Fate of Naphthyl-*1*-^{14}C Carbaryl in Laying Chickens

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The fate of naphthyl-*1*-^{14}C carbaryl in laying chickens was studied by oral administration of nonradiolabeled carbaryl for 17 days followed by ^{14}C-carbaryl for 14 days at dosages corresponding to 7, 21, and 70 ppm in the diet. Seven days after dosing, 94.5, 0.153, and 0.05% of the dose were recovered in the excrement, eggs, and carcass, respectively. Radiolabeled residues in the excrement, eggs, and tissues were proportional to the dosage level. Residues reached maximum levels within 1 day in the excrement, 2 days in egg white, and 6 to 8 days in egg yolk. After discontinuation of dosing, the half-life of ^{14}C residues was <1 day in the excrement and egg white, 2 to 3 days in egg yolk, and 5 days in the carcass. A metabolite, tentatively identified as 1-naphthyl sulfate, constituted 39.1% of the egg residues.

In recent years considerable emphasis has been placed on the potential transfer of pesticide residues from feeds to meat and eggs of poultry. After oral intake a compound is absorbed from the gastrointestinal tract into the blood or lymph, by which it is distributed to the various body tissues and ultimately excreted (Loomis, 1968). During these processes biodegradable pesticides are subjected to a variety of biochemical attacks which result in the formation of metabolic products having chemical structures different from the parent molecule. Thus, in evaluating the potential transfer of residues into meat and eggs, the determination of the parent compound as well as its metabolites should be considered. Because of the lack of sensitive analytical methods capable of detecting all possible products, radiochemical analysis is often used as an alternative.

Carbaryl has been used to control ectoparasites on poultry (Furman and Pieper, 1962; Harrison, 1960, 1961; Kirkwood, 1966; Kraemer, 1959; Kraemer and Furman, 1959). Oral intake of carbaryl may result through the use of the compound directly on the birds or through the diet. Reference is made here to the use of carbaryl on forage and grain crops for control of insect pests. Such a use may leave minute residues of carbaryl on poultry feed (Prudich, 1962). Previous studies relating to the fate of carbaryl in chickens have shown only limited transfer of residues to tissues and eggs (Furman and Pieper, 1962; Johnson *et al.*, 1963: Khmelevskii, 1968; McCay and Arthur, 1962; Nir *et al.*, 1966; Prudich, 1963). Following administration of a single oral dose of naphthyl-*1*-^{14}C carbaryl, Paulson and Feil (1969) have demonstrated that carbaryl is rapidly metabolized and excreted by laying chickens. The urine was found to be the primary route of excretion, with small amounts excreted in the feces. This rapid elimination resulted in only a small portion of the dose being deposited in the eggs and tissues. Total ^{14}C residues reached a maximum and dissipated at a much faster rate in egg white than in egg yolk.

Metabolic studies using a single oral dose of carbonyl-^{14}C carbaryl demonstrated extensive hydrolysis of the applied chemical, since as much as 55% of the dose was accounted for as respiratory $^{14}CO_2$ by 48 hr after treatment (Paulson and Feil, 1969). Although there was a continuous expiration of $^{14}CO_2$ by the treated hens during the 48-hr test period, approximately 90% of the total $^{14}CO_2$ was accounted for in the first 6 hr after treatment. Hydrolysis of carbaryl resulted in the liberation of 1-naphthol which was conjugated rapidly to form primarily 1-naphthyl sulfate and to a lesser extent 1-naphthyl glucuronide. 1-Naphthyl sulfate constituted one half of the total residues in the urine at 6 hr after treatment (Paulson *et al.*, 1970). Ring hydroxylation of carbaryl resulted in the formation of 4-hydroxy-, 5-hydroxy-, and 5,6-dihydroxycarbaryl. Hydrolysis products of the carbamates, *i.e.*, 1,4-dihydroxy-, 1,5-dihydroxy-, and 1,5,6-trihydroxynaphthalene, were also formed. The carbamate metabolites and their phenols were never found in their free form in the urine. They rapidly conjugated to form sulfates and glucuronides, which were excreted primarily in the urine of the treated hens (Paulson *et al.*, 1970).

This report describes the extent of transfer of total carbaryl residues to tissues and eggs from the continual consumption of carbaryl by laying hens and the nature of residues in the eggs.

METHODS AND MATERIALS

Chemicals. 1-Naphthyl-^{14}C carbaryl used in this study was purchased from Mallinckrodt Nuclear, Orlando, Fla. The compound had a specific activity of 4.0 mCi/mmol and a radiochemical purity greater than 99%, as determined by thin-layer chromatography, radioautography, and liquid scintillation counting. Nonradioactive carbaryl and suspected metabolites were synthesized and authenticated by Union Carbide Corp., South Charleston, W. Va.

Test Birds, Treatment, and Sampling. Two separate experiments, short-term and continuous feeding, were conducted to determine the fate of 1-naphthyl-^{14}C carbaryl in laying chickens. In both experiments White Leghorn hens were used. The birds were housed singly in standard two-bird wire cages installed in an air-conditioned room. Daily photoperiod in the room was programed to provide 18 hr of light and 6 hr of dark. Standard laying mash formulation (obtained through the Farmers Co-Op Exchange, Clayton, N. C.) and water were provided *ad libitum* from separate containers in each cage to prevent possible cross-contamination.

For treatment of birds, the daily doses were divided into two equal portions of which one was given at 8 a.m. and the other at 5 p.m. This schedule allowed maximum availability of the toxicant during the active feeding hours of the chickens. For preparation of doses the appropriate amount of carbaryl dissolved in 5% polyethylene glycol 400 in chloroform was introduced into No. 1 gelatin capsules (Eli Lilly & Co., Indianapolis, Ind.) containing approximately 0.3 g of feed. The solvent was allowed to evaporate from the feed at room temperature and then the capsules were capped and stored in a

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freezer until used. At each dosing period each hen was treated with one capsule introduced into the bird's mouth and then forced with a glass rod through the esophagus into the crop.

Droppings from each hen were collected in preweighed pans made from heavy duty aluminum foil. Prior to and during the treatment periods daily records were maintained on feed consumption, feces elimination, egg production, and egg weights, while body weights were determined at 3- to 4-day intervals. None of the birds included in the two experiments displayed any toxic manifestations during the course of the experiment. At autopsy, no gross pathological conditions could be observed. In addition, analysis of variance showed no significant difference between the treated and the control hens in body weights, egg weights, egg production, food consumption, and feces elimination.

Short-Term Feeding. One-year-old White Leghorn hens (DeKalb 131) having an average weight of 1786 ± 118 g and an egg production of 0.65 egg per day were used in this experiment. After a conditioning period of 10 days, three hens were treated twice daily with 1-naphthyl-^{14}C carbaryl for a period of 4 days. Each hen received 8.7 mg per day of radio-labeled carbaryl fortified with nonradio-labeled material (specific activity of the mixture equaled 1.36 mCi/mmol). Based on an average feed consumption of 125 g per day, this treatment corresponded to a level of 70 ppm of toxicant in the feed. Capsules containing untreated feed were administered to three control chickens. Eggs and feces were collected from each hen and analyzed separately for total ^{14}C-carbaryl equivalents. Two of the treated birds were sacrificed at 16 hr and the third one at 7 days after the last treatment. The hens were decapitated and blood was collected in heparinized beakers. Immediately after each slaughter, various tissues and organs were excised under conditions which would prevent cross-contamination by blood, body fluids, or dissecting instruments. Each sample was weighed and then kept frozen until analyzed.

Continuous Feeding. Six-month-old White Leghorns (DeKalb 161) having an average weight of 1669 ± 174 g and egg production of 0.85 ± 0.04 egg per day were used in this experiment. The test consisted of four dosage levels, equivalent to 0, 7, 21, and 70 ppm of carbaryl in the diet, with two replicates at each level and three hens per replicate. The hens were housed singly in the wire cages, leaving one empty cage to separate different groups. After the birds had been allowed to adjust to their environment for 10 days, they were given their respective doses of nonradio-labeled carbaryl for 17 days to establish a metabolic equilibrium. At the end of this period, 1-naphthyl-^{14}C carbaryl (specific activity of 0.91 mCi/mmol) was administered at the same levels as the nonradio-labeled material for 14 days. The daily doses were calculated on the basis of a mean daily feed consumption of 140 g per hen.

