glucuronides. Base hydrolysis helps support this conclusion in that methyl and carbonyl labels are lost but the base hydrolyzed metabolite containing the naphthyl label chromatographs with the original metabolite. Silica gel chromatography of glucuronides found in the rat urine does not adequately resolve glucuronides from each other but does resolve glucuronides from other urinary metabolites. Finally, β-glucuronidase treatment of the metabolite results in 90% or greater hydrolysis as determined by DEAE–cellulose analysis, while acid hydrolysis converts the metabolite to a new anionic compound chromatographing with a known glucuronide.

It must be concluded from these studies that conjugation is to the ring, with the majority of the chromatographic peaks being a conjugate of 5,6-dihydro-5,6-dihydroxycarbaryl. No evidence was found to support the proposed structure 1-naphthyl methylimidocarbonate *O*-glucuronide.

DISCUSSION

This study on the metabolism of carbaryl in the rat at 30 mg/kg as a single oral dose has qualitatively reproduced the chromatographic profile of urines of rats on diethylaminoethylcellulose columns of single oral and intraperitoneal dose studies obtained by Knaak *et al.* (1965). Quantitatively, the results in this study show a shift in distribution with an increase in the percentage of materials chromatographing as metabolite(s) D and a corresponding decrease in materials chromatographing as naphthyl glucuronide and naphthyl sulfate. A higher percentage of the methyl and carbonyl labels was found in the urine with first-day results in the present study showing 68, 70, and 75% of the dose for the naphthyl, methyl, and carbonyl labels, respectively. In an unpublished study in which nonlabeled carbaryl was incorporated into the diet at 15 mg/kg body wt/day, for at least 15 days, substitution of naphthyl or methyl-labeled carbaryl in one day's diet gave quantitative results duplicating the studies reported here. Peaks chromatographing as naphthyl glucuronide and naphthyl sulfate collectively accounted for 14.5% of the dose using the naphthyl label. Metabolite D in these feeding studies accounted for 28 and 30% of the dose for naphthyl and methyl labels, respectively. Thus, the single oral dose study reported here is probably representative of the results to be obtained when carbaryl is incorporated into the diet in chronic studies.

If the percentages of losses, unhydrolyzed glucuronide, etc., are distributed proportionately over all the aglycones, the 5,6-dihydro-5,6-dihydroxycarbaryl glucuronide would account for 40–50% of metabolite(s) D. The principal aglycone of metabolite(s) D on a proportionment of losses basis represents 10 to 11% of the dose as carbaryl equivalents with an additional 1.4% of the dose excreted as unconjugated 5,6-dihydro-5,6-dihydroxycarbaryl.

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Fly Control, Chronic Toxicity, and Residues from Feeding 2-Chloro-1-(2,4-dichlorophenyl)vinyl Diethyl Phosphate to Hens

Martin Sherman* and Raymond B. Herrick

Technical grade Compound 4072 [2-chloro-1-(2,4-dichlorophenyl)vinyl diethyl phosphate] was administered in the feed of laying hens for 52 weeks at 50 and 200 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. No hen mortality occurred due to treatment but blood plasma cholinesterase activity was inhibited. Feed consumption, weight gain, shell thickness, egg weight, egg flavor, and odor were normal, but treatment affected egg production. Glc methods were developed to measure nanogram amounts of Compound 4072 and 2,2′,4′-trichloroacetophenone by phosphorus and electron capture detection, respectively. The limits of detectability in the various tissues ranged from 0.002 to 0.020 ppm for Compound 4072 and from 0.002 to 0.009 ppm for 2,2′,4′-trichloroacetophenone. No residues of Compound 4072 were detected, but low levels of 2,2′,4′-trichloroacetophenone were found in liver, fat, and egg yolk.

The oral administration of a number of insecticides over a lengthy period of time by means of treated feed was found to have little detrimental effect on poultry, yet it prevented the development of fly larvae in the droppings (Sherman *et al.*, 1972). Sherman *et al.* (1967a) found the acute toxicity of Compound 4072 to 10- to 12-day-old cockerels to be 29 mg/kg and that an admixture of 800 ppm in the feed given for 2 weeks had no detrimental effect on cockerels other than causing between 45 and 25% plasma cholinesterase inhibition. Droppings from these treated chicks were highly toxic to the larvae of four species of flies

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(Sherman *et al.*, 1967b). This paper reports on the effects on laying hens of technical grade Compound 4072 [2-chloro-1-(2,4-dichlorophenyl)vinyl diethyl phosphate] administered in the feed at 50 and 200 ppm for 52 weeks.

