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

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Carbaryl-naphthyl-C^{14} (1-naphthyl *N*-methylcarbamate) administered in the feed to a lactating cow at single doses of 0.25 and 3.05 mg. per kg. resulted in radiolabeled residues in the milk for as long as 60 hours after treatment. Approximately 0.35% of each dose was detected in the milk. Maximum concentrations were found in 6-hour samples which, following the two treatments, were 0.063 and 0.950 p.p.m., respectively. The major chloroform-extractable metabolite from the milk was tentatively identified as 5,6-dihydro-5,6-dihydroxy-1-naphthyl *N*-methylcarbamate. Approximately 30% of all residues in the 6-hour samples was this product. After the 0.25 mg. per kg. treatment, 70% of the dose was detected in the urine and 11% in the feces. The percentages of the 3.05 mg. per kg. dose excreted in the urine and feces were 58 and 15, respectively. Analysis of 27 tissue samples, taken 6 days after the 3.05 mg. per kg. treatment, revealed that residues were highest in the liver, kidney, and ovaries, although these residues were present only in trace amounts.

Carbaryl (1-naphthyl *N*-methylcarbamate) is widely used for control of various pests which attack forage crops. These crops often make up a major portion of the diet of dairy cows and could result in the consumption of carbaryl by the animals. Estensive studies have been conducted to determine the concentration of carbaryl that could be ingested by cattle without residues appearing in the milk or tissues. Based on the findings of these studies, carbaryl can be present on some forage crops, such as alfalfa, at levels up to 100 p.p.m. at the time of harvest.

A preliminary study (*6*) indicated that feeding carbaryl at 450 p.p.m. in the diet of dairy cattle for 2 weeks did not result in carbaryl residues in the milk. In a more thorough study (*11*) carbaryl also was fed in the diet of lactating cows at levels up to 450 p.p.m. for 14 days. The milk was analyzed for carbaryl, 1-naphthol, and conjugates of 1-naphthol. The authors concluded that no detectable residues were encountered before, during, or after the exposure period. Also, 1-naphthol or its conjugates were not found in milk from a cow fed daily doses of 1-naphthol at the rate of 12 p.p.m., based on body weight, for 3 days. Tissues, likewise, have been reported to be free from carbaryl residues after cattle were fed a diet containing 200 p.p.m. of the carbamate for 27 days (*3*).

Direct dermal applications of carbaryl sprays and dusts to cattle have been shown by several workers to result in little, if any, residues in meat or milk from the treated animals (*1, 3, 5, 9*).

In each of the aforementioned studies, a colorimetric method using *p*-nitrobenzenediazonium fluoborate as a chromogenic reagent was used to detect carbaryl and its metabolites. The material actually measured by this procedure was 1-naphthol, the hydrolysis product of carbaryl. The efficiency of this method in detecting metabolites resulting from ring modifications that would yield something other than 1-naphthol upon hydrolysis has not been established. The likelihood that such metabolites may be formed has been reported (*4*). Several carbamate metabolites were tentatively identified as ring-hydroxylated products. One of these, 3,4-dihydro-3,4-dihydroxy-1-naphthyl *N*-methylcarbamate, appeared in milk from a goat treated orally with carbaryl-C^{14} labeled in the carbonyl position. This metabolite would not respond to the fluoborate color test. Other metabolites that could not be extracted with organic solvents made up the majority of the radioactivity in the milk. These products were not identified, and no attempt was made to quantitate them using a colorimetric method.

This paper reports on the magnitude and nature of residues in milk, tissues, urine, and feces following administration of carbaryl-naphthyl-C^{14} in the feed of a dairy cow. Results from studies designed to evaluate the efficiency of colorimetric methods in detecting the radiolabeled residues also are included.