Droppings were collected twice daily, morning and evening, and each collection was weighed and pooled by replicate for analysis. All the fecal samples were kept frozen until analyzed. Eggs were collected shortly after they were laid, stored at 5°C, and then pooled by replicate to compose the daily samples for analysis. At 1, 3, and 7 days after the last dose was given, one hen from each replicate was sacrificed and various tissues and organs were removed for analysis. Each tissue was weighed and then kept frozen until analyzed.

Methods of ^{14}C Residue Determination. Radioactivity was determined by a LS-150 Beckman liquid scintillation spectrometer with its carbon-14 channel adjusted to count a nonquenched ^{14}C-hexadecane standard (Beckman Instruments) at 90% efficiency. Two scintillator solutions were used to count solubilized biological samples and various extracts. One consisted of 4 g of 2,5-diphenyloxazole (PPO) and 50 mg of 1,4-bis[2-(5-phenyloxazolyl)] benzene (POPOP) per liter of toluene (hereafter referred to as solution A) and the other was composed of 100 ml of xylene, 300 ml of dioxane, 300 ml of methyl cellosolve, 7.0 g of PPO, 350 mg of POPOP, and 56 g of naphthalene (Bruno and Christian, 1961; hereafter referred to as solution B). Ten milliliters of the mixture was used for counting each sample. All samples were counted until enough counts accumulated to give a statistical error of 3.0% or less. Quench corrections were made using the external standard channels ratio method. Counting efficiency of 50% was considered as the minimum acceptable level for quench corrections. Background samples were prepared by processing samples from control hens using identical procedures to those used for samples from treated birds. All samples and backgrounds were prepared in duplicate and the average of the two determinations was taken as the activity for the individual sample. If the analyses did not agree within the counting error, additional samples were analyzed until satisfactory results were obtained. The determination limit of carbon-14 counting was established at 5 D/M above background.

Eggs. Egg samples were processed for analysis of total ^{14}C residues within 1 day after collection. Egg white was separated from yolk and the weights were determined to the nearest 0.1 g. Each fraction was blended with an equal amount of water in a Virtis homogenizer at a medium speed for 10 min. Duplicate subsamples (200 to 250 mg of homogenate, weighed to the nearest 1.0 mg) were transferred to 20-ml glass counting vials whose foil-lined caps were provided with $^{13}/_{16}$-in. Teflon liner (The Chemical Rubber Co., Cleveland, Ohio). One-and-one-half milliliters of NCS solubilizer (Amersham/Searle, Des Plaines, Ill.) was added and the vial was covered with the cap. The preparation was incubated at 50 ± 5°C for 20 to 24 hr with occasional shaking. Complete digestion of the material was evident when a clear solution was obtained. In a few cases, an additional 0.5 ml of the reagent, followed by incubation for 8 to 10 hr, was necessary to achieve complete digestion of the sample. After cooling to room temperature, 10 ml of the liquid scintillation mixture (A) was added and the samples were allowed to remain at room temperature for 1 day before counting. For determination of the percent recovery, samples from untreated birds were fortified with known counts of ^{14}C-carbaryl and processed in the same manner just described. Mean recoveries of added material were 95.0 ± 1.0% from yolks and 100.5 ± 0.8% from whites. The determination limit of this procedure for eggs was established as 5.0 ppb of ^{14}C-carbaryl equivalents.

Tissues. Individual tissues were minced coarsely using safety razor blades or a Hobart food chopper. Duplicate subsamples of 100 to 150 mg (weighed to the nearest 1 mg) were transferred from the minced muscle (leg, thigh, and breast), gizzard, heart, kidney, skin, fat, brain, pancreas, oviduct, and intestinal wall into liquid scintillation vials. Two milliliters of the NCS reagent was added and each sample was incubated and radioassayed as described for the eggs. Radioactivity in the developing eggs was determined using the same steps described for egg analysis. Blood, liver, lung, spleen, remaining carcass, and intestine plus contents were analyzed for ^{14}C-carbaryl equivalents by the oxygen combustion technique previously described (Andrawes *et al.*, 1967). In this method sample size ranged between 1.2 to 1.5 g. Liquid scintillation system (B) was used to count radioactivity in the trapping solution. The determination limit of the NCS solubilizer and oxygen combustion methods for tissues was established at 5.0 ppb of ^{14}C-carbaryl equivalents.

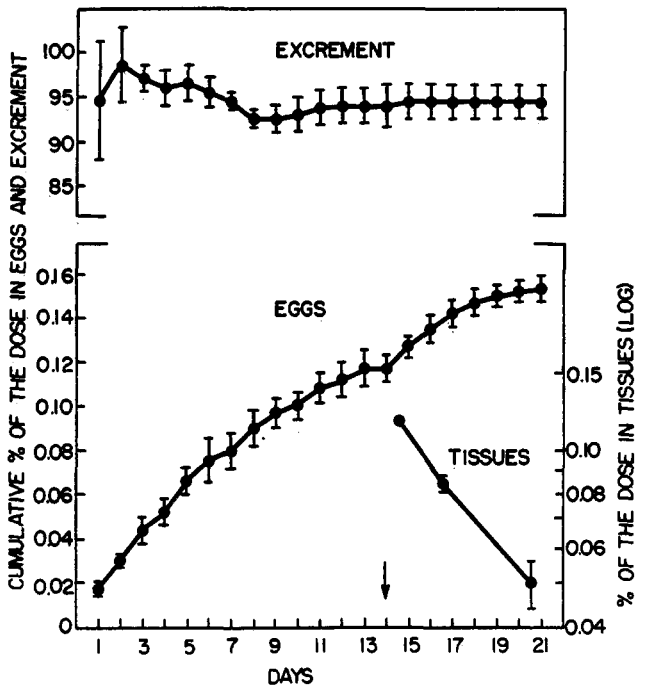

Figure 1. Elimination in the excrement and eggs and depletion of tissue residues after feeding 1-naphthyl-^{14}C carbaryl to laying hens for 14 days (average of three dosage levels)

Excrement. Frozen samples were allowed to thaw at room temperature and then each sample was mixed thoroughly with a spatula. Approximately 500-mg subsamples (weighed to nearest 1 mg) were transferred into 20-ml standard test tubes containing Teflon-coated magnetic stirring bars (10 × 3 mm). The fecal material was carefully deposited on the bottom of the tube to avoid smearing. Five milliliters of the NCS solubilizer was added to each sample and the tubes were stoppered with corks lined with 0.001-mm thick Teflon film. The preparations were incubated at 50 ± 5°C with continuous stirring for 24 hr. After cooling to room temperature the samples were centrifuged at 1500 rpm for 10 min to separate the insoluble particles. The liquid phase was transferred to a 25-ml graduated cylinder and the residue was washed successively with 5 ml each of toluene and toluene–ethanol (1:1, v/v). The washes were combined and the volume was adjusted to 15 ml with toluene. Duplicate 0.5-ml aliquots were removed for counting using the liquid scintillation solvent A. To eliminate possible interference from chemiluminescence, the prepared scintillation samples were allowed to remain at room temperature for 2 days prior to counting.

The efficiency of the above-outlined method in removing ^{14}C residues from droppings was determined by analyzing samples from the short-term feeding study for total ^{14}C-carbaryl equivalents. Mean recoveries were 98.2 ± 0.7% in the NCS solubilizer wash, 1.7 ± 0.7% in the toluene wash, and 0.11 ± 0.01% in the toluene–ethanol final wash. No radioactivity could be detected in the insoluble residues when counted directly or when 1 g of insolubles from several analyses was combusted in a Parr double-valved oxygen bomb. Total radioactivity recovered from fecal samples of treated hens, as determined by the NCS solubilizer method, was consistently 3–5% higher than that found by the oxygen combustion technique described by Andrawes *et al.* (1967). The determination limit of the solubilizer procedure was established as 0.03 ppm of ^{14}C-carbaryl equivalents.

