with Dursban, Sandoz-6626, Phosvel, lindane, or carbofuran. Furthermore, water from diazinon-treated plots did not affect the degradation of ethyl parathion or a solution of Dursban in alcohol. Only diazinon was appreciably degraded (Table VI). Since all three compounds possess a P–O–ester bond at which diazinon is hydrolyzed the diazinon-degrading factor appears to be highly specific.

LITERATURE CITED

Bray, G. A., *Anal. Biochem.* **1**, 279 (1960).
IRRI, International Rice Research Institute, Annual Report (1968).
IRRI, International Rice Research Institute, Annual Report (1969).
IRRI, International Rice Research Institute, Annual Report (1970).
Lichtenstein, E. P., *J. Econ. Entomol.* **59**, 985 (1966).
Pathak, M. D., *Intern. Pest Control* **10**(6), 12 (1968).
Sethunathan, N., Caballa, S., Pathak, M. D., *J. Econ. Entomol.* **64**, 571 (1971).
Sethunathan, N., MacRae, I. C., J. AGR. FOOD CHEM. **17**, 221 (1969).
Sethunathan, N., Pathak, M. D., *Canad. J. Microbiol.* **17**, 699 (1971).
Sethunathan, N., Yoshida, T., J. AGR. FOOD CHEM. **17**, 1192 (1969).
Yoshida, T., unpublished data (1971).

Received for review October 5, 1971. Accepted December 27, 1971

Presence of Residues in Eggs Laid by Chickens Receiving Decoquinate

Rudolph F. Kouba, Elliott M. Craine,* and William H. Ray

Decoquinate-*3*-^{14}C was administered at a level of 0.003% in feed to two laying hens (White Leghorn–Babcock 300) over a 19-day period. All eggs laid by the birds during the medication period and during a 15-day withdrawal period were collected and assayed for radioactivity. A plateau of radioactivity corresponding to 0.88 ppm equivalents of decoquinate was obtained after 10 days of continuous medication. Most of the residue was located in the yolk. The residues in the whites and yolks of eggs obtained in the plateau period were examined by thin-layer chromatography. Decoquinate plus one nondecoquinate component were detected in egg yolks, while decoquinate was the only residue detected in egg white.

Decoquinate [ethyl 6-(decyloxy)-7-ethoxy-4-hydroxy-3-quinolinecarboxylate] has high anticoccidial activity when administered to chickens in the diet at a low concentration (Ball *et al.*, 1968; Johnson *et al.*, 1968). When Craine *et al.* (1971) administered single oral doses of decoquinate-^{14}C to chickens, less than 2% of the radioactivity was excreted in urine. Only minor amounts of the decoquinate dose were converted to nondecoquinate metabolites. Filer *et al.* (1969) obtained a plateau of radioactivity in tissues of broiler chickens after administration of decoquinate-^{14}C through the feed. Using a fluorometric method of measure, Button *et al.* (1969) found residues in tissues of chickens medicated with decoquinate. In both cases a rapid disappearance of residue occurred when medication ceased. Using a tlc analytical method, Ferrando *et al.* (1971) found residues of decoquinate mainly in fatty tissues.

When birds were medicated on successive days with decoquinate-*3*-^{14}C, Craine *et al.* (1971) obtained a plateau of tissue residues within 3 days. The extracted radioactive residues were examined by thin-layer chromatography. Two nondecoquinate components in addition to decoquinate were detected in kidney, liver, and bile. In contrast, only decoquinate was present in muscle, skin, and fat. In the present report decoquinate-^{14}C was fed to laying hens at a recommended use level to determine whether residues accumulated in eggs.

EXPERIMENTAL

Chemicals and Materials. Decoquinate-*3*-^{14}C (Filer *et al.*, 1969) was obtained from May & Baker, Ltd. (Dagenham, England). Particle size of the compound was reduced as described previously (Craine *et al.*, 1971). The compound had a specific activity of 0.61 μCi/mg and radiopurity of 99.9% based on thin-layer chromatographic analysis. The sources of other chemicals were those described by Craine *et al.* (1971).

