactive content of the milk showed that only 0.31% of the Furadan-carbonyl-C^{14} dose was eliminated in the milk as Furadan-type metabolites.

Identification of the Furadan-type materials in the milk showed the presence of the parent carbamate, three carbamate metabolites, three hydrolytic products, and one unknown material (Table III). Although Furadan was readily detectable, especially in the 2-hour milk samples, its concentration was always less than 0.01 p.p.m. in the whole milk. Of the carbamate metabolites, 3-OH-Furadan and 3-OH-N-CH_2OH-Furadan occurred both in the free and conjugated forms. The metabolite 3-keto-Furadan was observed only in the free form, as was the hydrolytic product 3-OH-Furadan phenol. Hydrolytic products detected as water-soluble conjugates were 3-keto-Furadan phenol and Furadan phenol. The unknown metabolite had a 0.0 R_f value (Table I) and occurred in very small concentrations.

Three metabolites, 3-OH-Furadan, 3-keto-Furadan phenol, and Furadan-phenol, were the most predominant products, accounting for 70 to 80% of the radioactive content of the individual milk samples. Obviously 3-OH-Furadan was one of the most rapidly formed metabolites in the cow and 3-keto-Furadan phenol was one of the end products in the metabolism of Furadan.

Urine. The urine was the major route of elimination of Furadan-C^{14} residues from the cow (Figure 2). Following the Furadan-ring C^{14} treatment, 94% of the dose was detected in the urine after 72 hours. Although the higher specific activity of the Furadan-carbonyl-C^{14} allowed the quantitation of radiolabeled materials in the urine for 240 hours, only 21% of the dose was eliminated by this route. The difference between these two values should fairly accurately represent the amount of Furadan hydrolysis that occurred in the cow. Comparing the urine excretion curves in Figure 2, the rate of hydrolysis was very rapid during the first 4 hours and almost nil thereafter. Thus, a small portion of the Furadan-C^{14} was quickly converted into products which were no longer susceptible to hydrolytic attack and were slowly excreted from the body.

After extraction of the urine with ethyl acetate, over 90% of the carbonyl-C^{14} and ring-C^{14} residues remained in the aqueous layer (Table V). The small amount of radiolabeled materials which was extracted into ethyl acetate was composed of 3-OH-Furadan, 3-OH-N-CH_2OH-Furadan, and the hydrolytic products, Furadan-, 3-OH-

Table V. Nature of Radiolabeled Residues in Urine of a Cow Fed Furadan-Carbonyl-C[14] and Furadan-Ring-C[14]

Metabolites[a]	Per Cent of Total Radioactivity in Sample (Hours)					
	2		4		12	
	Carbonyl-C[14]	Ring-C[14]	Carbonyl-C[14]	Ring-C[14]	Carbonyl-C[14]	Ring-C[14]
Organoextractables	9.24	3.77	6.41	4.62	3.28	2.61
3-OH-Furadan	8.57	1.87	5.88	1.57	2.29	1.20
3-OH-N-CH₂OH-Furadan	0.67	0.29	0.53	0.11	0.99	0.38
3-OH-Furadan phenol	0	1.36	0	2.62	0	0.92
3-Keto-Furadan phenol	0	0.14	0	0.17	0	0.05
Furadan phenol	0	0.11	0	0.15	0	0.06
Water-solubles	90.76	96.23	93.59	95.38	96.72	97.39
3-OH-Furadan	47.68	14.32	47.95	13.58	1.94	9.14
3-OH-N-CH₂OH-Furadan	3.49	3.20	4.35	9.48	5.16	2.16
3-OH-Furadan phenol	0	5.58	0	16.95	0	10.90
3-Keto-Furadan phenol	0	11.97	0	18.06	0	15.12
Furadan phenol	0	22.82	0	20.69	0	34.36
Unknown II	0	0	0	0	4.87	0
Unknown III	0.93	0	0.71	0	27.25	0
Water-solubles[b]	38.66	38.34	40.58	16.62	57.50	25.71

[a] Metabolites designated as organoextractables were extracted directly with ethyl acetate. The water-solubles were extracted from the urine in the same manner following acid hydrolysis.
[b] Radiolabeled residues remaining in the aqueous layer after acid hydrolysis and extraction with ethyl acetate.

Furadan-, and 3-keto-Furadan phenol. These same metabolites, plus unknowns II and III, were present in the urine as water-soluble conjugates. There was insufficient production of the unknown metabolites for studying their chemical identity.

