differences in the metabolites isolated from different samples in the same animal species, no qualitative differences were observed except that desmethyl dehydro was found only in milk and methyl formyl only in hen excreta.

The metabolic pathway of buthidazole in cows and hens is shown in Figure 3. This pathway involved hydroxylation, oxidation, demethylation, dehydration, ring opening, and hydrolysis.

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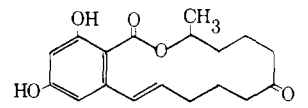
Metabolism of [^{14}C]Zearalenone in Laying Hens

Robert E. Dailey,* Ruth E. Reese, and Emilio A. Brouwer

A single dose of 10 mg of [^{14}C]zearalenone/kg was administered by gavage to White Leghorn laying hens, and its absorption, distribution, and excretion at 2, 4, 24, 48, and 72 h after dosage were studied. ^{14}C-Labeled residues in excreta, bile, egg yolk, clutch, and liver were partially characterized. About 94% of the administered ^{14}C activity was eliminated via the excreta within 72 h of dosing. About one-third of the dose was excreted as unchanged [^{14}C]zearalenone, while another one-third appeared as a polar metabolite. No major retention sites of ^{14}C activity were found in edible muscle tissues but persistent levels of lipophilic metabolite(s) were detected in egg yolk at a concentration of 195 μg equiv/100 g of wet weight (about 2 ppm) 72 h after dosing.

Zearalenone, a mycotoxin produced by some strains of



Fusarium roseum, *F. oxysporum*, *F. tricinctum*, and *F. moniliforme* (Steele et al., 1976), has been found in corn infested with these fungi. When the 1973 corn crop was surveyed, zearalenone was detected in 6% of the marketable corn from the Corn Belt area, which includes nine midwestern states (Stoloff et al., 1976). Zearalenone and its uterotropically active derivatives are classified as estrogens since they produce estrus, i.e., cornification of the vagina in adult mice. The biological effect of zearalenone on the metabolism of various animal species has been reviewed (Mirocha et al., 1977). Swine, poultry, and cattle appear to be affected by the presence of zearalenone in the diet. Swine are possibly most sensitive to the estrogenic activity of this compound, which can contribute to infertility in sows by its effect on the ovaries. Since chickens are relatively resistant to the physiological effects of zearalenone (Mirocha et al., 1977), the likelihood that contaminated grain might be used as a feedstock for them is increased. Therefore zearalenone might enter the human food chain via chicken meat and eggs.

This study was undertaken to determine the distribution of zearalenone and its metabolites in eggs and edible tissues of laying hens dosed with [^{14}C]zearalenone and to partially characterize some of the metabolites.

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MATERIALS AND METHODS

Radiolabeled Zearalenone. The uniformly labeled [^{14}C]zearalenone was prepared by Anver Bioscience Design, Inc., Sierra Madre, CA. Purity, reported to be 99%, was verified by the high-pressure liquid chromatographic procedure of Ware and Thorpe (1978). A total of 400 mg (61.6 μCi), having a sp act. of 0.154 μCi/mg, was available for this study.

Experimental Animals. White Leghorn laying hens (Truslow Farms, Chestertown, MD) were not less than 26 or more than 39 weeks of age and had an egg-laying efficiency of 70% or more. Hens were housed individually in metal cages in a room in which the temperature was maintained at 70–72 °F, relative humidity at 30–50%, and a light/dark cycle of 16/8 h. The hens were fed laying mash (Truslow Farms, Chestertown, MD) and water ad libitum. Each hen was weighed weekly, and daily egg production was recorded.

At 8:00 a.m. on the day that a hen was selected for use in the metabolic study it was fasted for 1 h before being dosed (by gavage into the crop) with 10 mg/kg (1.54 μCi/kg) of [^{14}C]zearalenone dissolved in propylene glycol at a concentration of 10 mg/mL. The hen was then placed immediately in a Delmar-Roth metabolic chamber (Delmar Scientific Glass Products of Coleman Instruments, Maywood, IL) from which excreta and expired CO_2 could be collected quantitatively. Feed and water were available at all times. Each hen was kept in the metabolic chamber until the selected time interval after dosing had elapsed. Four hens were used for each of five different time in-

tervals, i.e., 2, 4, 24, 48, and 72 h.