Dosage Preparation. The decoquinate-*3*-^{14}C (107 mg) was placed in a plastic bag containing 3600 mg of oiled corn meal, mixed to a uniform consistency by kneading and transferred to a 10-l. screw-cap plastic bottle. Laying hen ration was mixed with the decoquinate–corn meal mixture at a level so that each laying hen would receive approximately 3 mg of labeled decoquinate per day. Portions (5 g) of the meal were extracted with chloroform and radioactivity was measured in two aliquots of each extract to determine homogeneity.

Radioactivity Measurement. Radioactivity was measured in a scintillation spectrometer in glass counting vials using a colloidal silica gel suspension system (Green, 1970). Counting efficiency for individual samples was determined with an external γ source. Portions (1.0 ml) of the ethanolic or chloroform–ethanolic extracts of eggs were placed in counting vials which were heated in a water bath to evaporate the solvent. Portions of the lyophilized egg yolk or white (100 mg) were weighed into counting vials. One milliliter of Soluene 100 (digestion fluid) was added and the vials were heated at 50°C for 8 hr. To reduce color a few drops of 50% hydrogen peroxide were added. After 15–20 hr, 1 ml of methanol and 15 ml of the counting system were added and the vials counted. The specific activity of the compound allowed detection of 0.1 ppm of decoquinate.

Birds. Laying hens 36 weeks of age (White Leghorn–Babcock 300—replacements) were housed in a laying battery. Six birds were given a laying hen mash *ad libitum* for a 10-day

Hess & Clark, Division of Rhodia, Incorporated, Ashland, Ohio 44805.

Table I. The Amounts of Mash and Decoquinate-3-^{14}C Consumed by Each Hen

Days on medication	Mash consumed		Decoquinate consumed	
	Hen #1, g	Hen #2, g	Hen #1, mg	Hen #2, mg
1	80	38	2.2	1.1
2	55	86	1.5	2.4
3	94	95	2.6	2.7
4	120	108	3.4	3.0
5	76	77	2.1	2.2
6	94	106	2.6	3.0
7	90	108	2.5	3.0
8	95	99	2.7	2.8
9	101	101	2.8	2.8
10	102	134	3.1	3.8
11	66	79	2.0	2.2
12	102	51	2.9	1.4
13	104	93	2.9	2.6
14	94	95	2.6	2.7
15	115	109	3.2	3.0
16	107	105	3.0	2.9
17	127	113	3.6	3.2
18	107	100	3.0	2.8
19	55	70	1.5	2.0

Table II. Concentration of Total Radioactive Residue[b] in the Eggs of Chickens Medicated with Decoquinate-^{14}C

Day	Yolk Residue		White Residue		Total Egg Residue	
	Hen #1	Hen #2	Hen #1	Hen #2	Hen #1	Hen #2
1	0.0	*	0.0	*	0.0	*
2	*[a]	0.1	*	0.0	*	0.0
3	0.5	0.3	0.1	0.1	0.2	0.1
4	0.6	0.6	0.1	0.1	0.2	0.2
5	1.0	1.0	0.1	0.1	0.4	0.4
6	1.5	1.5	0.1	0.1	0.6	0.5
7	1.6	1.9	0.1	0.1	0.6	0.7
8	1.8	1.4	0.1	0.1	0.7	0.8
9	1.8	2.5	0.1	0.1	0.7	0.9
10	*	2.6	*	0.1	*	0.9
11	2.1	*	0.1	*	0.8	*
12	2.1	2.5	0.1	0.1	0.8	0.9
13	2.1	3.2	0.1	0.1	0.8	1.2
14	2.2	2.6	0.1	0.1	0.8	1.0
15	2.0	2.4	0.1	0.1	0.8	0.9
16	2.2	2.4	0.1	0.1	0.9	0.9
17	2.2	2.5	0.1	0.1	0.9	0.9
18	2.2	2.5	0.1	0.1	0.9	0.9
19	2.0	2.5	0.0	0.1	0.8	1.6
20	*	2.4	*	0.1	*	0.9
21	2.0	2.2	0.0	0.0	0.7	0.8
22	1.6	*	0.0	*	0.6	*
23	1.1	1.6	0.0	0.0	0.4	0.6
24	1.0	*	0.0	*	0.3	*
25	0.5	0.9	0.0	0.0	0.2	0.3
26	0.3	0.6	0.0	0.0	0.1	0.2
27	0.2	0.4	0.0	0.0	0.1	0.2
28	*	0.3	*	0.0	*	0.1
29	0.1	0.2	0.0	0.0	0.1	0.1
30	0.1	0.1	0.0	0.0	0.0	0.1
31	0.1	0.1	0.0	0.0	0.0	0.1
32	0.1	0.1	0.0	0.0	0.0	0.0
33	0.1	*	0.0	*	0.0	*
34	0.0	0.1	0.0	0.0	0.0	0.0
35	0.0	0.1	0.0	0.0	0.0	0.0