Materials and Methods

Synthesis of Carbaryl-Naphthyl-C^{14}. Carbaryl-naphthyl-C^{14} was synthesized by reacting 4 mc. of 1-naphthol-1-C^{14} (specific activity 3.76 mc. per mmole, Chem-Tract, Cambridge, Mass.) with an excess of methylisocyanate for 16 hours at 40° C. in a sealed glass ampoule. The radiolabeled product was purified using thin layer chromatography (TLC). Evidence that the radioactive purity of the product was in excess of 99% was as follows: The infrared spectrum was identical with that of an authentic sample; the product cochromatographed with known carbaryl on TLC when developed two-dimensionally; and only a single product, carbaryl, was evident after applying 1.0×10^6 c.p.m. to TLC, developing two-dimensionally, and exposing the chromatogram to x-ray film for 7 days. This technique was capable of detecting readily as little as 500 c.p.m. if impurities of this magnitude had been

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present. The specific activity of the final product was 21,000 c.p.m. per μg.

All samples in this study were radioassayed using liquid scintillation counting (Packard Tri-Carb Model 3365). The instrument, equipped with automatic external standardization, was adjusted so that the counting efficiency for carbon-14 was 70%. All values have been corrected for quenching.

Standards. Analytical samples of carbaryl and 1-naphthol were supplied by the Union Carbide Chemical Co. Metabolites of carbaryl were biologically synthesized using a described rat liver microsome system (*4*). The identity of metabolites reported here is based on findings of these workers and of a study (*8*) indicating that the metabolite tentatively identified as 3,4-dihydro-3,4-dihydroxy-1-naphthyl *N*-methylcarbamate was probably 5,6-dihydro-5,6-dihydroxy-1-naphthyl *N*-methylcarbamate.

Carbaryl-naphthyl-C^{14} of low specific activity was incubated with the microsomes and each chloroform-extractable product was isolated in a sufficient quantity for detection using chromogenic reagents (*4*). Cochromatographic studies on TLC were conducted by locating the unknown radiolabeled metabolite by radioautography and then spraying with a chromogenic reagent to detect the standards added to the extract before spotting.

Treatment and Sampling. A 500-kg. Jersey cow was placed in a metabolism stall several days prior to treatment for preconditioning. A rubber tube, 3 inches in diameter, was attached to the vulva to collect the urine separate from the feces and the animal was exercised twice daily for 30 minutes to 1 hour in an attempt to keep the cow as normal as possible. Alfalfa hay was provided free-choice and 4 pounds of crushed grain were fed to the cow at the morning and evening milking.

For the first treatment, 126 mg. of the labeled carbaryl in ether were added to 1 pound of grain and mixed thoroughly until the ether odor had completely disappeared. This treated grain was placed in a feed container in small portions until the cow had consumed the entire amount. Three pounds of untreated grain were then fed to the cow from the feeder. The feeding container was removed and washed with acetone, and the amount of unconsumed carbaryl determined by counting aliquots of the wash. The cow consumed 125.7 mg. (0.25 mg. per kg. body weight). Total radioactivity consumed was 2.64×10^9 c.p.m. The same procedure was used for a second treatment of the same cow 6 days later, except nonlabeled carbaryl was added to the radioactive material to give a total dosage of 3.05 mg. per kg. of body weight (3.05×10^9 c.p.m.).

Following each treatment, milk, urine, and feces were collected for analysis. The first urine samples were taken 2 hours after treatment, after which all samples were taken at 6, 12, and 24 hours, and then continuously at 12-hour intervals through 144 hours. Urine and fecal samples were frozen until time of analysis, but the milk was analyzed immediately.

Extraction and Measurement of Residues. Aliquots (0.2 ml.) of whole milk were radioassayed directly, and if they contained labeled residues, the cream was separated by centrifugation. The cream was analyzed by combustion and the skim milk by direct counting. The skim milk was extracted by adding 100 ml. of acetone to 50 ml. of milk and shaking thoroughly until the milk solids precipitated completely on standing. The solids were removed by filtering through Whatman No. 3 filter paper with the aid of a slight vacuum. An additional 50 ml. of acetone were used to wash the extracting flask and solids. The acetone was removed under vacuum at 40° C. and the remaining water transferred to a 250-ml. separatory funnel. The evaporating flask was washed twice with 25-ml. portions of acetonitrile, and the washes were added to the water extract. Most of the oily materials present at this point were eliminated by extracting the water-acetonitrile mixture with heptane that had been previously saturated with acetonitrile. Radioassay of the heptane was made routinely, but this fraction was always discarded because no labeled materials were detected.