Eggs laid during the selected time interval after dosing were immediately removed from the cage and weighed. Yolks and whites were separated and weighed, lyophilized, and stored for later analysis. Whole eggs found in the oviduct at the time of sacrifice were treated in the same manner. Hens were killed by exsanguination via cardiac puncture, using heparin as an anticoagulant. Skin, with feathers intact, was removed from the carcass. The alimentary canal was divided into sections consisting of crop, proventriculus, gizzard, and intestinal tract. Each segment was weighed and placed in a beaker for immediate analysis. The comb and wattles, oviduct, gall bladder, bile, pancreas, spleen, adrenals, muscle tissue from wing, breast, and leg, brain, heart, liver, kidney, lung, clutch (ovary), and fat were removed, weighed, and stored at −15 °C until they could be processed for analysis. The feet, which in most cases had been contaminated with ^{14}C from excreta in the cage, were removed and analyzed separately. The remainder of the carcass was then placed in a beaker, digested, and the ^{14}C-content determined.

Measurement of Radioactivity. All samples were counted and dpm computed (by reference to appropriately stored quench curves) using a Mark III Model 6880 Searle liquid scintillation spectrometer (Searle Analytical Inc., Des Plains, IL). It was not found necessary to use decolorizing agents in the preparation of any tissue samples for counting except whole blood samples, which were decolorized with hydrogen peroxide (Dailey et al., 1977). The scintillation fluid consisted of 4.0 g of PPO and 0.10 g of DMPOPOP (Packed Instrument Co., Downers Grove, IL) dissolved in 700 mL of toluene and 300 mL of methanol. All samples were counted until a 1% counting error was attained or for 100 min, whichever occurred first.

Preparation of Samples. *Red Blood Cells.* The amount of radioactivity found in the red blood cells was computed by subtracting the ^{14}C content of the plasma contained in 1 mL of whole blood, as determined from the hematocrit (packed cell volume), from the ^{14}C content of 1 mL of whole blood and dividing that number by the hematocrit value (see Dailey et al., 1977).

Eggs. Aliquots of lyophilized egg white and yolk were weighed into scintillation vials and digested by gentle heating (40 °C) with 2 mL of Soluene-350 tissue solubilizer (Packard Instrument Co., Downers Grove, IL). After digestion was complete, 15 mL of scintillation fluid was added to each vial, and the vials were placed in the dark at 8 °C for at least 16 h before counting.

Egg yolks to be analyzed in the metabolite characterization study were lyophilized, grouped by time after dosing (i.e., 1–10, 21–26, 43–50, and 69–72 h), and thoroughly mixed.

Gall Bladder, Pancreas, Spleen, Adrenals, and Wing, Breast, and Leg Muscle. Weighed samples (about 200 mg) of each tissue were processed as described for eggs. The gall bladder was washed thoroughly with water before counting.

Brain, Heart, Liver, Kidney, Lung, and Clutch. The whole organs were weighed and homogenized with measured volumes of distilled water. Aliquots (about 200 mg) were processed as described for eggs. The remainder of the liver and clutch homogenates was stored at −15 °C until processed for metabolite characterization studies, at which time the samples were grouped according to time after dosing, lyophilized, and mixed.

Fat. Most of the fat depots found in the abdominal cavity at autopsy were removed, weighed, placed in a beaker, and heated on a hot plate to melt the solid fat. On cooling, while still liquid, 0.2-mL aliquots of the melted fat were placed in scintillation vials and treated as described for eggs.