There was some indication that carbon-14 dioxide incorporation from the Furadan-carbonyl-C[14] treatment was responsible for a portion of the radioactivity in the urine. When the urine was acidified and extracted with ethyl acetate, twice as much of the ring-C[14] products were converted to organoextractables than from the carbonyl-C[14] residues. This was similar to the situation found in the Furadan-C[14] milk residues, although the ring-C[14] milk water-solubles were converted much more completely. Incorporation of the carbon from carbon-14 dioxide into commonly occurring products in the urine was further indicated by the elimination of 3.5% of the dose in the urine following treatment of the cow with sodium bicarbonate-C[14] (Figure 3).

Feces. Less than 1% of the Furadan-carbonyl-C[14] and Furadan-ring-C[14] doses were eliminated from the cow in the feces (Figure 2). Though the amounts excreted were very close, 0.9 and 0.7% of the two treatments,

respectively, there was a definite difference in the nature of the materials in the feces. Data from the analysis of the 8- and 24-hour samples (Table VI) demonstrate these differences. Very large proportions of the carbonyl-C[14] residues, 62% of the 8-hour and 85% of the 24-hour feces, could not be extracted with water or acetonitrile. Ring-C[14] materials were more effectively extracted, the unextractables accounting for 22 and 60% of the radioactivity in the 8- and 24 hour samples. These data indicate that some of the carbonyl-C[14] residues resulted from carbon-14 dioxide incorporation, although their identity was not ascertained. This incorporation could explain the fact that a larger percentage of the Furadan-carbonyl-C[14] treatment was detected in the feces. Otherwise, one would have to assume that sufficient carbamate metabolites were eliminated to more than make up for the quantity of hydrolysis products detected in the ring-C[14] feces. This was not supported by the data in Table VI.

The major organoextractable metabolite in the feces, 3-OH-Furadan, constituted up to three times more of the radioactive content of the feces from the cow treated with Furadan-ring-C[14]. Also, the relative concentration of 3-OH-N-CH₂OH-Furadan was greater in the ring-C[14]

Table VI. Nature of Radiolabeled Residues in Feces of Cow Fed Furadan-Carbonyl-C[14] and Furadan-Ring-C[14]

Metabolites	Per Cent of Total Radioactivity in Feces Sample (Hours)			
	8		24	
	Carbonyl-C[14]	Ring-C[14]	Carbonyl-C[14]	Ring-C[14]
Unextractables	61.83	21.55	84.76	59.92
Water-solubles	7.27	8.96	8.55	10.69
Organoextractables				
Furadan	2.85	0	0.53	0
3-OH-Furadan	26.23	47.93	3.84	10.76
3-OH-N-CH₂OH-Furadan	0.36	9.83	2.32	9.47
3-Keto-Furadan	1.46	0	0	0
3-OH-Furadan phenol	0	4.86	0	3.83
Furadan phenol	0	6.87	0	5.33

feces. Because of the low specific activity of Furadan-ring-C^{14}, Furadan and 3-keto-Furadan were detected only in the carbonyl-C^{14} feces. However, their combined concentrations were far less than the levels of the hydrolysis products, 3-OH-Furadan phenol and Furadan phenol.

Since the total radioactivity in the feces was low, and because only 7 to 10% of these were water-solubles, it was impossible to conduct studies of their chemical nature. However, the carbonyl-C^{14} feces water-solubles being just slightly less predominant than the ring-C^{14} water-solubles suggested that carbon-14 dioxide incorporated products were present in this fraction of the feces.

The ultimate fate of Furadan following oral administration to a lactating cow is summarized in Table VII and the pathway of metabolite formation is shown in Figure 4. The values in Table VII are based on the Furadan-ring C^{14} treatment since hydrolytic products could not be accounted for when using Furadan-carbonyl-C^{14}. Also, the natural body chemicals containing carbon-14 which occurred as a result of the Furadan-carbonyl-C^{14} dose prevented the absolute quantitation of true Furadan metabolites.

Total recovery of the Furadan-ring-C^{14} dose was approximately 95%. Only trace amounts of the administered radioactivity, 0.001% of the dose, was eliminated in the form of the administered compound, and all of this was found in the milk. The quantities of metabolites given in Table VII represent both the free and conjugated forms of the materials. Only 23% of the administered dose was excreted as metabolites known to contain the carbamate moiety and 49% of the materials was identified as products of hydrolysis. The unknown radioactive excretory products, 23% of the administered radioactivity, were located almost entirely in the urine. It is likely that those unknowns are a mixture of carbamate and hydrolytic metabolites since they were present in the urine after both Furadan treatments.