TOXICOLOGICAL PROCEDURES

Seventy-five pullets of a commercial strain of single-comb White Leghorns were used in this study. The experiment was initiated when the birds were 36 weeks of age. The pullets were leg banded, weighed, and distributed at random into three treatment groups of 25 birds each. Each group was divided into five replicates of five birds each. These birds were all caged individually. The basal diet was a standard University of Hawaii layer ration (Sherman *et al.*, 1969). Technical grade Compound 4072 was added to the basal ration at the rates of 50 and 200 ppm and given to two of the treatment groups. The third group of birds was designated as the control and continued to receive untreated feed and fresh water. The birds were placed on the insecticide-treated diet for a total of 52 weeks.

Manure was collected, approximately biweekly, from dropping pans set beneath the cages and the toxicity to first-instar larvae of the housefly, *Musca domestica* L., an anthomyid, *Fannia pusio* (Weidemann), a blowfly, *Chrysomya megacephala* (F.), and a flesh fly, *Parasarcophaga argyrostoma* (Robineau-Desvoidy), was determined by methods described by Sherman and Ross (1960a,b) and Sherman *et al.* (1962). In order to determine whether there was any significant difference between larval mortality and adult emergence, the larval mortality was determined after 3 days and then the surviving larvae were replaced in the droppings and held for adult emergence. *F. pusio* mortality was determined after adult emergence only because the larvae were so well camouflaged in the manure that they were difficult to detect.

Egg production was recorded daily for all birds, while feed consumption and individual body weights were obtained every 4 weeks during the course of the experiment. For measuring egg weight, albumen height (calculated as Haugh units), and shell thickness, eggs were collected on 1 day every 4 weeks throughout the course of the experiment. More than 400 eggs from treated hens were involved in these determinations. Organoleptic tests on eggs as described by Sherman *et al.* (1972) were conducted after the birds had been on experiment for 14 and 37 weeks.

One of the replicates from each treatment group was used to determine the effect of treatment of blood plasma cholinesterase activity. Blood was obtained by venipuncture prior to treatment 1, 2, 5, 7, 14, and 21 days after the start of treatment, and biweekly for the duration of the experiment. Blood was also sampled 1, 2, 4, and 14 days after the hens were withdrawn from treated feed and placed on the untreated control ration. The blood plasma was analyzed colorimetrically as described by Cook (1954) and Fallscheer and Cook (1956).

Statistical analyses were made where applicable using Abbott's formula (Abbott, 1925), analysis of variance (Snedecor, 1946), and Duncan's multiple range test (Duncan, 1955).

Eggs were collected periodically throughout the course of the experiment. Whites and yolks were separated and quick frozen. One year after the start of the experiment, eight hens from each of the treatments and control were slaughtered; the liver, breast muscle, and peritoneal fat were removed, placed separately in plastic bags, and stored at $-10°$ for future analysis. All remaining animals were removed from treated feed and placed on untreated feed. An additional three to eight animals from each of the treated and control groups were slaughtered 7, 15, and 21 days after removal from the treated feed. During the period that the hens were on untreated feed eggs were taken from the surviving hens and collected for analysis.

ANALYTICAL PROCEDURES

Compound 4072 was detected by analytical procedures consisting of sample extraction, cleanup, and glc analysis, utilizing a phosphorus-sensitive thermionic detector. The metabolite, 2,2′,4′-trichloroacetophenone, was detected by similar methods using the electron capture detector.

Solvents were reagent grade and redistilled shortly before use; other common chemicals also were reagent grade. The Florisil, 60/100 mesh, obtained from Matheson Coleman and Bell was activated at 600° for 3 hr. Standardized aluminum oxide was prepared as follows. Baker aluminum oxide for chromatographic work was activated at 800° for 4 hr. After cooling, 5% distilled water was added, distributed by shaking the bottle until no lumps appeared and rolling the bottle on a jar mill overnight. The aluminum oxide was used within 2 weeks after standardization. The reference standard solutions were prepared in hexane with analytical standards (99+ %) of 2-chloro-1-(2,4-dichlorophenyl)vinyl diethyl phosphate and 2,2′,4′-trichloroacetophenone.