Crop, Proventriculus, Gizzard, Intestinal Tract, Comb and Wattles, Oviduct, Feet, Carcass, and Skin and Feathers. Each weighed tissue (except carcass and skin and feathers) was placed in a beaker to which 150–200 mL of distilled water and 10 g of NaOH pellets were added. The beakers were placed on a steam bath in a hood and stirred until digestion was complete. A volume of ethanol equal to the volume of the digest was added and the solution reheated. On cooling, the volume of the digest was measured and 0.2-mL aliquots of each were added to scintillation vials and dissolved in 15 mL of scintillation fluid. The total dpm found in feet was added to that found in excreta when accounting for percent of administered dose recovered.

Carcass and skin and feathers were treated in the same manner except that 20 g of NaOH and about 1 L of distilled water were used in digestion. The carcass consisted of all of the body components remaining (including bones) after removal of the tissues and organs used in sampling.

Excreta. Excreta (with washings) was collected from the metabolism cages and the total volume measured. The mixture was placed in a Waring blender and homogenized for at least 2 min. Half of the homogenate was digested with NaOH on a steam bath as described above and counted. The other half was stored at −15 °C until it could be further processed for metabolite characterization studies, at which time the samples were grouped according to time after dosing, lyophilized, and mixed.

$^{14}CO_2$. Expired CO_2 from each hen was collected in a measured volume of trapping solution (33% monoethanolamine–67% ethylene glycol monomethyl ether). Aliquots (1–3 mL) of this solution were dissolved in 15 mL of scintillation fluid and counted. Fresh trapping solution was prepared every 24 h.

Bile. After removal of the gall bladder, the bile was withdrawn into a syringe and the volume measured. A 0.25-mL aliquot was added to 1.75 mL of distilled water in a centrifuge tube, mixed thoroughly, and 0.20-mL aliquots of this solution were added to a scintillation vial. Two milliliters of Soluene-350 was added to solubilize any proteinaceous material before addition of 15 mL of scintillation fluid. The remainder of the undiluted bile was added to a pool of bile collected from each hen at autopsy and was lyophilized, mixed, and frozen for later metabolite characterization.

Metabolite Characterization. Certain groups of the pooled liver, clutch, excreta, bile, and egg yolk samples were selected for extraction, solvent fractionation, and chromatography depending upon the level of radioactivity they contained, the availability of material, and time interval after dosing. Four aliquots from each selected group were processed as shown in Figure 1, and the solubility characteristics and chromatographic behavior of the ^{14}C-labeled compounds in these groups were compared with those of 99% pure [^{14}C]zearalenone.

Extraction and Solvent Fractionation (Figure 1). The basic methodology used in the extraction and fractionation procedures has been described by Mirocha et al. (1974) and Ware and Thorpe (1978) with a few modifications. Emulsions tended to form in most of the samples. Care was exercised throughout to minimize this problem.

By washing the chloroform extract with 0.154 M pH 4 phosphate buffer before extracting with NaOH, the recovery of [^{14}C]zearalenone added to clutch and yolk samples was increased from 71 to 80%. Therefore, this step