Extraction of Eggs. Homogenates of egg white or yolk containing approximately 200,000 dpm were used directly for the subsequent steps; lower activity samples were combined to obtain sufficient activity for further analysis. A volume of acetonitrile equal to that of the homogenate was added to precipitate egg proteins. The solids were separated by centrifugation at 1500 rpm for 20 min and the supernatant was filtered. Egg solids were extracted twice more with acetonitrile–water (1:1, v/v) using approximately 3 ml of solvent per gram of solids for each extraction. After filtration the extracts were combined and the volume was reduced under vacuum at 40°C. The final volume was adjusted to contain an equivalent of 0.5 g of sample per milliliter of solution. Aliquots were removed for radioassay and determination of the efficiency of the extraction method. Analysis of egg samples showed a mean recovery of 79.0% for the white and 76.9% for yolk.

Organosoluble metabolites were removed from egg extracts by partitioning the aqueous phase three times with an equal volume of diethyl ether. The ether washes were combined, dried over sodium sulfate, and then filtered. Radioactivity in both the ether fraction (organosolubles) and in the aqueous phase (water-solubles) was determined by direct counting.

Chromatography. Organic extracts of egg yolk and white were subjected to column chromatography for separation of the metabolic products present in the eggs. A 2 × 45 cm chromatographic column was packed to a height of 30 cm with 60 to 100 mesh Florisil (Floridine Co., Hancock, W.Va.) in hexane. Ether extracts of yolk or white were concentrated under reduced pressure to approximately 5 ml, a small amount of Florisil was added, and the residue solvent was evaporated under a stream of dry nitrogen. The Florisil was placed on the top of the column and the column eluted with the following solvent sequence: 400 ml of 1:1 ether–hexane; 400 ml of 2:1 ether–hexane; 200 ml of 3:1 ether–hexane; 200 ml of ether; and 200 ml of methanol. Ten-milliliter fractions were collected and aliquots (0.5 to 1.0 ml) removed for radioassay. Chromatographic fractions comprising the individual radioactive peaks were combined for further characterization. Each peak was concentrated under reduced pressure and aliquots were removed for cochromatography with authentic standards on thin-layer chromatography. Recoveries of radioactivity from the column ranged between 90–95%.

The aqueous phase of egg white or yolk was concentrated to 10 ml and excess methanol added to precipitate additional egg solids. After separation by centrifugation, the precipitate was extracted twice with methanol using 100 ml of solvent for each extraction. The extracts were combined and then concentrated in a flash evaporator at 40°C. Ten milliliters of a 5:1 mixture of acetone–methanol was used to dissolve the residues for further analysis by gel column chromatography. Sephadex LH-20 (Pharmacia Fine Chemicals, Inc., Piscataway, N.J.), swollen overnight in a 5:1 mixture of acetone–methanol, was used for this purpose. Sufficient gel in the acetone–methanol mixture was added to a 2 × 45 cm column containing the same solvent to give a bed 30-cm high after allowing it to settle under 4 psi air pressure. A solution containing the water-solubles was placed on the top of the bed and the pressure again applied to achieve a flow rate of 2 ml per min. The column was then developed with the following solvent sequence: 200 ml of 5:1 acetone–methanol; 200 ml of 3:1 acetone–methanol; 200 ml of 1:1 acetone–methanol; and 200 ml of methanol. Ten-milliliter fractions were collected and aliquots (0.5 to 1.0 ml) were removed for radioassay. Fractions within individual radioactive peaks were combined for

further characterization. Recovery of added radioactivity from the column ranged between 85–90% in different samples. To indicate the nature of the water-soluble metabolites, peaks from the Sephadex gel column were evaporated to dryness and then incubated for 24 hr with Glusulase enzyme (Endo Laboratories, Inc., Garden City, N.Y.) in 0.1 *M* sodium acetate buffer (pH 5.0) followed by extraction with diethyl ether. The aqueous phase was subjected to acid hydrolysis and extraction as described by Dorough and Wiggins (1969). Enzyme and acid reaction products were characterized by thin-layer chromatography.

Thin-layer glass plates (20 × 20 cm) coated 0.3-mm thick with silica gel G (Brinkmann Instruments, Inc., Westbury, N.Y.) were used for thin-layer chromatography (tlc) throughout this investigation. Solvent systems used to develop the chromatograms were: diethyl ether–hexane (3:1 and 1:1); chloroform–acetonitrile (5:1 and 3:1); methylene chloride–ethyl acetate (7:1 and 1:1); ethyl acetate–benzene (3:1); and isopropyl ether–acetonitrile (5:1). The solvents were mixed on a volume/volume basis. Radioactive metabolites were located on the chromatograms by radioautography for 2 weeks and then scraped from the plates for direct radioassay. Liquid scintillation system B was used for counting silica gel samples. For identification of the unconjugated metabolites and the aglycones of the conjugates, the radioactive material was mixed with authentic standards dissolved in acetone and the mixture chromatographed two-dimensionally in several solvent pairs. Radio-labeled materials were located on the chromatograms by radioautography and the standards were located by spraying with *p*-nitrobenzenediazonium fluoborate, as described by Krishna *et al.* (1962).

RESULTS AND DISCUSSION

Elimination and Retention of ^{14}C-Carbaryl by Laying Hens. The behavior of 1-naphthyl-^{14}C carbaryl applied twice daily for 14 days to hens at levels equivalent to 7, 21, and 70 ppm in the feed is shown in Figure 1. To draw an overall picture of the fate of carbaryl in laying hens, the mean percent-of-applied for the three treatment levels were averaged during and after discontinuation of dosing.

Most of the applied radio-labeled carbaryl was eliminated rapidly in the excrement of the treated hens. Fifteen hours (morning collection) after the initial treatment, radioactivity in the excrement accounted for 100.1 and 105.3% of the dose in the 7- and 21-ppm treatments, respectively. A slower rate of elimination was initially obtained in the 70-ppm treatment which resulted in 80.8% in the excrement at 15 hr after the first dose was given and 86.9% at 9 hr after the second dose. Excrement weights and food consumption in this treatment did not differ significantly from the other treatments or the control. By the end of the second day, however, the cumulative percent in the 70-ppm treatment increased to 93.9%. By the end of the third day the value had risen to 98.5%, which was within the range of the other two treatments (97.4–95.6%). Between that time and the end of the 14-day feeding period, the variation among treatments became less evident, as measured by the low value of the standard deviation among treatments which ranged between 0.9 and 2.3% at different sampling times. Elimination through the excrement was essentially complete shortly after the last dose was given. At the end of the 14-day dosing period (or 9 hr after the last dosing) a total of 94.1 ± 2.3% of the cumulated dose was recovered in the droppings. An additional 0.4% of the total dose was eliminated during the following 7 days, which resulted in a total recovery of 94.5 ± 2.3% being excreted by this route.

Table I. Concentration of ^{14}C Residues in Eggs of Hens Treated with 1-Naphthyl-^{14}C Carbaryl at a Level Equivalent to 70 ppm in the Feed for 4 Days

Days	ppb ^{14}C-carbaryl equivalents			Cumulative % of dose
	Yolk	White	Yolk + white	
	Days fed ^{14}C-carbaryl			
1.5	21.1	44.6	35.4	0.015
2.5	143.8	78.2	100.7	0.029
4	267.5	64.9	135.0	0.048
	Days after last treatment			
1	366.8	41.4	156.0	0.059
3	360.7	7.1	139.8	0.087
4	308.5	<5.0	119.8	0.106
5	182.3	<5.0	69.9	0.116
7	48.6	<5.0	17.3	0.119

The rapid rate of elimination of orally applied carbaryl to hens is in agreement with published results on the fate of carbaryl in laying hens (Paulson and Feil, 1969). A similar pattern of excretion was also found in our short-term feeding study with 1-naphthyl-^{14}C carbaryl. After dosing three laying hens twice daily for 4 days at a level equivalent to 70 ppm in the feed (or 4.87 mg/kg/day), a total of 84.3% of the cumulated dose was eliminated in the excrement at the end of the dosing period. An additional 1.7% was recovered during the following 6 days. Although the total recovery in the excrement in the latter test was lower than that found in the single dose (Paulson and Feil, 1969) and in the continuous feeding previously discussed, it is quite evident that the majority of the applied carbaryl was eliminated mainly in the excrement within 1 day after dosing.