[a] Asterisk indicates no eggs laid. [b] Values are calculated as equivalents of decoquinate in ppm (wet basis) assuming that compounds forming the residue have the same specific activity as decoquinate.

period before experimentation to allow adjustment to environmental conditions. Two birds were selected to receive the labeled medication based on their consistent feed consumption and egg production.

Schedule. The two hens received medicated mash containing 0.0028% decoquinate-^{14}C, which is a recommended use level to control coccidiosis. From the daily consumption during the 10 days before experimentation this level would have given the birds about 2 mg of decoquinate per day. However, their daily consumption of feed increased after the experiment started and they actually received about 2.6 mg per day. The feed was weighed every day to determine the actual amount of decoquinate consumed.

Table III. The Amount of Radioactivity Deposited in Chicken Egg *in vivo* during the 19 Days of Continuous Medication

Chicken no.	Amount of radioactivity administered, μCi	Amount of radioactivity in eggs		Percent deposited in egg
		White, $\mu Ci \times 10^{-2}$	Yolk, $\mu Ci \times 10^{-2}$	
1	30.62	2.81	37.12	1.3
2	30.26	3.01	44.73	1.6

Collection of Eggs. Each egg collected after the first administration of medicated feed was analyzed for its radioactive content. The eggs were collected for the entire 19-day medication period and for an additional 15 days, during which time nonmedicated ration was given to the hens. Each egg collected after administration of medicated feed was separated into white and yolk portions which were weighed, freeze-dried, and assayed for radioactivity. The yolk and white portions of eggs comprising the plateau period from the tenth through the twentieth day were pooled and stored in sealed plastic containers at −25°C.

Egg Processing. Extraction of the pooled egg yolk and white material was carried out in a manner similar to that for tissues (Craine *et al.*, 1971). A 5-g sample of lyophilized powder (yolk or white) was placed in a 125-ml Erlenmeyer flask containing 20 ml of absolute ethanol. The mixture was stirred for 15 min and then transferred to a centrifuge tube, covered, and centrifuged at 680 × *g* for 20 min. This process was repeated with an additional 10 ml of absolute ethanol. The supernatant was decanted directly into a 125-ml separatory funnel.

The radioactive components were then transferred from the primary ethanol extract by the addition of 50 ml of 2% HCl and 10 ml of chloroform. After shaking, the chloroform phase was separated and transferred to a 300-ml round-bottomed flask. The aqueous phase was washed with successive 5-ml portions of chloroform until free of radioactivity.

The chloroform extracts were combined and concentrated to dryness on a rotary evaporator. The oily material was dissolved in 2–5 ml of hexane, assayed for radioactivity, and subsequently analyzed for radioactive components by thin-layer chromatography. An additional extraction of the egg yolk residue was performed using a chloroform–ethanol (1:1) mixture. The procedure followed for this step was identical to that for the primary ethanol extraction. Samples of control eggs were processed by the same procedure after the addition of standard decoquinate-^{14}C.