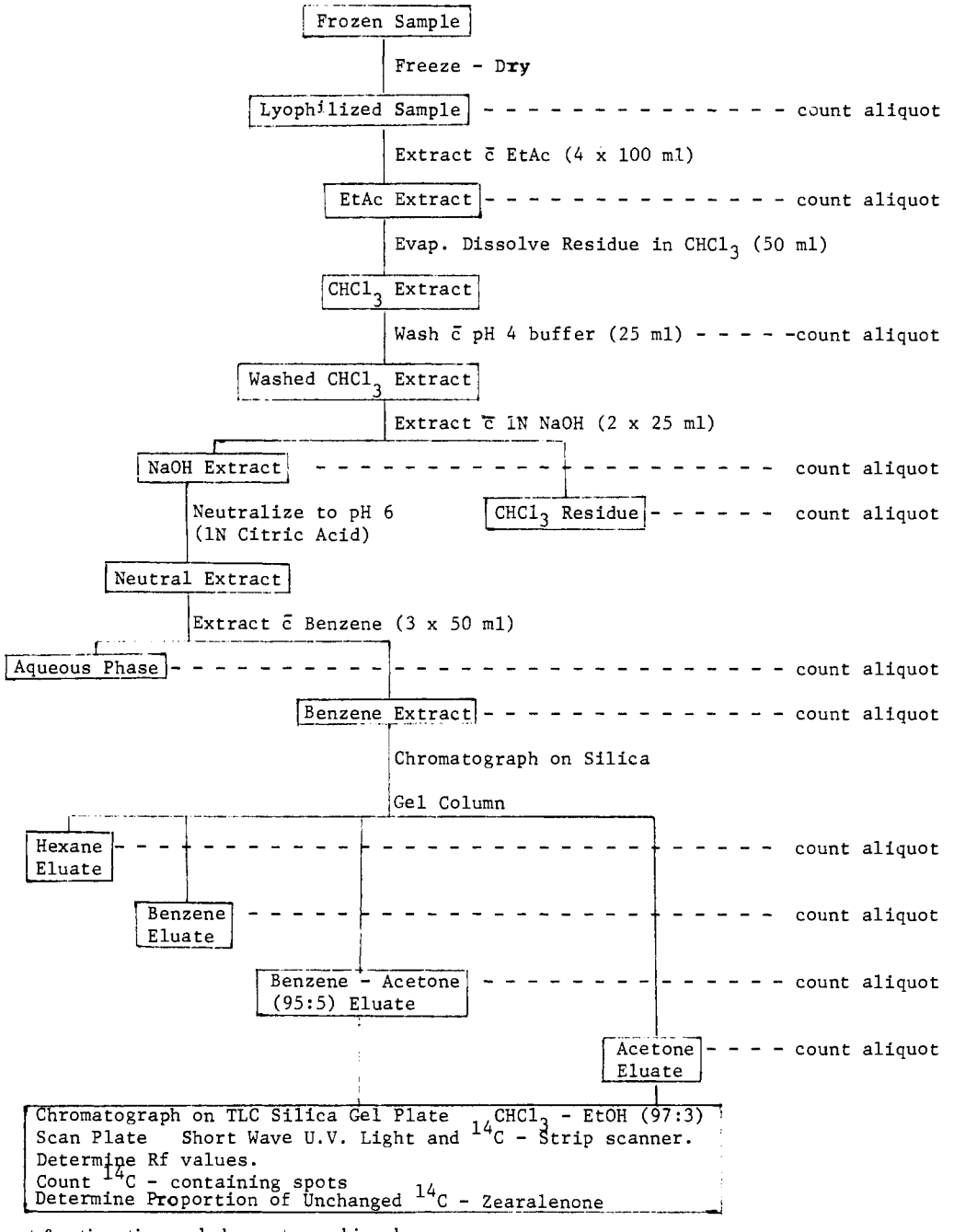

Figure 1. Solvent fractionation and chromatographic scheme.

was incorporated into the scheme used for all of the samples.

Silica Gel Column Chromatography. A modification of the method described by Eppley (1968) for chromatography of zearalenone on silica gel columns was used in this study. A chromatographic column (9 mm i.d.) was prepared using 6 g of silica gel 60 (Brinkmann Instruments, Inc., Westbury, NY) slurried with hexane. The residue from the benzene extracts was placed on top of the column and eluted successively with 50 mL of hexane, 50 mL of benzene, 100 mL of benzene–acetone (95:5), and 50 mL of acetone. Standard [^{14}C]zearalenone eluted in the benzene–acetone fraction.

Thin-Layer Chromatography (TLC). Both the benzene–acetone and the acetone fractions contained radioactivity. Aliquots were spotted on TLC plates (silica gel 13181, Eastman Kodak Co., Rochester, NY) which were developed in chloroform–ethanol (97:3). The plates were viewed under shortwave ultraviolet light (256 nm). Both light blue, fluorescent spots (e.g., zearalenone) and dark, absorbing spots were marked and R_f values computed. The plates were then cut into 2 in. wide strips and scanned for ^{14}C activity by using a Model 7201 Packard radiochromatogram scanner (Packard Instrument Co., Downers Grove, IL). The ^{14}C-active spots were marked and R_f values determined. All spots (both ultraviolet reactive and ^{14}C active) were cut out and placed in scintillation vials to which 1 mL of dimethylformamide and 15 mL of scintillation fluid were added. The ^{14}C activity in each spot was determined and the percentage of the total dpm on the strip was computed for each spot.