Although egg white and yolk samples were analyzed separately, only values for the total egg are shown in Figure 1. Deposition of ^{14}C-carbaryl and its metabolic products in the eggs was very limited. After a continuous administration of nonradioactive carbaryl for 17 days followed by 1-naphthyl-^{14}C material, only 0.017 ± 0.002% of the radioactivity was detected in the eggs at 1 day after treatment. The percentage of the cumulated dose in eggs progressively increased with time and probably reached a plateau by the thirteenth day. The continued rise in the cumulative percent for eggs (Figure 1), after equilibration had been reached at 6 to 8 days (Table III), has its origin in the nature of the arithmetic calculation involved. As is evident from Table III, the ppb concentration in the eggs does not increase after 6 to 8 days. At various sampling times during and after termination of dosing, the percentage of the applied ^{14}C-carbaryl found in the eggs was independent of the treatment levels. Total radioactivity in the eggs accounted for 0.117 ± 0.006% of the cumulated dose at the end of 14 days. Eggs laid after discontinuation of dosing contained radioactive residues which brought the percent of the dose up to 0.153 ± 0.006% at the end of 7 days after the last dose was given. The continued appearance of radioactivity in the egg during the latter period appears to be related to the nature and the period required for completion of the developmental processes of egg formation. Accordingly, eggs laid during the 7 days after termination of treatments began their formation and were exposed to ^{14}C-carbaryl applied during the treatment period.

Deposition in the eggs of ^{14}C residues in the short-term feeding with 1-naphthyl-^{14}C carbaryl at a level equivalent to 70 ppm in the feed is shown in Table I. Although the hens

Table II. Elimination in the Excrement of Radioactivity by Hens Fed 1-Naphthyl-^{14}C Carbaryl for 14 Days at Levels Equivalent to 7, 21, and 70 ppm in the Diet

	ppm ^{14}C-carbaryl equivalents[a]					
	7 ppm		21 ppm		70 ppm	
Days	M	E[b]	M	E	M	E
			Days fed ^{14}C-carbaryl			
1	4.14 ± 0.04	5.27 ± 1.05	13.69 ± 2.61	17.18 ± 1.18	29.33 ± 7.55	38.81 ± 3.80
2	4.25 ± 0.81	5.68 ± 0	13.63 ± 5.14	13.81 ± 5.16	40.51 ± 0.35	48.64 ± 3.34
3	3.89 ± 0.33	6.25 ± 0.01	12.67 ± 1.71	11.95 ± 4.18	43.44 ± 4.14	49.50 ± 2.35
5	3.56 ± 0.55	4.29 ± 0.06	12.28 ± 0.02	12.80 ± 0.49	38.16 ± 0.49	39.47 ± 1.72
7	3.49 ± 0.28	5.71 ± 1.24	14.28 ± 2.67	13.39 ± 4.64	35.20 ± 0.23	53.55 ± 16.12
10	3.77 ± 0.13	3.79 ± 1.41	10.76 ± 2.04	15.41 ± 0.60	40.09 ± 1.99	44.16 ± 3.13
14	3.42 ± 0.26	5.14 ± 0.08	11.85 ± 1.84	14.34 ± 0.59	38.98 ± 7.33	37.06 ± 9.09
			Days after last treatment			
1	0.20 ± 0.03	0.04 ± 0.01	0.63 ± 0.08	0.14 ± 0.04	2.03 ± 0.86	0.30 ± 0.04
2	<0.03	<0.03	0.07 ± 0.01	0.05 ± 0.01	0.23 ± 0.01	0.18 ± 0.06
3	<0.03	<0.03	0.05 ± 0.01	0.03 ± 0.01	0.15 ± 0.04	0.13 ± 0.06
4	<0.03	<0.03	0.04 ± 0.01	<0.03	0.07 ± 0.01	0.06 ± 0.01
5	<0.03	<0.03	<0.03	<0.03	0.06 ± 0	0.05 ± 0.01
6	<0.03	<0.03	<0.03	<0.03	0.06 ± 0.01	0.03 ± 0.01
7	...	...	<0.03	...	0.04 ± 0.02	...

[a] Mean ± standard deviation. [b] M, Morning collection; E, evening collection.

used in this test were not equilibrated with nonradio-labeled carbaryl prior to administration of the labeled material, the rate of transfer of ^{14}C residues to the eggs was identical to that determined in the continuous feeding experiment using equilibrated birds. Egg radioactivity in the former test accounted for 0.015 and 0.048% of the dose at 1.5 and 4 days, respectively. This compares well with 0.017 and 0.052% of the dose at 1 and 4 days, respectively, in the continuous feeding experiment.

To determine the magnitude of retention of ^{14}C-carbaryl equivalents and the overall rate of depletion of body residues, the radioactivity found in the various hen parts was combined to derive the percentage of the accumulated dose at various times after cessation of dosing. The values obtained for the three dosage levels in the continuous feeding study were averaged and the standard deviation of the treatments was computed. As shown in Figure 1, only 0.116 ± 0.001% of the accumulated ^{14}C dose remained in the body at 1 day after the last dose was given. At that time the gastrointestinal tract and its contents (G.I.) contained an average of 22.8% of the total radioactivity remaining in the hen. The liver, blood, and remaining carcass contributed 17.7, 18.3, and 20.3%, respectively. Radioactivity found in the other tissues was low and never exceeded 7% in any given organ or tissue. Detailed assessment of the data is discussed under the residue section of this report.

Once the supply of radioactivity in the G.I. had passed out in the droppings, there was a notable decline in the total ^{14}C materials found in the hen's body. Only 0.084 ± 0.004% of the dose was found at 3 days and 0.050 ± 0.006% at 7 days after the last dosing. From 50–60% of the recovered radioactivity at 3 and 7 days was associated with the blood and the remaining carcass. The G.I. contributed 5.7% at 3 days and 3.2% at 7 days after the last treatment.

The small standard deviation observed between the mean percentages of the dose remaining in the total carcass of the three dosage levels indicated that the rate of tissue retention and depletion was independent of the levels fed. By plotting these percentages against time on a semilogarithmic scale, a straight line relationship was obtained. This rate of depletion of carbaryl residues from the body resembles that of a single component system and follows first-order reaction kinetics. From the graph, the half-life of total residues equaled 5 days. In the short-term feeding experiment radioactivity found in the carcass accounted for 0.52% of the dose at 16 hr after the last treatment and declined to 0.059% at the end of 7 days. The shorter half-life (1.7 days) in the latter experiment may reflect the fact that the level of residues in the tissues had not yet reached equilibrium; however, the limited number of chickens involved does not allow a firm conclusion.

Summation of the total recovered residue in feces, eggs, and carcass at the end of 7 days after the last dose in the continuous feeding study averaged 92.3, 94.9, and 96.8% in the 7-, 21-, and 70-ppm treatments, respectively.

Residues of ^{14}C-Carbaryl in Excrement, Eggs, and Tissues. Parts per million of ^{14}C residues in the excrement of selected days after treatment are presented in Table II. Concentration of ^{14}C-carbaryl equivalents leveled off within the first 15 hr (the first morning collection) in the 7- and 21-ppm treatments and by the end of the first day (evening collection) in the 70-ppm treatment. This rapid rate of equilibration between the intake and excretion is related to the rapid rate of elimination of carbaryl and its metabolites, as discussed above. The data in Table II demonstrate that within biological variation the concentration of ^{14}C-carbaryl equivalents in the excrement was directly proportional to that of ^{14}C-carbaryl fed.