Extraction and Cleanup. The acetonitrile and hexane used in back extraction procedures were saturated with each other.

For analysis of egg yolk, 20.0 g was blended with 200 ml of 95% ethanol at low speed until uniform, 10 ml of hexane was added, and the mixture was blended at high speed for 1 min. The mixture was transferred to a 500-ml separatory funnel, vigorously shaken, and allowed to separate overnight. The water phase was extracted five times with 20 ml of hexane and the combined hexane fractions were back extracted four times with 50 ml of acetonitrile (containing 2% distilled water, v/v). The hexane phase was discarded; 200 ml of aqueous 2% sodium sulfate solution was added to the acetonitrile, shaken vigorously for 2 min, and back extracted three times with 50 ml of hexane. The hexane was passed through a 3-cm anhydrous sodium sulfate layer in a funnel (5 mm top i.d.), combined, and the filtrate was evaporated to 2–3 ml on a Rotovapor. The residue was chromatographed on 10 g of Florisil in a 450 × 16 mm glass column containing a 1.5-cm layer of anhydrous sodium sulfate above and below the Florisil. The column had previously been wetted with 20 ml of hexane, which was discarded. 250 ml of hexane was used as an initial eluant and discarded. The receiver was changed and the column was eluted with 200 ml of 1.5% acetone in hexane. The extract from this eluate contained the trichloroacetophenone and was evaporated to 2–3 ml and chromatographed on 20 g of standardized aluminum oxide in a 450 × 16 mm glass column containing a 1.5-cm layer of anhydrous sodium sulfate above and below the aluminum oxide using 150 ml of hexane after first rinsing the column with 50 ml of hexane, which was discarded. This eluate was evaporated to 2–3 ml, transferred to a 10-ml volumetric flask, brought to volume, and analyzed for 2,2′,4′-trichloroacetophenone by glc. The Florisil column was further eluted with 200 ml of 1% acetone in methylene chloride; the eluate was evaporated to 2–3 ml in the Rotovapor, then to dryness with dry air to eliminate the methylene chloride, taken up in hexane, transferred to a 10-ml volumetric flask, brought to volume, and analyzed for Compound 4072 by glc.

For analysis of egg white, 50.0 g were ground in a mortar with 300 g of anhydrous sodium sulfate and packed in a 600 × 40 mm glass column. The column was eluted slowly with 500 ml of hexane and the eluate was evaporated to 2–3 ml and chromatographed on a Florisil column with: 300 ml of hexane, 150 ml of 1.5% acetone in hexane, and 150 ml of 1% acetone in methylene chloride. The three eluate fractions were treated as with egg yolks except that the Compound 4072 eluate was brought to a 5-ml volume prior to glc.

For analysis of liver, 25.0 g were ground in a mortar with 300 g of anhydrous sodium sulfate and packed in a 600 × 40 mm glass column. The column was eluted with 500 ml of hexane and the eluate was evaporated to 50 ml. The eluate was extracted five times with 20 ml of acetonitrile and the hexane fraction was discarded. The combined acetonitrile fractions were partitioned two times with 20 ml of hexane. The acetonitrile was transferred to a 500-ml separatory funnel; the 40 ml of hexane was back extracted two times with 20 ml of acetonitrile and separated, the hexane fraction was discarded, and the acetonitrile was combined with the acetonitrile in the separatory funnel. 300 ml of 2% sodium sulfate solution was added to the acetonitrile and shaken vigorously for 2 min. The upper hexane layer was passed through a funnel containing a 3-cm layer of anhydrous sodium sulfate into a boiling flask. The lower acetonitrile–water phase was back extracted four times with 50 ml of hexane. After separation, the acetonitrile–water phase was discarded, the hexane phases were combined, evaporated to 2–3 ml, chromatographed on Florisil, and treated as described for egg white.

Peritoneal fat was melted in an oven at 65°, passed through filter paper (Whatman No. 1), and 10.0 g was dissolved in 20 ml of hexane and transferred to a 250-ml separatory funnel by rinsing three times with 10 ml of hexane and twice with 10 ml of acetonitrile. After partitioning the hexane and acetonitrile phases, the fat–hexane solution was extracted four times with 20 ml of acetonitrile. The hexane phase was discarded; 400 ml of 2% sodium sulfate solution was added to the acetonitrile in a 500-ml separatory funnel and shaken vigorously for 2 min. After 20 min, the separated hexane phase was saved and the water phase was extracted twice with 10 ml of hexane and discarded. The combined hexane phases were passed through a 3-cm layer of anhydrous sodium sulfate in a funnel, chromatographed, and treated as described for egg white.