RESULTS AND DISCUSSION

Table I shows the percentage of the administered dose of [^{14}C]zearalenone recovered in the various fractions at the indicated time intervals after dosing. Most of a single oral dose of 10 mg of mycotoxin/kg was excreted within 72 h. Less than 1% of the administered dose of 99% pure

Table I. Percentage of Administered Dose of [^{14}C]Zearalenone Recovered[a]

sample	time interval after dosing, h				
	2	4	24	48	72
tissues, blood, bile	2.69 ± 0.75	4.20 ± 1.15	7.04 ± 1.16	4.54 ± 1.89	1.05 ± 0.26
excreta	7.82 ± 2.92	18.05 ± 8.03	76.16 ± 4.06	88.66 ± 4.24	93.61 ± 2.57
crop and contents	41.28 ± 8.92	28.05 ± 11.10	0.40 ± 0.14	0.11 ± 0.04	0.09 ± 0.03
proventriculus and contents	4.21 ± 2.04	2.68 ± 0.53	0.24 ± 0.07	0.16 ± 0.11	0.07 ± 0.02
gizzard and contents	15.15 ± 3.23	14.72 ± 2.32	4.15 ± 0.64	1.48 ± 0.80	0.35 ± 0.06
gastrointestinal tract and contents	29.28 ± 4.28	29.98 ± 4.90	7.38 ± 1.76	3.04 ± 1.90	0.55 ± 0.11
carcass	1.13 ± 0.36	1.40 ± 0.28	0.76 ± 0.13	0.59 ± 0.08	0.75 ± 0.31
skin and feathers	0.64 ± 0.24	0.67 ± 0.15	1.14 ± 0.21	0.47 ± 0.09	0.95 ± 0.24
expired $^{14}CO_2$	0.28 ± 0.07	0.58 ± 0.16	0.98 ± 0.13	0.85 ± 0.22	0.80 ± 0.04
eggs and clutch	0.06 ± 0.02	0.10 ± 0.03	0.96 ± 0.04	0.97 ± 0.24	0.96 ± 0.17
total recovered	102.54 ± 2.95	100.43 ± 2.01	99.21 ± 1.43	100.87 ± 1.71	99.18 ± 3.65

[a] Average ± standard error of four values at each time interval.

Table II. Concentration of [^{14}C]Zearalenone Residues in Eggs and Clutch[a]

time after dosing, h	egg		clutch
	white	yolk	
1-10	7 ± 1.6 (11)	23 ± 3.5 (11)	19 ± 4.2 (8)
21-26	13 ± 2.1 (9)	29 ± 6.9 (10)	256 ± 13 (4)
43-50	22 ± 4.6 (7)	112 ± 15 (7)	186 ± 37 (4)
69-72	7 ± 0.3 (4)	195 ± 17 (4)	201 ± 31 (4)

[a] Expressed as microgram equivalents of zearalenone/100 g of wet weight. Values are average ± standard error of number of samples in parentheses.

[^{14}C]zearalenone was recovered as expired $^{14}CO_2$. This would usually indicate that at least some fragmentation and oxidation of the molecule had occurred. In this case, however, some doubt exists that the $^{14}CO_2$ collected actually came from the parent compound or from the unknown 1% impurity in the preparation. In three pilot experiments (not shown in the tables), when a sample of approximately 95% pure [^{14}C]zearalenone was administered, 3.05% of the total dose was recovered as expired $^{14}CO_2$ within 4 h and an average of 6.13% (from two hens) within 24 h after dosing. The nature and composition of the impurity(s) were not investigated further.