After discontinuation of feeding of ^{14}C-carbaryl, the residue in the excrement declined very rapidly. No detectable residues (<0.03 ppm) were found in the samples collected the second day in the 7-ppm treatment and the fifth day in the 21-ppm treatment. Demonstrable levels of ^{14}C residues were found in the 70-ppm samples until the end of 1 week after discontinuation of dosing. When the ppm of ^{14}C-carbaryl equivalents were plotted against time on a semilogarithmic scale, the depletion curve appeared to be one of a triphasic pattern. This suggested three sequential elimination processes, each having different rates. The first portion with the greatest slope fell between the end of the fourteenth day of feeding and the end of the following day. During that time a 100-fold reduction in the concentration of ^{14}C residues in the excrement was noted in all the treatments. In the following 3 days, the ^{14}C residues in the 21- and 70-ppm treatments were reduced by a factor of 5 and 7, respectively. A third phase between the fourth and the seventh day at the 70-ppm level

Table III. Deposition of ^{14}C Residues in the Eggs of Hens Fed 1-Naphthyl-^{14}C Carbaryl for 14 Days at Levels Equivalent to 7, 21, and 70 ppm in the Diet

	ppb of ^{14}C-carbaryl equivalents[a]								
	7 ppm			21 ppm			70 ppm		
Days	Yolk[b]	White	Y + W	Yolk	White	Y + W	Yolk	White	Y + W
					Days fed ^{14}C-carbaryl				
1	ND	5.8 ± 1.4	3.8 ± 0.9	6.1 ± 1.3	19.7 ± 8.7	15.4 ± 6.6	26.1 ± 12.4	61.0 ± 30.9	49.2 ± 24.5
2	15.9 ± 1.3	9.6 ± 3.5	11.7 ± 2.7	51.3 ± 5.2	24.0 ± 1.0	32.8 ± 0.8	113.9 ± 0.8	93.5 ± 27.1	100.8 ± 16.9
3	36.7 ± 10.9	9.1 ± 4.0	18.5 ± 5.4	95.5 ± 35.7	22.0 ± 1.6	45.4 ± 11.7	281.6 ± 31.9	101.3 ± 12.7	158.6 ± 21.6
4	57.5 ± 18.1	7.6 ± 2.1	25.5 ± 5.3	133.4 ± 39.8	22.0 ± 0	58.4 ± 12.7	488.4 ± 46.6	97.2 ± 5.5	228.7 ± 23.5
5	79.3 ± 14.1	10.5 ± 1.8	33.5 ± 5.9	198.8 ± 42.0	26.3 ± 2.6	80.6 ± 13.4	658.1 ± 54.7	95.5 ± 8.6	272.4 ± 22.3
6	91.1 ± 17.3	10.2 ± 0.4	36.4 ± 5.0	255.5 ± 21.4	24.4 ± 1.8	99.2 ± 10.7	688.2 ± 0	103.2 ± 0	293.1 ± 0
7	104.5 ± 22.3	10.0 ± 0.4	40.5 ± 4.7	329.1 ± 12.2	34.7 ± 2.5	128.5 ± 6.0	862.2 ± 33.4	99.8 ± 22.9	347.7 ± 26.1
8	102.4 ± 13.2	8.8 ± 0	39.4 ± 2.7	291.4 ± 23.3	22.9 ± 3.5	112.1 ± 9.2	1000.6 ± 54.0	118.7 ± 20.2	418.3 ± 37.1
9	107.5 ± 13.8	7.0 ± 0.3	40.6 ± 5.0	300.0 ± 44.8	26.8 ± 6.7	115.5 ± 20.2	973.3 ± 48.2	112.8 ± 21.4	407.4 ± 10.0
10	103.2 ± 7.4	6.3 ± 5.7	39.6 ± 5.3	267.0 ± 4.6	26.0 ± 2.6	106.7 ± 3.7	999.9 ± 58.1	120.0 ± 1.2	419.2 ± 10.2
11	103.7 ± 9.1	9.6 ± 0.5	41.1 ± 1.0	317.4 ± 16.3	29.2 ± 4.8	126.6 ± 4.9	962.9 ± 17.6	94.8 ± 14.8	379.2 ± 23.1
12	101.5 ± 5.4	8.2 ± 1.1	39.3 ± 1.6	334.6 ± 80.2	28.6 ± 9.3	126.8 ± 28.2	995.8 ± 53.7	105.6 ± 3.6	400.5 ± 13.3
13	108.1 ± 7.8	12.0 ± 0.2	42.5 ± 1.6	310.2 ± 34.7	26.8 ± 2.8	113.8 ± 7.1	997.2 ± 19.5	91.1 ± 7.5	346.4 ± 39.2
14	107.2 ± 2.1	5.7 ± 2.5	39.1 ± 1.1	310.6 ± 71.6	29.5 ± 9.6	123.9 ± 30.4	1097.0 ± 157.7	96.5 ± 11.8	418.4 ± 65.0
					Days after last treatment				
1	103.4 ± 8.4	6.5 ± 1.3	36.9 ± 0.7	310.7 ± 61.9	14.1 ± 2.8	107.9 ± 9.3	948.1 ± 53.2	42.9 ± 17.2	331.0 ± 29.0
2	84.2 ± 0.1	<5.0 ± 0	27.1 ± 3.3	317.6 ± 120.1	<5.0 ± 0	102.6 ± 37.1	898.2 ± 16.4	7.1 ± 1.3	281.3 ± 17.0
3	68.8 ± 4.2	<5.0 ± 0	22.2 ± 1.7	229.2 ± 23.9	<5.0 ± 0	72.2 ± 7.1	708.8 ± 43.1	<5.0 ± 0	223.4 ± 3.7
4	34.9 ± 0	<5.0 ± 0	15.4 ± 0	172.9 ± 3.7	<5.0 ± 0	57.5 ± 1.3	429.4 ± 0	<5.0 ± 0	146.3 ± 0
5	21.2 ± 0	<5.0 ± 0	6.9 ± 0	101.1 ± 27.7	<5.0 ± 0	35.2 ± 18.3	403.3 ± 226.1	<5.0 ± 0	126.0 ± 69.2
6	22.2 ± 3.3	<5.0 ± 0	7.5 ± 1.3	36.6 ± 14.5	<5.0 ± 0	12.4 ± 2.5	291.3 ± 0	<5.0 ± 0	88.7 ± 0
7	7.8 ± 3.6	<5.0 ± 0	<5.0 ± 0	...	...	...	95.2 ± 18.7	<5.0 ± 0	33.2 ± 10.5

[a] Mean ± standard deviation. [b] Y = Yolk; W = White.

was poorly defined because the ^{14}C residues were very low and approached the lower limit of the analytical method (0.03 ppm).

Shown in Table I are the results of egg analyses for the short-term feeding experiment. Eggs collected the second day after dosing contained very low residues. At that time the hens had already received three doses equivalent to 70 ppm of ^{14}C-carbaryl in the feed. The concentration of ^{14}C residues in the yolk was about one-half of that in the white. Total ^{14}C-carbaryl equivalents gradually accumulated in the yolk and exceeded those in the white at 2.5 days after initiation of treatment. Maximum residue level in the yolk (366.8 ppb) occurred at 1 day after discontinuation of dosing, after which it declined gradually to 48.6 ppb at the end of 7 days. Peaking of residues in the white was reached earlier than in the yolk. At 2.5 days the white had the highest ^{14}C residue level of 78.2 ppb and remained practically unchanged in the 4-day samples. After discontinuation of treatments, ^{14}C residues in the white declined rapidly and approached the lower limit of determination of the radiometric method by the end of 3 days after the last dose was given. Based on the total egg mass, a peak concentration of ^{14}C residues (156.0 ppb) was reached at 1 day after discontinuation of treatment and it coincided with that of yolk maximum residues. A tenfold decrease in the level of total egg ^{14}C residues was evident at the end of 7 days after discontinuation of dosing.

A more detailed picture of the distribution of ^{14}C residues in the eggs was obtained from the continuous feeding experiment (Table III). In general, the concentration of ^{14}C residues in the yolk, white, or total egg at various times after dosing was directly proportional to the amount of ^{14}C-carbaryl fed. When the concentrations of ^{14}C-carbaryl equivalents were plotted against time on a semilogarithmic scale, the resulting curves for the three treatment levels were of a similar shape. Although the hens were equilibrated with nonradio-labeled carbaryl for 17 days prior to feeding the radio-labeled material, a second equilibration began with the labeled compound. In all the three treatments, the ^{14}C residues began to appear in the egg from the first day of feeding of ^{14}C-carbaryl and reached a plateau by the second day in the white and between the sixth to the eighth day in the yolk and in the total egg. Once the plateau was established, the egg residues remained level until the end of the 14-day feeding period.