For analysis of breast muscle, 100.0 g were cut into small pieces, blended at low speed for 30 sec in a Waring blender with 20 g of anhydrous sodium sulfate and 250 ml of acetone, transferred to a Buchner funnel, and filtered under slight vacuum into a liter flask. The blender was rinsed with acetone and the rinse was poured over the filter cake and combined with the acetone. The filter cake was returned to the blender, blended for 30 sec with 150 ml of hexane, and filtered through the Buchner funnel. The acetone extract was evaporated to 50 ml, transferred to a 500-ml separatory funnel, and diluted with 200 ml of 5% sodium sulfate solution. This solution was extracted twice with 75 ml of the filtered hexane extracts and once with 75 ml of pure hexane. The hexane was combined, filtered through a funnel containing a 3-cm layer of anhydrous sodium sulfate, evaporated to 50 ml, transferred to a 250-ml separatory funnel, and extracted five times with 20 ml of acetonitrile. The hexane was discarded; the acetonitrile was evaporated to 10 ml, transferred to a 250-ml separatory funnel, diluted with 30 ml of 5% sodium sulfate solution, and back extracted two times with 10 ml of hexane. The diluted acetonitrile was discarded and the separatory funnel was rinsed three times with 5 ml of hexane, which was passed over anhydrous sodium sulfate and combined with the other hexane. The hexane was evaporated to 2–3 ml, chromatographed, and treated as described for egg yolk.

Gas Chromatography. The presence of Compound 4072 was determined by the use of an Aerograph Autoprep Model 705 with a thermionic phosphorus detector using a cesium bromide pellet. This Autoprep was used as an analytical instrument by plugging the effluent splitter unit so that all the carrier gas went through the detector. The operating parameters follow: detector voltage, 285 V; column length, 1.5 m × 3.18 mm i.d., borosilicate glass; column packing, 2% diethylene glycol succinate (DEGS) on 60/80 mesh Dow-Corning Gas Chrom Q; purified nitrogen carrier gas, 21–27 ml/min; air, 190–210 ml/min; hydrogen, 22–26 ml/min; injector temperature, 203–225°; column temperature, 168–195°; detector temperature, 190–215°; recorder, 1 mV; full chart deflection, 25.4 cm; and chart speed, 4.23 mm/min.

In analyzing for 2,2′,4′-trichloroacetophenone, a combination of Aerograph instruments was used. A Model 600 electrometer was combined with a Model 550 B oven which was monitored by a Linear Temperature Programmer Model 326. The instruments were equipped with a tritium foil electron capture detector. The operating parameters follow: detector voltage, 90 V; column length, 1.5 m × 3.18 mm i.d., all Pyrex glass; column packing, 2% SE30 on 100/120 mesh Gas Chrom Q; purified nitrogen carrier gas, 25–35 ml/min; injector temperature, 210–220°; column temperature, 178–205°; detector temperature, 170–210°; recorder, 1 mV; full chart deflection, 12.7 cm; and chart speed, 12.7 mm/min.

All samples and standard solutions of Compound 4072 were injected with a volume of either 5 or 10 μl and the acetophenone was injected with a constant volume of 2 μl. The retention time for Compound 4072 was between 1.4 and 7.8 min and a linear dynamic range was shown between 0.3 to 3 ng. The retention time for 2,2′,4′-trichloroacetophenone was between 0.6 and 1.25 min and a linear dynamic range was shown between 0.03 to 0.3 ng. Peak heights were found to be directly proportional to concentration and were used for quantitation. Peak heights were measured to an accuracy of 0.5 mm.

Each series of analysis was composed of a reference standard, a tissue control sample, a fortified tissue control sample, and four to six unknown tissue samples. Injections of each sample were repeated until the maximum peak height variation was less than 2%. Each series of analysis was initiated and ended with the injection of the reference standard solution and, if more than 5% variation occurred between the initial and final reference standard peak heights, the series of analysis was repeated.