Approximately 1% of the administered radioisotope was found in the eggs and clutch within 24 h of dosing and this level persisted for 72 h even though about 94% of the ^{14}C activity had been eliminated via the excreta by that time. The situation in eggs and clutch, presented in greater detail in Table II, shows a rather dramatic increase in the ^{14}C activity in the yolks of eggs laid 43–50 and 69–72 h after dosing. An even greater increase in the ^{14}C activity was found in the developing yolks in the clutch 1 day earlier than in eggs that were laid and that increased level persisted for 72 h after dosing. The accumulation of radioactivity in the yolks could be the result of the layered development of the yolk which would impede elimination rather than attachment to specific binding sites within the developing eggs. However, the net effect would produce a source of contamination to the human food supply.

Radioactivity levels in tissues and body fluids presented in Table III are expressed as microgram equivalents of zearalenone per 100 g of wet tissue. The highest levels of ^{14}C residues in the tissues and body fluids examined were found in the bile, which appears to be a major excretory pathway for zearalenone.

Radioactivity in edible tissues such as leg, wing, and breast muscles was low after a single dose of [^{14}C]zearalenone. ^{14}C activity in fat tissue was low but appeared to be persistent. The administration of serial doses of labeled zearalenone might reveal that apidose tissue is a storage compartment.

The solubility characteristics and chromatographic behavior of ^{14}C residues extracted from selected samples of excreta and the pooled bile are compared with those of 99% pure [^{14}C]zearalenone in Table IV. Zearalenone was readily extracted by ethyl acetate, reacted with NaOH to form a water-soluble salt which was extracted by benzene

Table III. Tissue Concentration of [^{14}C]Zearalenone Residues[a]

sample	time interval after dosing, h				
	2	4	24	48	72
bile	17 100 ± 6540	17 200 ± 6190	56 300 ± 12 500	29 600 ± 16 600	3460 ± 1330
whole blood	88 ± 23	95 ± 15	86 ± 23	88 ± 28	39 ± 10
plasma	74 ± 19	82 ± 21	39 ± 0.9	23 ± 6.3	12 ± 3.0
red blood cells	136 ± 61	138 ± 68	213 ± 94	269 ± 90	125 ± 44
gall bladder	746 ± 365	1280 ± 435	4080 ± 1920	2300 ± 1320	386 ± 133
liver	341 ± 70	397 ± 82	164 ± 24	66 ± 19	50 ± 11
kidney	144 ± 29	120 ± 8.7	62 ± 4.2	53 ± 13	31 ± 5.3
spleen	45 ± 15	37 ± 13	64 ± 17	47 ± 8.7	28 ± 6.2
heart	57 ± 25	45 ± 14	38 ± 13	25 ± 4.9	37 ± 8.7
lung	59 ± 25	40 ± 3.0	40 ± 23	63 ± 17	26 ± 9.6
pancreas	38 ± 6.6	34 ± 12	30 ± 4.4	22 ± 4.1	15 ± 3.0
brain	18 ± 5.6	26 ± 13	19 ± 4.0	17 ± 2.9	11 ± 4.6
oviduct	39 ± 15	66 ± 27	68 ± 29	41 ± 8.5	49 ± 18
comb	57 ± 20	112 ± 32	183 ± 50	82 ± 33	94 ± 25
adrenals	52 ± 27	32 ± 12	79 ± 36	53 ± 8.2	38 ± 16
breast muscle	7 ± 3.0	6 ± 2.2	8 ± 4.8	10 ± 0.9	12 ± 5.5
leg muscle	9 ± 3.7	16 ± 5.4	14 ± 7.9	18 ± 3.4	12 ± 5.0
wing muscle	8 ± 3.7	8 ± 2.5	12 ± 1.3	11 ± 1.5	8 ± 1.6
fat	26 ± 7.7	30 ± 13	38 ± 12	38 ± 16	41 ± 4.2

[a] Expressed as microgram equivalent of zearalenone/100 g of wet tissue. Values are average ± standard error of four samples at each time interval.