Fifty milliliters of chloroform were added to the separatory funnel containing the water-acetonitrile mixture and shaken vigorously. The bottom acetonitrile-chloroform layer was removed and the aqueous layer re-extracted with 50 ml. of chloroform. After drying with anhydrous sodium sulfate, the organic solvent phase was evaporated to 10 ml., and aliquots were taken from both the aqueous and organic phases for counting. The organic solvent phase was reduced to approximately 0.1 ml. in a graduated centrifuge tube. In most instances the extract was ready for application to TLC. When oils were present in concentrations too great for direct chromatography, the sample was diluted to 5 ml. with acetonitrile, extracted with heptane, and again evaporated to about 0.1 ml.

Urine was extracted directly with chloroform and the amount of radioactivity in each layer determined. The chloroform extract was dried with anhydrous sodium sulfate and filtered, and the filtrate applied to TLC after concentration.

For feces extraction, 10 grams were homogenized in 50 ml. of acetonitrile and, after filtration, the residues were rehomogenized in 50 ml. of a 1 to 1 mixture of chloroform and acetonitrile. The filtered extracts were combined, and 10 ml. of water were added. Fifty milliliters of chloroform were added, and the mixture was shaken. After separation of the phases, the water layer was extracted twice more with 25-ml. portions of chloroform and the acetonitrile-chloroform layer extracted with 15 ml. of distilled water.

The chloroform-acetonitrile extract required further cleanup before spotting on TLC. Ten grams of Filter-Cel (Johns-Manville, Houston, Tex.) and 100 mg. of Darco G-60 carbon (Atlas Chemical Industries, Wilmington, Del.) were added to the extract, shaken, and then filtered. To prevent retention of the radioactivity, the carbon was washed with 1*N* HCl and dried at room temperature before use. The volume of the filtrate was reduced to approximately 50 ml.; most of the chloroform had been removed by the time the volume had been reduced to this level. The remaining acetonitrile was extracted with heptane until the heptane

extracts were colorless. The acetonitrile was then evaporated to a volume suitable for spotting on TLC.

In addition to direct assay of liquid samples, a wet combustion technique was used to determine the total radiolabeled residues in milk, urine, feces, and tissue samples. Using Van Slyke's combustion reagents (*10*), 1-gram samples were analyzed, except for fat and cream where 300 mg. were used. Recovery of the labeled carbon added to control samples was greater than 90%.

The combustion apparatus consisted of a 500-ml. three-necked distilling flask with a dropping funnel inserted into one of the side joints and a carbon dioxide trap attached to the other. In the center joint were a thermometer and a glass tube, held in place with a Neoprene stopper, extending downward into the combustion mixture. The glass tube was connected to a nitrogen source. The combustion flask was placed in a heating mantle situated over a magnetic stirrer. The carbon dioxide trap was constructed by sealing a gas-dispersion tube, with a 12-mm. fritted cylinder, to a connection tube and bending the dispersion tube downward so that the fritted cylinder could be submerged in a trap solution. Sixteen milliliters of a 1 to 2 solution of ethanolamine–ethylene glycol monomethyl ether were used to trap the carbon dioxide (*7*). The flask containing the sample and combustion reagents was heated to 2000° C. over a 15-minute period and cooled for 20 minutes, and aliquots were removed from the carbon dioxide trap for radioassay.

Chromatography. Thin layer chromatography using silica gel G (Brinkmann Instruments, Inc., Great Neck, N. Y.) was used for resolving carbaryl and its metabolites. All chromatograms were prepared 0.3 mm. thick except those used in purification of the carbaryl-C^{14} which were 1.0 mm. thick. For single dimensional chromatography, the 8 × 8 inch plates were developed with 2 to 1 ether-hexane and when developed two-dimensionally, a 4 to 1 mixture of benzene and acetonitrile was used as the second system.