RESULTS AND DISCUSSION

Fly Mortality. Table I summarizes the toxicity of droppings from the hens to larvae of *M. domestica*, *F. pusio*, *C. megacephala*, and *P. argyrostoma* 3 days after placement into the droppings and after adult emergence. The feeding of Compound 4072 at 200 ppm gave far better control of the maggots than at 50 ppm. But even at 50 ppm, the manure was highly toxic to the housefly and blowfly if the flies were allowed to remain until adult emergence.

Hen Mortality. No mortality occurred in the hens that could be attributed to the administration of Compound 4072. During the experimental period, 16% mortality occurred in animals receiving the untreated control diet and 200 ppm of Compound 4072, while 32% mortality occurred in the hens

Table I. Toxicity to Fly Larvae of Droppings from Hens Receiving Compound 4072-Treated Feed

Compound 4072 concentration in feed, ppm	Corrected % mortality[a]			
	M. domestica	*F. pusio*[b]	*C. megacephala*	*P. argyrostoma*
	After 3 days			
200	95.2	...	96.9	88.3
50	66.6	...	67.9	47.5
0	10.5	...	10.2	10.0
	After adult emergence			
200	100	92.0	100	100
50	96.7	36.2	93.9	74.8
0	12.8	9.4	17.8	17.2

[a] Mean values based on 78 replications of ten larvae each. Corrected for natural mortality by Abbott's formula. [b] Mortality determined only after adult emergence.

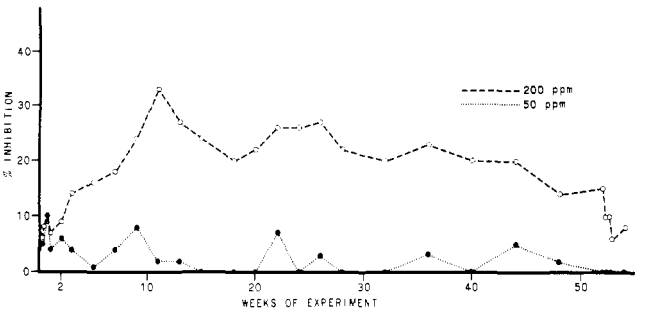

Figure 1. Inhibition and recovery of blood plasma cholinesterase in White Leghorn hens treated with Compound 4072. Hens removed from treated feed after 52 weeks

on the 50-ppm Compound 4072 diet. Autopsy showed that death was attributable to avian lymphoid leucosis in 47% of the cases.

In Vivo **Cholinesterase Inhibition.** Figure 1 summarizes the effect on blood plasma cholinesterase activity of treatment with Compound 4072 and withdrawal from treatment. Ingestion of feed containing 50 ppm of Compound 4072 had a negligible effect on activity. On the other hand, hens receiving 200 ppm of Compound 4072 showed a significant reduction in cholinesterase activity. Maximum inhibition, 33%, occurred 11 weeks after placement on treatment. Inhibition dropped and fluctuated between 14 and 27% during the rest of the experimental period. Recovery of enzyme activity was rapid; within 4 days of removal of Compound 4072 from the diet, normal levels of activity were reached.

Body Weight. There was no detrimental effect on body weight caused by ingestion of Compound 4072. There were appreciable differences in the average initial body weight of birds in the three treatment groups: 1794, 1699, and 1879 g for the birds fed 0, 50, and 200 ppm of Compound 4072, respectively. The untreated control hens gained 48 g during the experimental period, while the hens fed 50 ppm of Compound 4072 lost 2 g and those fed 200 ppm gained 72 g. These differences in weight gain were not statistically significant.

Feed Consumption. Birds receiving the 50-ppm level of Compound 4072 consumed significantly less ($p < 0.01$) feed than control birds, but birds receiving 200 ppm of Compound 4072 consumed significantly more ($p < 0.01$) feed than control birds. These differences in feed consumption appear to be