Table IV. Solvent Fractionation and Chromatographic Separation of ^{14}C Residues Recovered from Hens Administered [^{14}C]Zearalenone by Gavage[a]

sample/fraction	[^{14}C]zearal. std.	excreta 2 h[b]	excreta 4 h[b]	excreta 72 h[b]	pooled bile	gluc. hydrol. of pooled bile[c]
		Ethyl Acetate Extraction Efficiency				
dpm/sample, total	173 000 ± 1710	122 000 ± 1530	161 000 ± 577	363 000 ± 5930	313 000 ± 4570	285 000 ± 5550
dpm extracted	173 000 ± 1710	99 700 ± 5690	143 000 ± 2780	303 000 ± 3230	13 000 ± 527	235 000 ± 3800
dpm extracted, %	100.3 ± 1.1	81.8 ± 4.7	88.5 ± 1.7	83.4 ± 0.9	4.2 ± 0.2	82.3 ± 1.3
		Recovery (%) of Extracted dpm[d]				
pH 4 buffer wash	0.5 ± 0.15	13.5 ± 0.4	13.1 ± 0.3	5.8 ± 0.3	63.5 ± 7.9	3.1 ± 0.3
$CHCl_3$ residue	4.8 ± 0.6	13.7 ± 1.6	5.4 ± 0.2	7.6 ± 0.2	13.8 ± 2.6	3.2 ± 0.2
NaOH extract	83.2 ± 1.4	73.8 ± 2.8	74.5 ± 1.5	79.3 ± 1.5	40.3 ± 1.0	90.2 ± 1.9
aqueous phase	4.1 ± 0.7	9.3 ± 0.5	4.9 ± 0.3	8.3 ± 0.4	29.6 ± 3.6	8.9 ± 0.4
benzene extract	83.3 ± 1.1	65.4 ± 2.9	70.9 ± 2.4	71.8 ± 0.7	21.1 ± 0.2	82.2 ± 1.7
hexane eluate	0.1 ± 0.05	0.2 ± 0.20	0.0	0.0	0.1[e]	0.0
benzene eluate	0.1 ± 0.08	0.0	0.0	0.0	0.1[e]	0.3 ± 0.21
benzene-acetone (95:5) eluate	77.0 ± 0.8	33.0 ± 1.1	31.6 ± 1.1	30.8 ± 0.1	8.6[e]	25.1 ± 0.8
acetone eluate	1.5 ± 0.2	28.6 ± 1.2	29.9 ± 1.2	36.7 ± 0.4	9.5[e]	53.8 ± 1.2

[a] Each value is the average of four replicate samples ± standard error. [b] Time after administration of [^{14}C]zearalenone. [c] β-Glucuronidase hydrolysate of extracted pooled bile. [d] See Figure 1 for fractionation and chromatographic analytical scheme. [e] Pooled sample.

Table V. Thin-Layer Chromatographic Separation of Benzene–Acetone and Acetone Eluates from Silica Gel 60 Columns

column chromat. eluate	[^{14}C]zearal. std.			excreta 2 h[a]			excreta 4 h[a]			excreta 72 h[a]			pooled bile			gluc. hydrol. of pooled bile[b]		
	R_f	UV	%[c]	R_f	UV	%[c]	R_f	UV	%[c]	R_f	UV	%[c]	R_f	UV	%[c]	R_f	UV	%[c]
benzene-acetone (95:5)	0.00	D[d]	0.4	0.00	D	0.3	0.05	D	3.0	0.05	D	0.6	0.11	D	0.1	0.00	D	0.1
	0.59	L[d]	75.8[e]	0.48	L	32.0[e]	0.49	D	1.6	0.46	D	0.8	0.38	D	0.1	0.32	D	0.1
	0.77	D	0.8	0.70	D	0.6	0.59	L	27.0[e]	0.57	L	29.5[e]	0.57	D	0.2	0.60	L	24.5[e]
													0.63	L	7.9[e]	0.83	D	0.5
													0.82	D	0.3			
acetone	0.00	D	0.8	0.00	D	1.1	0.02	D	3.3	0.01	D	1.0	0.09	D	0.8	0.00	D	0.5
	0.81	D	0.7	0.09	D	1.0	0.17	D	0.5	0.09	D	1.1	0.42	L	8.6[f]	0.07	L	1.9
				0.15	L	0.5	0.25	L	0.4	0.26	D	1.3	0.88	D	0.1	0.15	D	0.6
				0.33	L	26.1[f]	0.44	L	25.7[f]	0.42	L	33.7[f]				0.36	D	17.1[f]
				0.79	D	0.0										0.45	L	33.7[f]