Radioactive areas on the chromatograms were detected by radioautography, and the gel containing the labeled materials was placed in vials for scintillation counting. When compounds on the TLC plates were to be recovered, the gel was transferred to the top of a Florisil column and the material eluted with ether.

Results and Discussion

Milk. Radiolabeled residues were detected in the milk after the cow had received doses of both 0.25 and 3.05 mg. per kg. in the feed (Table I). Maximum concentrations of carbaryl-C^{14} equivalents were present in samples taken 6 hours after treatment where almost 1.0 p.p.m. was found after the 3.05 mg. per kg. dose and 0.06 p.p.m. following the lower dose. Residues of only slightly lower magnitudes were detected in the 12-hour milk samples but then declined rapidly until none were present after 60 hours. The per cent of each dose detected in the milk was similar after both treatments, 0.32% of the first dose and 0.37% of the second.

After the whole milk was separated into skim milk and cream by centrifugation, analysis by combustion showed that the radioactive materials remained entirely in the skim milk. Fractionation of the radioactivity into chloroform-extractables, water-solubles, and unextractables revealed that the unextractables constituted an increasing percentage of the total residues detected as the radioactivity in the milk declined. All of the residues in the 60-hour samples were of an unextractable nature.

Table I. Residues in Whole Milk after Feeding Carbaryl-Naphthyl-C^{14} to a Cow

Hours after Indicated Dose	P.P.M. Carbaryl-Naphthyl-C^{14} Equivalents Present as			
	Chloroform-extractables	Water-solubles	Unextractables	Total
	0.25 Mg./Kg.			
6	0.041	0.015	0.007	0.063
12	0.010	0.008	0.033	0.051
24	0.003	0.005	0.008	0.016
36	0.002	0.003	0.004	0.009
48	0.001	0.002	0.011	0.014
60	0.000	0.000	0.003	0.003
	3.05 Mg./Kg.			
6	0.480	0.410	0.060	0.950
12	0.277	0.379	0.154	0.810
24	0.058	0.058	0.076	0.192
36	0.005	0.007	0.036	0.048
48	0.000	0.001	0.017	0.018
60	0.000	0.000	0.010	0.010

Certain studies (*6*, *11*) indicated no residues were detected colorimetrically in milk from cows fed carbaryl in the diet for 14 days. Since the presence of radiolabeled residues in milk following a single oral dose of carbaryl-C^{14} was now established, the effectiveness of reported colorimetric techniques in detecting these products was determined. A study (*11*), similar to the present one, used the fluoborate colorimetric method for detecting residues of carbaryl and its metabolites; therefore, attempts were made to determine the radiolabeled materials in the milk using techniques similar to those employed by these workers.

Skim milk from the 6-hour, second-treatment (3.05 mg. per kg.) sample was extracted and fractionated as described above. An aliquot containing 100 μg. of carbaryl-C^{14} equivalents was removed from the acetonitrile-chloroform layer and color developed using the fluoborate method. Comparing the absorbance with standard curve values for carbaryl showed that a maximum of 3 μg. of the radioactive products was detectable. Radioassay of the chromogenic mixture revealed that over 90% of the added carbaryl equivalents were still present. Thus, the failure of the colorimetric procedure in detecting the residues was not caused by a loss of material by evaporation, etc., but rather by the presence of carbaryl metabolites which would not respond to the color test.

Carbaryl-C^{14} metabolites remaining in the water layer were processed exactly as previously described (*11*)

for the determination of conjugates of 1-naphthol in skim milk. Briefly, this procedure involved acid hydrolysis of the conjugates, extraction with methylene chloride, cleanup of the extract on a Florisil column, and detecting residues with *p*-nitrobenzenediazonium fluoborate. After processing 40 ml. of the aqueous fraction containing 20 μg. of radiolabeled carbaryl equivalents, only 2-μg. equivalents were detected colorimetrically. Six-microgram equivalents were lost—i.e., remained in the water layer following extraction with methylene chloride—because of incomplete hydrolysis of the conjugates. Additional 12-μg. equivalents were lost during subsequent extraction and column cleanup. Since the amount of residues recovered from the column was so small, eluates from several columns were combined before final color development.