Table VII. Nature of the Total Radioactivity Eliminated from the Body of a Lactating Cow Fed Furadan-Ring-C^{14}

Metabolites	Per Cent of Administered Dose as Indicated Metabolites			
	Milk	Urine	Feces	Total
Furadan	0.001	0.000	0.000	0.001
3-OH-Furadan	0.046	16.710	0.109	16.865
3-OH-N-CH$_2$OH-Furadan	0.004	5.938	0.052	5.994
3-Keto-Furadan	0.002	0	0	0.002
Furadan phenol	0.016	23.803	0.031	23.850
3-OH-Furadan phenol	0.001	10.944	0.023	10.968
3-Keto-Furadan phenol	0.071	14.179	0	14.250
Unknowns[a]	0.016	22.339	0.468	22.823
Total	0.157	93.913	0.683	94.753

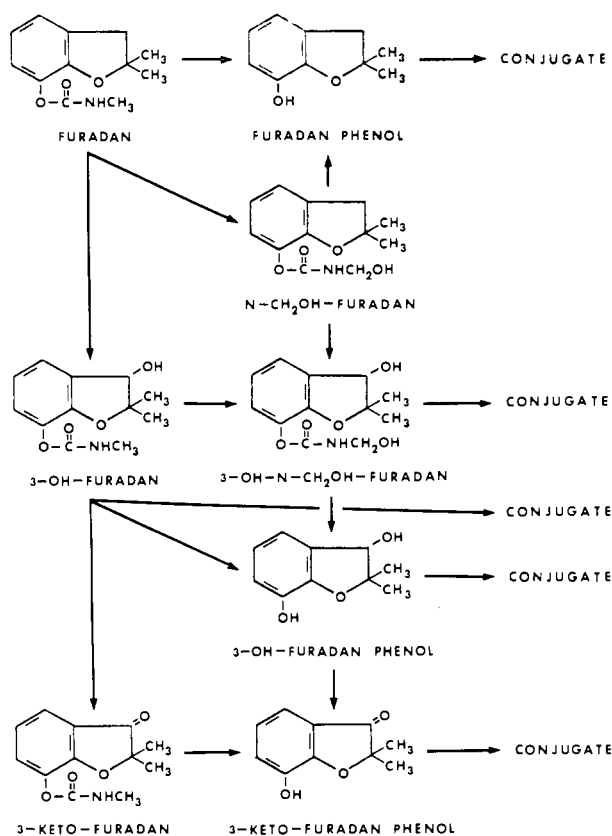[a] Includes organoextractable, water-soluble, and unextractable materials.

Figure 4. Metabolic pathway for Furadan in a lactating cow

LITERATURE CITED

Andrawes, N. R., Dorough, H. W., Lindquist, D. A., *J. Econ. Entomol.* **60**, 879 (1967).
Armbrust, E. J., Gyrisco, G. G., *J. Econ. Entomol.* **59**, 801 (1966).
Broersma, D. B., *J. Econ. Entomol.* **60**, 819 (1967).
Dorough, H. W., *J. Agr. Food Chem.* **15**, 261 (1967).
Dorough, H. W., *J. Agr. Food Chem.* **16**, 319 (1968).
Dorough, H. W., Ivie, G. W., *J. Agr. Food Chem.* **16**, 460 (1968a).
Dorough, H. W., Ivie, G. W., *Science* **159**, 732 (1968b).
Forsythe, H. Y., Jr., *J. Econ. Entomol.* **59**, 1413 (1966).
Johnson, D. P., *J. Assoc. Offic. Agr. Chemists* **47**, 283 (1964).
Moffitt, R. A., "Analytical Methods for Pesticides, Plant Growth Regulators, and Food Additives," p. 545, G. Zweig. Ed., Academic Press, New York, 1963.
Pass, B. C., *J. Econ. Entomol.* **59**, 1232 (1966).
Rawlins, W. A., Gonzalez, D., *J. Econ. Entomol.* **59**, 288 (1966).
Timmerman, J. A., Dorough, H. W., Buttram, J. R., Arthur, B. W., *J. Econ. Entomol.* **54**, 441 (1961).
Wood, H. G., Lifson, N., Lorber, V., *J. Biol. Chem.* **159**, 475 (1945).