The faster equilibration of residues in the white than in the yolk can be related to the fact that it takes the hen approximately 9 days to produce a mature yolk as compared with 1

Table IV. Concentration of ^{14}C Residues in Hen Tissues after Feeding 1-Naphthyl-^{14}C Carbaryl at the Level of 70 ppm for 4 Days

Sample	ppb at indicated times after last treatment	
	16 hr	7 days
Brain	17.3	6.8
Heart	47.5	20.9
Kidney	405.5	80.7
Pancreas	69.6	15.6
Skin	86.6	22.4
Fat	25.2	5.2
Gizzard	43.0	13.9
Thigh	28.6	10.1
Breast	25.9	12.7
Leg muscle	29.1	9.0
Blood	197.2	152.2
Lung	138.5	122.5
Liver	332.6	33.4
Spleen	108.7	74.2
Intestine and contents	300.1	<5.0
Intestinal wall	44.0	11.1
Oviduct	50.5	6.7
Developing egg (small)	534.3	7.4
Developing egg (large)	508.1	35.5
Remaining carcass	35.5	<5.0

day for the white (Romanoff and Romanoff, 1949). Thus yolks that had begun their active development several days before the feeding of ^{14}C-carbaryl began apparently had residues in the outermost layers only, while those that began their formation during the feeding period probably had residues more evenly distributed from the center to the outside.

During the state of equilibrium discussed above, the concentrations of ^{14}C-carbaryl equivalents in the egg were very small, when compared to the concentration of ^{14}C-carbaryl in the diet. Separate calculations of the ratio between the ppm of ^{14}C residues found daily in the egg to that of ^{14}C-carbaryl in the diet resulted in an average ratio of 1 to 1000 for the white, 14 to 1000 for the yolk, and 6 to 1000 for the total egg. As shown in Table III, deposition of ^{14}C residues in the yolk was much greater than that in the white. When the residues in the egg reached the plateau level, the concentration of residues in the white was approximately one-tenth of that in the yolk. However, due to difference in the mass of yolk and white, the total μg of ^{14}C-carbaryl equivalents in the white was approximately one-fifth of that in the yolk. This difference in the magnitude of residues between yolk and white suggests that different mechanisms for the deposition of ^{14}C residues in the two parts of the egg were in operation. Since it took at least 1 week for residues to reach a plateau in the egg, misleading conclusions on the degree of residue deposition in the eggs can be drawn from studies involving a single dose of carbaryl or a short-term feeding to laying hens.

Dissipation of total ^{14}C residues was studied in the eggs laid during the week after discontinuation of dosing (Table III). ^{14}C-Carbaryl equivalents declined more rapidly in the white than in the yolk. In the white radioactivity reached background by the second day after the last dose was given in all three treatments and resulted in a half-life of less than 1 day. The delayed decrease in yolk residues results from the same factors in the development of the egg that caused a lag in the appearance of residues in the yolk at the beginning of the feeding period. The lack of appearance of residues in the white after the second day after the last dose was given indicates that transfer of residues from the blood to the egg has ceased shortly after discontinuation of dosing. Yolk residues steadily declined throughout the 7-day period after discontinuation of dosing and resulted in a half-life of 2 to 3 days. At the end of 7 days yolk residues declined to below the lower limit of determination of the radiometric method (5 ppb) in the 7-ppm treatment and to 40 and 100 ppb in the 21- and 70-ppm treatments, respectively. Based on the total egg, the corresponding 7-day residue values in the latter two treatments were 10 and 30 ppb, respectively. It should be noted that these values represent the total of several metabolic components, as discussed under the egg metabolites section. Therefore, it is doubtful that conventional analytical techniques would be capable of detecting each of the individual components in the 7-day eggs even at the 70-ppm dosage level.

Distribution of total ^{14}C-carbaryl equivalents in various hen tissues after discontinuation of dosing in the short-term and the continuous feeding experiments is shown in Tables IV and V. Although the two tests are not strictly comparable, there was a good agreement in the relative distribution and the overall rate of dissipation of ^{14}C residues found in individual tissues during the 1-week period after the last dose. In addition, the pattern of distribution in various tissues was similar among the different dosage levels used in the continuous feeding test. Within 1 day after discontinuation of dosing the highest residue levels were found in the kidney, liver, blood, lung, spleen, developing eggs, and the intestinal contents. Muscle tissues, skin, fat, gizzard, heart, oviduct, pancreas, brain, intestinal wall, and the remaining carcass contained very low levels of residues, even at the 70-ppm treatment. The total ^{14}C residue contents in the latter tissues further declined and in some cases reached below the lower limit of determination of the radiometric method in the second and third slaughters (3 and 7 days after discontinuation of dosing, respectively). Total ^{14}C-carbaryl equivalents remained practically unchanged or declined only slightly in the spleen, lung, and blood. A faster rate of dissipation occurred in the liver, kidney, and the intestinal contents.

Separation and Characterization of the Metabolic Residues in the Eggs. To provide a possible explanation for the difference in total ^{14}C residues in yolk and white and also to provide a basis for evaluating the toxicological significance of the difference, the components of each egg part were analyzed. Only the egg samples collected from the 70-ppm level of the continuous feeding experiment contained sufficient radioactivity for analysis. Even then it was necessary to pool collections of yolk or white from several days. Egg radioactivity was first fractionated into organosoluble and water-soluble fractions by partitioning the aqueous extract three times with equal volumes of diethyl ether. The organic fraction was analyzed by Florisil column chromatography and the water-solubles were analyzed by the Sephadex LH-20 gel column chromatography. Since there was considerable overlap between the adjacent peaks eluted from the gel columns, the data showing the relative percentages of these peaks should be considered semiquantitative. The data showed differences in the relative distribution of the metabolites found in the two parts of the egg (Table VI). No significant change was observed in the respective distribution of the metabolic components of either the white or yolk during the 14-day feeding period; therefore, figures averaged over the entire feeding period were used to construct Table VI.

Organosoluble metabolites of yolk and white consisted of four distinct components which were separated very nicely by the Florisil column. Peaks F_1, F_2, and F_3 were separately characterized by cochromatography with authentic standards

Table V. Distribution of Radioactivity in Various Tissues of Hens Treated Twice Daily with 1-Naphthyl-^{14}C Carbaryl for 14 Days[a]