Thin-Layer Chromatography (tlc). Tlc adsorbent was silica gel G spread on glass plates (5 × 20 cm) with a Brinkmann High-Capacity Adjustable Applicator at a setting of 750 μ. Air-dried plates were activated at 110°C for 2 hr and stored in a desiccator. To obtain uniform solvent fronts, a 1-mm strip of adsorbent was removed from each edge of the plates (Davies, 1963). Development was ascending in circular tanks (5.5 × 23 cm) at room temperature with two systems: toluene–absolute ethanol–glacial acetic acid (5:1:1), and normal butanol–concentrated ammonium hydroxide (5:1).

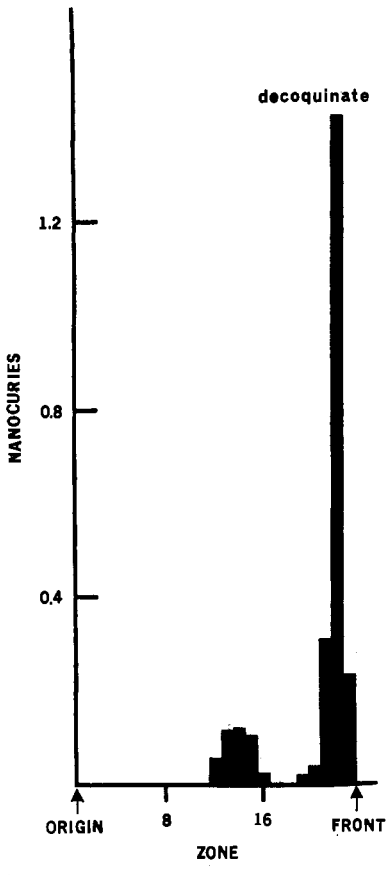


Figure 1. The distribution of radioactivity on a tlc chromatogram of an extract of egg yolk. Development system: 1-butanol–ammonium hydroxide (5:1)

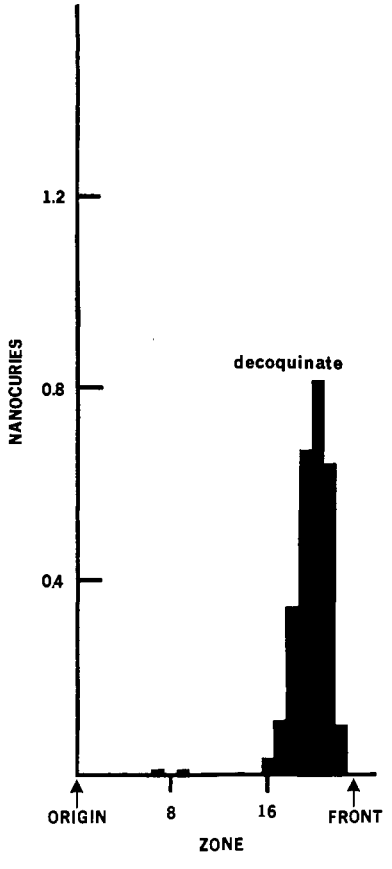


Figure 2. The distribution of radioactivity on a tlc chromatogram of an extract of egg white. Development system: 1-butanol–ammonium hydroxide (5:1)

Due to the high lipid content of the yolk extracts, predevelopment of the plates in ether was necessary.

Decoquinate was detected in all extracts examined. To determine position of the decoquinate zone, a duplicate plate was run with added standard decoquinate-^{14}C. Plates were examined under an ultraviolet light (254 nm and 366 nm) to locate fluorescent zones. Radioactivity on the plates was detected by a direct count of the silica gel from cross-sectional zones (0.5 cm) on each plate (Snyder and Stephens, 1963).