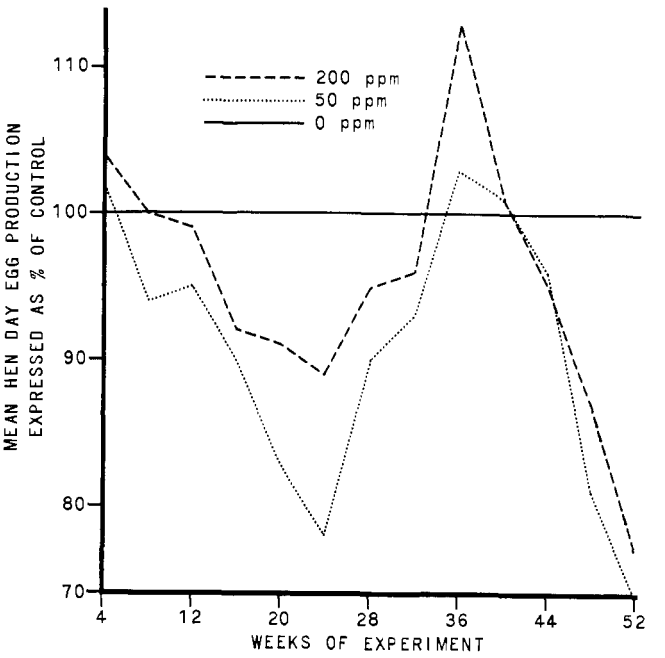

Figure 2. Mean egg production of White Leghorn hens receiving Compound 4072-treated feed compared with the untreated control hens

related to the differences in body weight and not due to treatment. The mean feed consumed per hen per day was 103.5 g for the control birds, 94.9 g for the birds receiving 50 ppm of Compound 4072, and 105.8 g for the birds receiving 200 ppm. The mean daily intake of Compound 4072 among birds receiving 50 and 200 ppm in the diet was 4.7 mg/hen and 71.2 mg/hen, respectively.

Egg Production. Figure 2 expresses egg production of the insecticide-treated hens compared with that of the control hens. Hens on both Compound 4072-treated diets produced significantly less ($p < 0.01$) eggs than did the hens on the untreated control diet. The lower production of Compound 4072-treated hens would seem to indicate a detrimental treatment effect. However, this detrimental effect does not appear to be dosage-related since birds receiving 50 and 200 ppm in the diet produced at the rate of 61.4 and 64.5%, respectively, while control hens produced at the rate of 68.0%.

Economic Factors. Table II summarizes the effects of Compound 4072 on various factors of economic importance. The continuous administration of the insecticide-treated feed had no detrimental effect on shell thickness. Although the eggs laid by hens fed 50 ppm of Compound 4072 weighed significantly less than the control eggs, the eggs laid by the birds receiving 200 ppm were significantly heavier than the control eggs. However, these differences probably are due to the differences in hen body weight rather than to the treat-

Table II. Mean Shell Thickness, Weight, and Interior Quality of Eggs[a] Produced by White Leghorn Hens Receiving Different Levels of Compound 4072-Treated Feed for 52 Weeks

Compound 4072 concentration in feed, ppm	Shell thickness, 0.001 cm	Egg weight, g	Albumen height, Haugh units
200	33.8	60.9[b]	78.4[c]
50	33.8	57.4[c]	79.1
0	34.0	59.0	81.1

[a] Collections made 1 day every 4 weeks during the experiment. 430 eggs were analyzed. [b] Significantly greater than control ($p < 0.05$). [c] Significantly less than control ($p < 0.05$).

Table III. Organoleptic Evaluation[a] of Hard-Cooked Eggs from Hens Receiving Compound 4072-Treated Feed

Compound 4072 concentration in feed, ppm	Flavor	Odor
	14 weeks on experiment	
200	5.61	5.61
50	5.50	5.56
0	5.64	5.39
	37 weeks on experiment	
200	5.48	5.42
50	5.67	5.61
0	5.52	5.42

[a] Mean values based on an 11-member taste panel scoring 6 for the highest quality and 1 for the lowest. Differences caused by treatment were not statistically significant.