Dose (ppm)	Days[b]	ppb of ^{14}C-carbaryl equivalents in tissues				
		Liver	Spleen	Lung	Blood	Breast
7	1	60.6 ± 14.6	33.8 ± 12.9	52.5 ± 0.6	70.1 ± 23.1	<5.0
	3	27.1 ± 4.3	34.5 ± 2.5	40.7 ± 8.3	64.6 ± 11.9	<5.0
	7	13.6 ± 0.9	49.7 ± 23.8	37.3 ± 0.7	51.1 ± 11.1	<5.0
21	1	257.8 ± 0	80.1 ± 8.1	120.3 ± 29.8	182.0 ± 3.2	8.5 ± 0.4
	3	90.3 ± 38.0	77.8 ± 4.3	101.4 ± 7.6	158.9 ± 12.7	8.7 ± 2.3
	7	41.8 ± 9.8	68.6 ± 34.1	85.8 ± 45.4	170.2 ± 124.2	5.2 ± 2.2
70	1	409.5 ± 9.9	298.1 ± 19.7	272.9 ± 27.5	516.7 ± 14.2	30.7 ± 3.3
	3	255.3 ± 35.4	364.2 ± 95.5	369.8 ± 154.6	700.5 ± 36.8	24.3 ± 4.2
	7	119.5 ± 38.5	247.2 ± 50.8	363.6 ± 54.4	578.6 ± 206.9	18.9 ± 1.9
		Kidney	Skin	Fat	Gizzard	Thigh
7	1	77.0 ± 0.5	<5.0	<5.0	<5.0	<5.0
	3	43.3 ± 7.6	<5.0	<5.0	<5.0	<5.0
	7	22.5 ± 1.8	<5.0	<5.0	<5.0	<5.0
21	1	222.2 ± 12.7	12.4 ± 3.0	6.7 ± 1.8	13.2 ± 1.7	10.7 ± 0.6
	3	117.7 ± 12.4	10.7 ± 1.1	5.8 ± 1.3	11.0 ± 1.1	12.1 ± 1.1
	7	67.7 ± 33.0	12.0 ± 2.8	7.4 ± 2.2	8.0 ± 1.3	<5.0
70	1	485.4 ± 18.1	42.6 ± 10.4	26.1 ± 5.1	39.8 ± 3.1	30.0 ± 0.8
	3	305.0 ± 75.0	28.9 ± 4.0	22.1 ± 9.1	32.2 ± 7.0	31.6 ± 6.2
	7	181.8 ± 32.3	31.4 ± 2.0	18.9 ± 5.3	23.5 ± 1.3	16.9 ± 3.1
		Heart	Brain	Pancreas	Leg	Oviduct
7	1	7.8 ± 0.1	<5.0	7.3 ± 0.8	5.5 ± 0.8	<5.0
	3	<5.0	<5.0	<5.0	<5.0	8.4 ± 2.2
	7	<5.0	<5.0	6.6 ± 0	<5.0	<5.0
21	1	18.7 ± 2.1	6.7 ± 0.9	21.6 ± 6.3	10.4 ± 2.3	17.2 ± 1.3
	3	13.3 ± 0.1	<5.0	16.5 ± 4.9	7.9 ± 2.5	16.8 ± 2.1
	7	11.9 ± 7.4	<5.0	18.3 ± 12.5	9.6 ± 5.9	11.6 ± 5.8
70	1	49.1 ± 1.9	17.2 ± 2.8	62.5 ± 6.4	32.2 ± 1.1	42.5 ± 1.6
	3	55.0 ± 2.3	16.8 ± 2.3	39.8 ± 2.9	27.1 ± 5.1	49.3 ± 4.9
	7	40.1 ± 8.8	11.1 ± 3.5	40.6 ± 29.2	25.2 ± 5.4	31.7 ± 4.4
		Developing eggs (large)	Developing eggs (small)	Intestinal wall	Intestine and contents	Remaining carcass
7	1	42.5 ± 13.5	28.0 ± 0.8	<5.0	24.7 ± 3.9	5.1 ± 0.9
	3	35.2 ± 3.8	9.4 ± 2.0	<5.0	<5.0	6.9 ± 4.5
	7	<5.0	<5.0	<5.0	<5.0	<5.0
21	1	125.1 ± 1.1	91.7 ± 4.2	20.7 ± 6.3	6.51 ± 33.4	22.6 ± 10.3
	3	104.2 ± 0	37.7 ± 5.9	10.7 ± 0.8	13.6 ± 5.4	14.5 ± 6.9
	7	6.5 ± 3.5	<5.0	9.7 ± 1.7	<5.0	15.1 ± 8.9
70	1	303.9 ± 18.8	259.0 ± 48.4	61.1 ± 4.9	261.5 ± 110.9	58.5 ± 10.8
	3	390.5 ± 33.1	127.1 ± 6.1	25.6 ± 0.7	39.8 ± 11.5	45.3 ± 8.3
	7	24.0 ± 12.3	11.1 ± 1.5	23.9 ± 3.7	20.9 ± 7.0	35.2 ± 2.4

[a] Hens were given nonradioactive carbaryl at the level of 7, 21, and 70 ppm for 17 days followed by 14 days with ^{14}C-carbaryl. [b] Days after last treatment.

using two-dimensional tlc in several solvent systems. Peak F_1 cochromatographed with 1-naphthol, peak F_2 with carbaryl, and peak F_3 with 1-naphthyl(hydroxymethyl)carbamate. Additional evidence for the identity of peak F_1 as 1-naphthol was obtained by its reaction with methylisocyanate to yield a component chromatographically identical to carbaryl. As expected, alkaline hydrolysis of peaks F_2 and F_3 resulted in the formation of 1-naphthol. Acid hydrolysis of peak F_3 resulted in the formation of 1-naphthyl carbamate. Peak F_4 eluted from the column in the methanol fraction. When analyzed by tlc, it was found that it consisted of several products which did not correspond to any of the available standards. Peak F_4 was pooled from several chromatograms and subjected to gel column chromatography as described for the water-solubles. It was found that peak F_4 contained metabolic products similar to those described later as water-solubles. Therefore, peak F_4 consisted of a small portion of the water-soluble metabolites which was carried over into organic solvent during the partitioning step. Subsequently peak F_4 was added to the water layer before analysis with the gel column.

Most of the radioactivity extracted from yolk (72.9%) and white (92.8%) remained in the aqueous phase after diethyl

Table VI. Metabolic Products Found in the Eggs of Hens Fed 1-Naphthyl-^{14}C Carbaryl for 14 Days at a Level Equivalent to 70 ppm in the Diet

Chromatographic fractions	Identity	% of the recovered radioactivity		
		Yolk (Y)	White (W)	Y + W
F_1	1-Naphthol	17.7	6.3	15.8
F_2	Carbaryl	4.6	0.7	3.9
F_3	1-Naphthyl(hydroxymethyl)-carbamate	4.9	0.2	4.1
S_1	...	2.1	3.5	2.4
S_2	Unknown A	3.1	8.4	4.0
S_3	Unknown B	7.0	46.3	13.8
S_4	1-Naphthol conjugate	2.7	5.6	3.2
S_5	1-Naphthyl sulfate	44.1	15.6	39.1
S_6	1-Naphthol conjugate	5.0	5.7	5.1
S_7	Unknown B conjugate	8.8	7.7	8.6
Average total μg of ^{14}C-carbaryl equivalents per egg[a]		16.3	3.4	19.7
Average ppm of ^{14}C-carbaryl equivalents[a]		1.0	0.1	0.4

[a] Based on eggs collected after equilibration was established; *i.e.*, between ninth and the fourteenth day of dosing.

Table VII. Thin-Layer Chromatographic Behavior of the Egg Metabolites Unknown A and B as Compared to the Standards

Chemicals	R_f values in solvent systems: Ethyl acetate–benzene (3:1)	Isopropyl ether–acetonitrile (5:1)
Unknown A	0.21	0.16
Unknown B	0.52	0.39
5,6-Dihydro-5,6-dihydroxycarbaryl	0.36	0.25
5,6-Dihydro-5,6-dihydroxy-1-naphthol	0.62	0.48

ether partitioning. Column chromatography with Sephadex LH-20 eluted with a series of acetone–methanol mixtures provided some cleanup and separation of the major water-soluble metabolites. Seven peaks were obtained in each of the yolk and white samples analyzed. Further examination of the radioactivity present in each peak was accomplished by tlc directly or after enzymatic (Glusulase) and acid hydrolyses.

Analysis of peak S_1 by tlc showed most of the radioactivity in this fraction was contamination from peaks S_2 and S_3 due to incomplete separation and the remainder as free 1-naphthol. The latter compound was probably generated from the other water-soluble metabolites during the workup of the sample.

Peaks S_2 and S_3 (hereafter referred to as Unknowns A and B, respectively) eluted from the gel column as two partially resolved peaks in the 5:1 acetone–methanol solvent. Sufficient quantity of these two products was obtained from several column runs and pooled for further analysis. Chromatography of the combined material on a Florisil column provided additional cleanup and separation of the two metabolites. The Florisil column was developed with the following solvents: 400 ml each of 1:1, 2:1, and 3:1 mixtures of diethyl ether–hexane; 1500 ml of diethyl ether; and finally with 500 ml of 9:1 ether–methanol. The two unknowns eluted in the ether–methanol fraction from the Florisil column as two distinctly separated peaks. It is noteworthy that the two unknowns reversed their order of elution from that observed with the Sephadex column. The two unknowns were subjected to the action of the enzyme Glusulase and the resulting products characterized by tlc. Based on their R_f values it was concluded that the enzyme did not attack the two compounds. However, trace amounts of material characterized by cochromatography on tlc as 1,4-naphthoquinone were formed in the enzyme as well as in the control (boiled enzyme) incubations of the two unknowns. Chromatography on tlc in several solvent systems showed Unknowns A and B to travel near, but not identically with, authentic samples of the known carbaryl metabolites 5,6-dihydro-5,6-dihydroxycarbaryl and 5,6-dihydro-5,6-dihydroxy-1-naphthol, respectively (Table VII). Also the chromatographic behavior of Unknown A relative to Unknown B was very similar to that of 5,6-dihydro-5,6-dihydroxycarbaryl relative to its phenol. Accordingly, it was thought that Unknown A could be the *N*-methylcarbamate derivative of Unknown B. However, attempts were not successful in generating Unknown B from Unknown A by alkaline hydrolysis (0.1 and 0.5 *N* aqueous NaOH at 50°C for 30 min). Under these conditions the two unknowns were degraded to material remaining at the origin of tlc plates developed in either diethyl ether–hexane (3:1), methylene chloride–acetonitrile (5:1), or methylene chloride–ethyl acetate (1:1).