The 6-hour, second-treatment milk sample contained a total of 0.950 p.p.m. of carbaryl-C^{14} equivalents (Table I). Of these, 0.480 p.p.m. were chloroform-extractables, and 0.410 p.p.m. were water-solubles. The fluoborate method detected 3% of the former and 10% of the latter residues, resulting in an efficiency of only 5.7% when total radioactive products were considered. It must be assumed that similar residues were present in the milk of cows receiving nonlabeled carbaryl treatments. The magnitude of residues in the milk of animals fed carbaryl for prolonged periods of time, therefore, remains largely unknown.

The material remaining on the Florisil after elution with methylene chloride was removed from the column by washing with methanol. A sample containing 20 μg. of carbaryl equivalents did not respond to the fluoborate color test. Another aliquot of this fraction was concentrated and splotted on TLC and developed two-dimensionally. Radioautography showed that all the radioactivity remained at the origin. This indicated that the product(s) was different from carbaryl or 1-naphthol, since these known compounds had R_f values greater than 0.6 when chromatographed in either of the solvent systems used.

Attempts also were made to detect the water-soluble metabolites in the milk collected after the second treatment using *N*-2,6-trichloroquinoneimine to develop color (*2*). Twenty milliliters of the water layer containing 10 μg. of carbaryl equivalents were added to 2 ml. of 10*N* HCl, and the mixture was boiled for 5 minutes. The aqueous mixture was extracted twice with 40-ml. portions of a 1 to 1 mixture of 1-butanol and chloroform. Ninety-eight per cent of the radioactive material was present in the butanol-chloroform extract. Color was developed after the solvent was removed. The average recovery for four such samples was 10.2-μg. carbaryl equivalents, while radiometric analysis of the 1-butanol just before addition of the chromogenic reagents indicated that 9.7-μg. equivalents of carbaryl were present.

The small quantities of water-soluble metabolites prevented substantiation of the quantitative recovery indicated using the *N*-2,6-trichloroquinoneimine method. However, this procedure should certainly merit further consideration in studies designed to develop an efficient method of carbaryl residue analysis. Because of a lack of material, the labeled chloroform extractables were not included in this particular study.

Chloroform-Extractable Metabolites in Milk. When the acetonitrile-chloroform-extractables from the 6-, 12-, and 24-hour skim milk samples were resolved by two-dimensional TLC, a maximum of seven metabolites was detected by radioautography (Table II). Identification of all products, except metabolite *A*, was made based on the fact that the labeled compound from the milk cochromatographed with the "known" metabolite that had been synthesized by rat liver microsomes.

Metabolite *A*, one of the major chloroform-extractable metabolites from milk, was detected during the first 24 hours after both treatments. This material remained at the origin after TLC was developed and its identity was not ascertained. The other major metabolite, metabolite *B*, also was present in milk samples taken within the first 24 hours. This compound was tentatively identified as 5,6-dihydro-5,6-dihydroxy-1-naphthyl *N*-methylcarbamate. The pure metabolite, after recovery from TLC, was subjected to the fluoborate color test. Fifty micrograms, based on the specific activity of carbaryl, could not be detected using this method. The same quantity of metabolite *A* likewise would not respond to the colorimetric test. Insufficient quantities of the other metabolites were present to determine if they could be detected colorimetrically. However, those metabolites containing an unchanged naphthyl moiety should certainly respond to the color test.

Urine. Determination of total radioactivity in the urine showed a rapid excretion of the administered doses by this route. Of the total labeled materials detected in the urine, over 95% could be accounted for in samples taken during the first 24 hours following treatment. After this the quantity of the labeled products in each urine sample, taken at 12-hour intervals, was low but could be detected in all samples collected through 120 hours. The cumulative per cent of the total dose found in the urine after 96 hours for the first and second treatment was 69.8 and 57.8, respectively (Table III). These values remained the same after 120 hours because of the small amount of detectable residues during this time period.