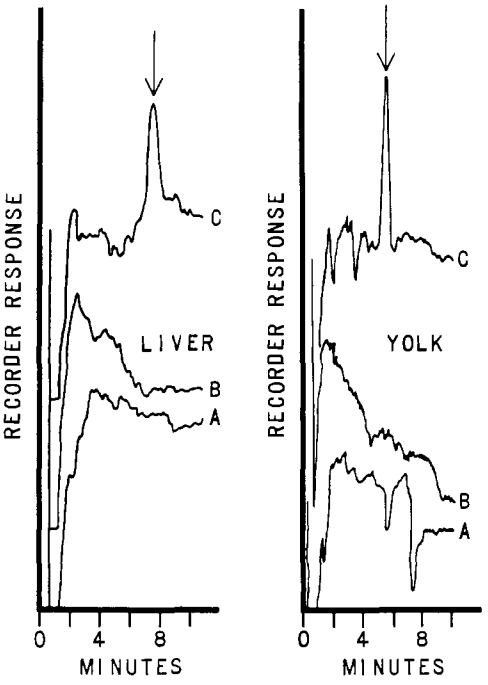

Figure 3. Typical chromatograms of Compound 4072 in liver and yolk using a thermionic phosphorus detector. A. From control hen. B. From hen receiving 200 ppm of Compound 4072 in feed for 52 weeks. C. From control liver fortified with 0.08 ppm of Compound 4072 and control yolk fortified with 0.1 ppm of Compound 4072. Arrow represents the retention time of Compound 4072

ments, since larger birds produce larger eggs (Funk, 1935). The albumen height of eggs laid by hens receiving 200 ppm of Compound 4072 was significantly lower than that from the untreated hens. Marketability, however, was not affected, since the Haugh unit scores for eggs laid by all hens were higher than required (72) for classification as Grade AA on the USDA scale.

Feed efficiency was not affected by treatment. Hens fed 200 and 50 ppm of Compound 4072 required 1.98 and 1.87 kg of feed per dozen eggs, respectively, while the untreated hens required 1.84 kg per dozen eggs. These differences were not statistically significant.

Organoleptic Evaluation. Table III summarizes the results of six taste panel sessions. No differences in flavor or odor were detected among hard-cooked eggs from birds receiving Compound 4072 in the diet and those from birds receiving no insecticide.

Residue Determinations. Claborn and Ivey (1965) stated that Compound 4072 could not be determined directly by glc and described a technique for its determination by electron capture after hydrolysis to 2,2′,4′-trichloroacetophenone with dilute sulfuric acid. However, Beroza and Bowman (1966) showed that Compound 4072 could be directly determined by glc, using either electron capture or flame photometry. In the present study, electron capture was more sensitive to

Table IV. Recovery of Compound 4072 and 2,2′,4′-Trichloroacetophenone from Fortified Tissues of Poultry

Tissue	Added µg	Added ppm	No. of samples	Recovery % Mean	Recovery % Range
		Compound 4072			
Egg white (50 g)	2	0.04	8	97.9	86.6–105.4
Egg yolk (20 g)	2	0.1	8	101.6	95.5–109.0
Liver (25 g)	2	0.08	11	97.1	82.9–104.9
Fat (10 g)	2	0.2	9	97.0	86.0–118.0
Muscle (100 g)	2	0.02	3	96.6	92.6–101.5
		2,2′-4′-Trichloroacetophenone			
Egg white (50 g)	0.4	0.02	5	103.3	87.1–117.2
Egg yolk (20 g)	0.4	0.008	5	80.0	73.9–89.7
Liver (25 g)	0.4	0.016	11	78.3	67.0–90.6
Fat (10 g)	0.4	0.04	4	47.5	39.3–72.0
Muscle (100 g)	0.4	0.004	4	38.2	31.3–43.0

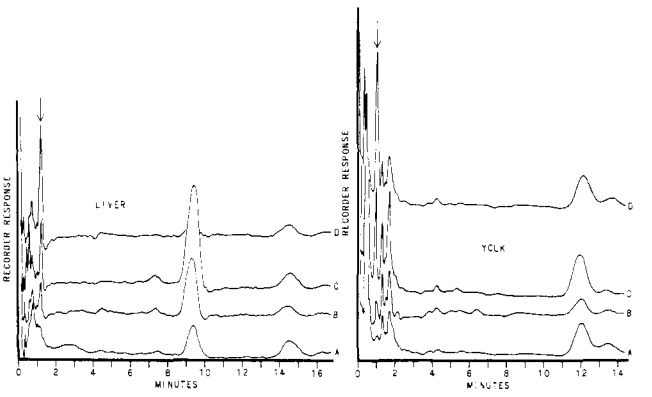

Figure 4. Typical chromatograms of 2,2′,4′-trichloroacetophenone in liver and yolk using an electron capture detector. A. From control hen. B. From hen receiving 50 ppm of Compound 4072 in diet. C. From hen receiving 200 ppm of Compound 4072 in diet. D. From control liver fortified with 0.016 ppm of 2,2′,4′-trichloroacetophenone and control yolk fortified with 0.020 ppm of 2,2′,4′-trichloroacetophenone. Arrow represents the retention time of 2,2′,4′-trichloroacetophenone