RESULTS

The Medication. The hen-laying ration containing the decoquinate was homogeneous with respect to radioactive content. The theoretical content of activity in the feed mixture was 17.20×10^{-3} μCi/g and the actual amount was 16.88×10^{-3} μCi/g with average deviation from the mean of 0.50×10^{-3} μCi/g. The consumption of labeled decoquinate was uniform throughout the 19-day medication period, averaging 2.6 mg per day for each hen (Table I).

Radioactivity in Eggs. Radioactivity arising from decoquinate-^{14}C consumed for 19 days appeared in both egg yolk and white (Table II). The radioactive residue was higher in the egg yolk throughout the entire medication period; therefore, most of the residue was located in the yolk. The data indicated that a plateau of residue occurred by the tenth day for egg yolk, whereas egg white showed a plateau by the fourth day. The difference of the onset of plateau in the egg yolk *vs.* the egg white can be attributed to the difference in the length of time required for yolk and white deposition (Sturkie, 1965). The residue for the total egg averaged 0.88 at plateau level. Of the total dose administered in 19 days, approximately 1.5% was deposited in the eggs *in vivo* (Table III).

Generally the radioactivity in the egg yolk was not completely extracted by simple homogenization with ethanol. This was probably due to the high lipid and lipoprotein content in the yolk. An additional extraction with chloroform–ethanol solvent (1:1) was necessary to extract the remaining radioactivity. However, radioactivity in egg white was completely extracted by ethanol.

Chromatography. The extracts obtained from the eggs at plateau were all analyzed by tlc using both solvent development systems. Two radioactive components, decoquinate and an unknown, were detected in egg yolk. Only one component, decoquinate, was found in egg white with each development system. Resolution was better with the 1-butanol–ammonium hydroxide than the other development solvent. Figures 1 and 2 show chromatograms of extracts of egg yolk and white where the development system was 1-butanol–ammonium hydroxide (5:1). The position of decoquinate was determined by a second chromatography with standard decoquinate-*3*-^{14}C added. About 17% of the radioactivity was associated with the unknown nondecoquinate component.

The possibility of modification of decoquinate during the course of extraction and tlc examination was discounted. The addition of decoquinate-^{14}C in control egg yolk and white revealed that decoquinate did not undergo a modification of molecular structure or decomposition as determined by tlc.

DISCUSSION

The results show that decoquinate-^{14}C appears in the eggs of birds fed a diet containing the drug. Within the limits of the

experiment (using two birds) it can be seen that for most of the eggs examined a plateau level of radioactive products was established within 10 days following the administration of decoquinate-^{14}C at the rate of 3 mg daily. This level, approximately 0.9 ppm (wet weight), remained constant until 2 days after the last administration of the drug, then declined rapidly to a level of 0.0 to 0.1 ppm on the fifteenth day after the last administration of the drug. These results correspond closely with those reported by Filer *et al.* (1969) and Button *et al.* (1969) where a plateau of radioactivity in tissues of broiler chickens was obtained after administration of decoquinate through the feed. In both cases a rapid disappearance of residue occurred when medication ceased.

The tlc examination of the egg residue showed that decoquinate-^{14}C was metabolized to at least one other compound in the egg yolk. This compound possessed chromatographic characteristics similar to those reported to be present in chicken tissues (Craine *et al.*, 1971). Whether the nondecoquinate component was metabolized *in situ* or deposited at the time of yolk formation was not established.

ACKNOWLEDGMENT

The authors thank Peter Resnis, Geraldine Liston, and Janice Carman for their technical assistance.