Only a small amount of the radioactivity in the urine was extractable into chloroform, indicating that most of the metabolites were conjugates of some type. Most were not present in sufficient quantities to isolate and further analyze. However, chloroform-extractables from each of the urine samples collected at 2, 6, 12, and 24 hours after both treatments were chromatographed two-dimensionally. There were a minimum of six radioactive areas on the chromatograms and a maximum of 18; the former was detected in 2- and 24-hour samples and the latter in 12-hour samples. Two of the metabolites were in concentrations high enough to allow isolation from TLC and rechromatography with individual metabolites derived from the rat liver microsome system. The major metabolite, accounting for 50 to 60% of the chloroform-extractables, was chromatographically the same as the

microsome metabolite tentatively identified as 5,6-dihydro-5,6-dihydroxy-1-naphthyl *N*-methylcarbamate (*8*). The other material was identified as its hydrolytic product, 1-hydroxy-5,6-dihydro-5,6-dihydroxynaphthalene. This material occurred at about one half the concentration of the carbamate.

The two colorimetric procedures described previously for attempted detection of carbaryl conjugates in skim milk also were tested for their efficiency in detecting the metabolites in the urine. Aliquots containing 100-μg. equivalents of carbaryl-C^{14} were taken from the second-treatment, 24-hour sample and treated the same as the water-solubles from the skim milk. Using the fluoborate method, only 16% of the total metabolites in the urine were detected. As in the milk experiments, the greatest loss occurred because of incomplete hydrolysis of the conjugated materials and because the Florisil column retained much of the radioactivity. With the *N*-2,6-trichloroquinoneimine procedure, 28% of the radioactive products in the urine were detected. Approximately 50% loss was attributed to incomplete hydrolysis while the remainder was attributed to compounds which would not respond to this particular colorimetric test. There was apparently some difference in the nature of the products in the urine and milk since, with the latter, almost quantitative recovery was obtained.

Feces. Eleven per cent of the administered dose was detected in the feces after the first treatment and 15% found in the feces following the second feeding of car-

Table II. Concentration and Tentative Identification of Chloroform-Extractable Carbaryl-Naphthyl-C^{14} Metabolites from Milk and Feces as Resolved by Thin-Layer Chromatography

	Per Cent of Radioactivity in Samples for Indicated Dose and Time after Treatment, Hours													
	Milk						Feces							
	0.25 Mg./Kg.			3.05 Mg./Kg.			0.25 Mg./Kg.				3.05 Mg./Kg.			
Metabolites	6	12	24	6	12	24	6	12	24	36	6	12	24	36
A Unknown[a]	25.7	11.4	14.9	19.5	10.3	23.9	11.0	2.4	5.1	15.6	15.9	8.1	9.1	9.7
B 5,6-Dihydro-5,6-dihydroxy-1-naphthyl *N*-methylcarbamate	38.4	8.4	5.9	26.0	11.1	6.5	50.3	49.9	13.3	18.8	47.4	31.5	19.6	15.2
C 1-Hydroxy-5,6-dihydro-5,6-dihydroxynaphthalene	0	0	0	2.4	1.0	0	0	4.5	3.5	5.6	13.9	4.7	5.7	4.4
D 1-Naphthyl *N*-hydroxymethylcarbamate	0	0	0	0.6	0.5	0	0	0	2.4	0	3.4	3.6	3.6	2.8
E 4-Hydroxy-1-naphthyl *N*-methylcarbamate	0	0	0	0.7	0.7	0	0	0	1.5	0	4.2	2.2	2.5	3.2
F Carbaryl	0	0	0	0.9	1.1	0	0	4.4	22.0	0	0	21.6	19.9	12.7
G 1-Naphthol	0	0	0	1.4	1.3	0	0	0	0.3	0	0	0	1.3	2.5

[a] Material remaining at origin after TLC is developed.