Table V. Apparent Detectability of Compound 4072 and 2,2′,4′-Trichloroacetophenone in Untreated Eggs and Tissues of Laying Hens

Tissue	No. of samples	Apparent,[a] ppm Mean	Apparent,[a] ppm Range	C_{ma}[b] (ppm)	C_{mc}[c] (ppm)
		Compound 4072			
Egg white	5	0.0097	0.0000–0.0014	0.003	0.002
Egg yolk	5	0.0048	0.0000–0.0074	0.017	0.012
Liver	6	0.0010	0.0000–0.0034	0.006	0.005
Fat	4	0.0033	0.0000–0.0073	0.023	0.020
Muscle	5	0.0002	0.0000–0.0007	0.007	0.006
		2,2′,4′-Trichloroacetophenone			
Egg white	4	0.0005	0.0000–0.0011	0.003	0.002
Egg yolk	5	0.0015	0.0000–0.0051	0.010	0.009
Liver	7	0.0011	0.0000–0.0028	0.005	0.004
Fat	4	0.0006	0.0000–0.0014	0.004	0.004
Muscle	4	0.0004	0.0000–0.0012	0.003	0.003

[a] From chromatographic response of control sample. [b] Apparent limit of detectability, 99% confidence level. [c] Corrected limit of detectability, 99% confidence level.

Table VI. Residues of 2,2′,4′-Trichloroacetophenone Found in Eggs and Tissues of Laying Hens Fed Compound 4072

Compound 4072 concentration in feed, ppm	Days after removal from treated feed	Residues in tissues, ppm[a] (range)		
		Liver	Fat	Egg yolk
200	0	0.020 (0.010–0.043)	0.019 (<0.004–0.040)	0.016 (<0.009–0.055)
	7	0.006 (<0.004–0.011)	<0.004	<0.009
	15	<0.004	...	...
50	0	0.010 (0.005–0.012)	0.005 (<0.004–0.010)	<0.009
	7	<0.004	...	...

[a] Less than 0.003 and 0.002 ppm found in muscle and egg white, respectively.

Compound 4072 than the thermionic detector. With electron capture, linearity was between 0.08 and 0.8 ng, compared to 0.3 to 3 ng for the thermionic detector. Difficulties arose with electron capture detection, however, when tissues were fortified and attempts were made at cleanup. Initial studies showed that the aluminum oxide chromatography necessary to eliminate the interfering chlorinated hydrocarbons present in these tissues caused partial hydrolysis of Compound 4072 to the trichloroacetophenone. Florisil chromatography alone did not clean up these contaminants, which interfered with the detection of Compound 4072 and trichloroacetophenone by electron capture. However, the use of the thermionic phosphorus-sensitive detector allowed for cleanup using both Florisil and aluminum oxide chromatography as described.

Compound 4072 and 2,2′-4′-trichloroacetophenone were added to portions of untreated hens' eggs and tissues prior to extraction, and recovery analyses were performed to check the analytical method (Table IV). Excellent recoveries of Compound 4072 ranging between 97 and 102% were obtained. Poor recovery of the trichloroacetophenone from fat and muscle was obtained. This was probably due to the aluminum oxide used during the cleanup of these tissues being insufficiently deactivated. Proper deactivation was necessary to release the trichloroacetophenone yet retain the contaminants on the aluminum oxide column. Longer deactivation of the aluminum oxide and overnight storage before use, as described earlier, resulted in the proper degree of deactivation.

Figures 3 and 4 show typical chromatograms obtained using the thermionic and electron capture detectors. It was necessary with the electron capture detector to allow the instrument to run at least 20 min before injecting the next sample, since there were a number of peaks caused by unknown materials in all the tissues analyzed.

The corrected limits of detectability based on the chromatographic response of untreated control samples were calculated at the 99% confidence level (Sutherland, 1965) and are given in Table V. No residues of Compound 4072 beyond the limits of detectability were found in any of the eggs or tissues analyzed, neither were residues of the metabolite 2,2′,4′-trichloroacetophenone beyond the limits of detectability found in the muscle or egg whites of hens administered Compound 4072 in the feed. Low levels of the trichloroacetophenone, however, were found in the liver, fat, and egg yolk from these hens (Table VI).

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