LITERATURE CITED

Ball, S. J., Davis, M., Hodgson, J. N., Lucas, J. M. S., Parnell, E. W., Sharp, B. W., Warburton, D., *Chem. Ind.* 56 (1968).
Button, R. G., Muggleton, D. F., Parnell, E. W., *J. Sci. Food Agr.* **20,** 70 (1969).
Craine, E. M., Kouba, R. F., Ray, W. H., J. AGR. FOOD CHEM. **19**(6), 1228 (1971).
Davies, B. H., *J. Chromatogr.* **10,** 518 (1963).
Ferrando, R., Laurent, M. R., Terlain, B. L., Caude, M. C., J. AGR. FOOD CHEM. **19,** 52 (1971).
Filer, C. W., Hiscock, D. R., Parnell, E. W., *J. Sci. Food Agr.* **20,** 65 (1969).
Green, R. C., in "Liquid Scintillation Counting," Chapter 19, E. D. Bransome, Ed., Grune and Stratton, New York, N. Y., 1970, p 189.
Johnson, C. A., Plank, H. E., Darling, M. A., *Poultry Sci.* **47,** 1685 (1968).
Snyder, F., Stephens, N., *Anal. Biochem.* **4,** 128 (1963).
Sturkie, P. D., "Avian Physiology," Second Ed., Cornell University Press, Ithaca, N. Y., 1965, p 447.

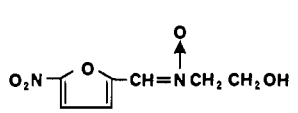
Received for review September 22, 1971. Accepted November 24, 1971.

Urinary Excretion of Nifuratrone Metabolites by Swine (Gilts)

Elliott M. Craine,* Janice Carman, and Dale L. Hobson

Gilts were given single oral doses of nifuratrone at a level of 6 or 12 mg/kg body weight. Urine was collected and assayed by two procedures which measure the compound: by reaction with phenylhydrazine and by extraction with nitromethane. Compounds measured by each procedure appeared in the urine rapidly and were not detected after 8 hr. A total of 6.4% of the dose was measured in the urine by the phenylhydrazone method and 1.0% was measured by the nitromethane assay. Ion exchange chromatography detected seven urinary components induced by nifuratrone medication. Five of the seven had absorption spectra similar to the parent compound and three of these responded positively to the phenylhydrazone method, indicating they contained a nitrofurfural moiety. One of the components was nifuratrone and was responsible for the nitromethane assay of the original urine. Thus, only 1.0% of the dose administered was excreted intact in the urine. The components measured by the phenylhydrazone assay procedure had a urinary excretory half-life of less than an hour. All metabolites detected by the chromatography, including those measured by the phenylhydrazone method, were eliminated at similar rates.

Nifuratrone (1) [*N*-(2-hydroxyethyl)-α-(5-nitro-2-furyl)nitrone] has antibacterial activity in swine (Kim and Bambury, 1969; Dainippon Pharmaceutical Co., 1968). In the present work the compound was given to female pigs in single oral doses. Urine was collected and examined by four procedures to determine whether nifuratrone or its metabolites could be detected.



(1)

In general, the group of compounds known as nitrofurans is rapidly metabolized by avians or mammalians (Paul and Paul, 1964). The degradation pathways vary from one compound to another but some generalities do seem to exist. Metabolites closely related to the parent compound are excreted in the urine in many cases. Also, with some compounds, metabolism is so extensive that carbon atoms of the molecule appear in the normal body constituents (Buzard, 1962; Tennent and Ray, 1971; Herrett *et al.*, 1967). Thus, in metabolic experiments with ^{14}C as a tracer, many labeled metabolites appear, including normal body components, and confuse the studies.

Most body tissues have enzymes capable of degrading the nitrofurans (Paul *et al.*, 1960). It is thus not surprising that no evidence has been obtained to show deposition or accumulation of nitrofuran residues in tissues. From a public health standpoint it is important to know whether metabolites closely related to the parent drug pose residue problems, *i.e.*, those which retain the furan ring. Where the molecule is extensively degraded to normal body components or small molecules, the significance from a residue standpoint becomes less important.

In earlier work Paul *et al.* (1960) detected drug-related metabolites of nitrofurans in urine by examining the ultraviolet absorption spectra. Tennent and Ray (1971) separated these drug related metabolites by ion exchange chromatog-

Hess & Clark, Ashland, Ohio 44805.