Table III. Radiolabeled Residues Recovered in the Urine and Feces of the Cow Fed Carbaryl-Naphthyl-C^{14} and Percentage of Residues Extractable into Chloroform

Hours after Treatment	0.25 Mg./Kg. Treatment				3.05 Mg./Kg. Treatment			
	Cumulative Dose, %		$CHCl_3$ Extr. Sample, %		Cumulative Dose, %		$CHCl_3$ Extr. Sample, %	
	Urine	Feces	Urine	Feces	Urine	Feces	Urine	Feces
2	25.6	—[a]	0.5	—	14.3	—	1.1	—
6	52.0	0.1	0.5	61.3	33.4	0.3	3.9	84.8
12	62.7	0.2	1.8	57.2	44.4	5.4	15.2	71.7
24	68.2	10.8	3.1	48.1	53.8	14.6	7.0	61.7
36	68.9	11.0	2.4	40.0	56.8	15.2	5.7	50.5
48	69.3	11.3	4.8	5.3	57.4	15.3	0.5	15.2
60	69.5	...[b]	2.5	...	57.6	15.4	0	3.1
72	69.6	...	2.9	...	57.7	...	0	...
84	69.7	...	3.1	...	57.8	...	0	...
96	69.8	...	1.5	...	57.8	...	0	...

[a] — No sample available.
[b] ... No residues could be detected.

baryl-C^{14} (Table III). Over 90% of that extracted in the feces was present in samples taken during the first 24 hours. Extraction and partitioning of the radiolabeled products into chloroform-extractables and water-solubles showed that both phases contained considerable quantities of radioactivity. The nature of the labeled residues in the feces indicated that carbaryl was metabolized fastest when given at 0.25 mg. per kg. This first treatment resulted in less water-soluble materials and less chloroform-extractable metabolites which were probably precursors to the water-solubles. Furthermore, no carbaryl was detected in the 36-hour feces sample, whereas in the higher dose, 3.05 mg. per kg., the parent compound accounted for almost 13% of the radioactivity in the feces at the same time interval. Chloroform-extractable metabolites from the feces were the same as those detected in the milk (Table II).

Tissue. Radiolabeled residues remaining in the tissues at the time the cow was sacrificed could have resulted from a combination of the two treatments. Therefore, expression of the residues in parts per million of carbaryl equivalents was impossible, and these experiments served only to indicate the relative concentrations of residues in various portions of the body.

None of the tissues contained large amounts of labeled residues. Highest levels were found in the liver, kidney, and ovaries, but the total activity in these tissues, based on wet weights, was only 200 to 300 c.p.m. per gram. Tissues having 100 to 200 c.p.m. per gram were the brain, spinal cord, and abomasum. Fifty to 100 c.p.m. per gram were detectable in the spleen, gall bladder, bile, adrenal gland, large intestine, small intestine, and skin. No residues could be detected in the tongue, udder, blood, bone, lungs, heart, neck muscle, leg muscle, loin muscle, omental fat, kidney fat, reticulum, omasum, or rumen wall.

Conclusions

Identification of a particular pesticide residue or its detection among various biological media is greatly facilitated by a method of analysis highly specific for the compound in question. Such a procedure, however, may fail to define the true residual nature of a chemical. Degradation or modification may occur to the extent that it no longer possesses characteristics necessary for response to the specific method. The present study indicates that carbaryl is a compound of this type.

Carbon-14 residues were secreted in the milk of a treated cow which were not detectable with the widely used fluoborate method for determining carbaryl residues. Analysis of a sample containing 0.950 p.p.m. carbaryl-C^{14} equivalents showed that the efficiency of the colorimetric method was less than 6%. The remainder of the residues was lost during sample preparation or failed to respond to the chromogenic reagent. Based on these findings, previous reports of "no residues" in the milk of carbaryl-treated animals can mean only that fluoborate-sensitive material was not present in measurable quantities. Residues of this nature do, indeed, occur in minute amounts in the milk; however, the presence of much larger amounts of fluoborate-insensitive metabolites has been established. It is essential that the toxicological significance of these metabolites now be evaluated.

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