Peaks S_4 and S_5 eluted from the gel column in the 3:1 acetone–methanol solvent. Peak S_4 was a minor component in both yolk and white samples and came off the column as a shoulder overlapping peak 5. Thus, most of the radioactivity in peak S_4 was a part of the larger peak S_5. A second run of the two peaks through the column resulted in a better separation of these two fractions. That peaks S_4 and S_5 were water-soluble conjugated metabolites became evident when they were chromatographed on tlc before and after enzyme glusulase hydrolysis. Peak S_4 was hydrolyzed poorly by the enzyme, however, yielding only 5% of the radioactivity as 1-naphthol. Most of the unhydrolyzed portion was cleaved by further treatment with 1.0 *N* HCl at 100°C for 1 hr to yield 1-naphthol as a product. In both cases the aglycone was characterized by cochromatography with standard 1-naphthol on two-dimensional tlc. Peak S_5, on the other hand, was hydrolyzed very efficiently by either the enzyme Glusulase and/or by 1.0 *N* HCl at 100°C for 1 hr to yield 1-naphthol as aglycone. Identity of peak S_5 was established as 1-naphthyl sulfate by cochromatography of the radioactivity in this fraction with an authentic sample of 1-naphthyl sulfate (potassium salt, Sigma Chemical Co., St. Louis, Mo.) on two-dimensional tlc using $CHCl_3$–methanol (3:1) plus 10% acetic acid and acetone–1-butanol–0.1 *M* boric acid (5:4:1). Since Paulson *et al.* (1970) reported several salt forms of 1-naphthyl sulfate were formed by hens treated with 1-naphthyl-^{14}C-carbaryl, the radioactivity in peak S_5 and the standard 1-naphthyl sulfate were dissolved in water and slightly acidified with 1 *N* HCl prior to chromatography on tlc.

Peaks S_6 and S_7 eluted from the gel column in the 1:1 acetone–methanol. Normally the two components chromatographed without complete resolution. A second pass through the column provided additional separation of the two peaks. That peaks S_6 and S_7 were conjugated metabolites was evident by their susceptibility to enzymatic (Glusulase) hydrolysis. From 20 to 30% of the radioactivity was cleaved by the enzyme to yield organosoluble aglycones. In the case of peak S_6 the aglycone was characterized by tlc as 1-naphthol. Peak S_7 yielded an aglycone similar to that of Unknown B.

To derive conclusions concerning the distribution of the above-mentioned metabolic products between yolk and white, the average absolute amount of ^{14}C-carbaryl equivalents in each part of the egg was calculated. These calculations were made on eggs collected from the 70-ppm treatment level after the equilibrium of ^{14}C residues was reached, *i.e.*, between the ninth day to the fourteenth day of dosing. On a per egg basis, total ^{14}C residues were 16.3 and 3.4 μg in the yolk and white, respectively. Because of this difference the metabolic products were compared on the basis of their relative percentages (Table VI). Carbaryl, 1-naphthol, and 1-naphthyl-(hydroxymethyl)carbamate totaled 27.2% of yolk radioactivity, as compared to 7.2% of that in the white. The low water solubilities of these compounds perhaps contributed to their preferential accumulation in the yolk over the white. Among these organosoluble products, 1-naphthol was the most abundant in both yolk and white. Its absolute amount in the 70-ppm treatment averaged 3 μg per egg, while carbaryl and 1-naphthyl(hydroxymethyl)carbamate each was approximately 0.8 μg per egg.

Naphthyl 1-sulfate (peak S_5) was the largest single component of egg residues and the determining factor in the higher concentration of residues in the yolk than in the white. It accounted for 44.1 and 15.6% of yolk and white, respectively. Based on the average micrograms of ^{14}C-carbaryl equivalents recovered from the egg in the 70-ppm dosing level, its absolute amounts were 7.2 μg in the yolk and 0.5 μg in the white. The mechanism leading to the preferential accumulation of this

metabolite in egg yolk has not been determined in the present study. One possible explanation is that 1-naphthyl sulfate ion may be attracted to phospholipids of yolk, or the permeability of the membrane surrounding the yolk in the oviduct may be such that it permits a one-directional movement of 1-naphthyl sulfate into the yolk. Similar factors may also be involved in the higher quantities of peaks S_6 and S_7 in the yolk than in the white.

Unknowns A and B represented a larger percentage of the recovered radioactivity in the white than in the yolk. In particular, Unknown B constituted 46.3% of the white residues, as compared to 7.0% of that in the yolk. Because of the much larger total ^{14}C residues in yolk, the large percentage of Unknowns A and B in the white had relatively little effect on the overall greater amount of total ^{14}C residues in the yolk.

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Chronic Toxicity and Residues from Feeding Nemacide [*O*-(2,4-Dichlorophenyl) *O,O*-Diethyl Phosphorothioate] to Laying Hens

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Technical grade Nemacide [*O*-(2,4-dichlorophenyl) *O,O*-diethyl phosphorothioate] was administered in the feed of laying hens for 55 weeks at 50, 100, 200, and 800 ppm. Excellent control of the housefly, *Musca domestica* L., and the blowfly, *Chrysomya megacephala* (F.), was obtained in droppings from the hens fed 50 ppm but higher levels were required for control of larvae of *Parasarcophaga argyrostoma* (Robineau-Desvoidy) and *Fannia pusio* (Wiedemann). No hen mortality occurred that could be attributed to treatment but there was a direct relationship between Nemacide concentration and blood plasma cholinesterase inhibition. Feed consumption, weight gain, egg production, shell thickness, egg weight, egg quality, and odor were normal but eggs from hens treated with 800 ppm had a less desirable flavor than eggs from untreated hens. A glc electron capture method was developed to measure nanogram amounts of Nemacide and 2,4-dichlorophenol. The limits of detectability were 0.005 to 0.086 ppm for Nemacide and 0.006 to 0.208 ppm for 2,4-dichlorophenol. Appreciable residues of Nemacide were found in the liver, muscle, fat, and yolk of treated hens, while 2,4-dichlorophenol residues were detected only in the liver and yolk.

The ingestion of certain insecticides over a prolonged period of time by means of treated feed or water was found to have little detrimental effect on poultry, yet prevented the development of fly larvae in the droppings (Ross and Sherman, 1960; Sherman and Ross, 1960b; Sherman *et al.*, 1963, 1969). Sherman *et al.* (1967b) found the acute toxicity of Nemacide to 10- to 12-day-old cockerels to be 148 mg/kg and that an admixture of 800 ppm in the feed administered over a 2-week period, although causing 60% plasma cholinesterase inhibition, had no other detrimental effects on cockerels. Droppings from these treated chicks were highly toxic to the larvae of four species of flies (Sherman *et al.*, 1967a). The present paper reports the effects on laying hens of technical grade Nemacide [*O*-(2,4-dichlorophenyl) *O,O*-diethyl phosphorothioate] administered in the feed at 50, 100, 200, and 800 ppm over a 55-week period.

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TOXICOLOGICAL PROCEDURES

One-hundred and fifty pullets of a commercial strain of single-comb White Leghorns were used in this study. At 27 weeks of age, the pullets were leg-banded, weighed, and distributed at random into five treatment groups of 30 birds each. Each group was divided into three replicates of ten birds each. The birds were housed in individual laying cages, two birds per cage. The basal diet was a standard University of Hawaii layer ration (Sherman *et al.*, 1969). Technical grade Nemacide was added to the basal ration at the rates of