[a] Time after administration of [^{14}C]zearalenone. [b] β-Glucuronidase hydrolysate of extracted, pooled bile. [c] Average percentage of extracted dpm accounted for from four separate chromatograms. [d] D = dark, absorbing spot and L = light blue fluorescence under shortwave ultraviolet light. [e] Spot had same R_f and ultraviolet light reaction as standard [^{14}C]zearalenone and cochromatographed with it. [f] More polar than standard [^{14}C]zearalenone; did not cochromatograph with it.

Table VI. Solvent Fractionation and Chromatographic Separation of ^{14}C Residues Recovered from Hens Administered [^{14}C]Zearalenone by Gavage[a]

sample/fraction	egg yolk 43–50 h[b]	clutch 24 h[b]	liver 2 h[b]	liver 4 h[b]
	Ethyl Acetate Extraction Efficiency			
dpm/sample, total	6690 ± 189	11800 ± 91	26100 ± 41	31800 ± 350
dpm extracted	4430 ± 193	9760 ± 373	15600 ± 1720	16000 ± 333
dpm extracted, %	66.2 ± 2.9	82.8 ± 3.2	59.6 ± 6.5	50.2 ± 1.0
	Recovery (%) of Extracted dpm[c]			
pH 4 buffer wash	5.0 ± 1.4	6.1 ± 0.8	4.1 ± 0.5	5.2 ± 0.7
$CHCl_3$ residue	91.6 ± 5.0	92.8 ± 1.5	72.0 ± 1.2	63.8 ± 0.7
NaOH extract	8.3 ± 1.6	8.9 ± 1.6	14.2 ± 0.6	20.6 ± 1.5
aqueous phase	4.9 ± 2.5	1.5 ± 1.5	8.5 ± 2.5	1.9 ± 1.1
benzene extract	3.5 ± 1.1	2.1 ± 0.3	5.7 ± 1.3	15.2 ± 1.3
hexane eluate	0.3[d]	0.2 ± 0.09	0.1[d]	0.1[d]
benzene eluate	1.7[d]	0.2 ± 0.14	0.5[d]	0.6[d]
benzene-acetone (95:5) eluate	1.6[d]	0.4 ± 0.20	1.0[d]	5.5[d]
acetone eluate	0.6[d]	0.2 ± 0.12	2.6[d]	6.2[d]

[a] Each value is the average of four replicate samples ± standard error. [b] Time after administration of [^{14}C]zearalenone. [c] See Figure 1 for fractionation and chromatographic analytical scheme. [d] Pooled sample.

after acidification to pH 6, and was eluted from a silica gel 60 column with benzene–acetone (95:5) (Figure 1). When the benzene–acetone eluate was chromatographed on a silica gel plate with chloroform–ethanol (97:3), approximately 76% of the original [^{14}C]zearalenone standard (Table V) was accounted for in a light-blue, fluorescent spot with an average R_f value of 0.59. There was some variation in the R_f value (0.48–0.63) of zearalenone in this system from day to day possibly due to differences in the degree of saturation of the chromatographic chamber. However, to assure reliability, at least two standard [^{14}C]zearalenone spots and one spot of sample plus

standard were applied to each TLC plate on each analysis.

When the 2-, 4-, and 72-h excreta samples were partitioned and chromatographed on columns, about 31–33% (Table IV) of the extractable ^{14}C activity was eluted with benzene–acetone, while another 29–37% eluted with the more polar solvent. Results in Table V show that most of the ^{14}C activity of the benzene–acetone eluates had the same R_f and ultraviolet light reaction as standard [^{14}C]-zearalenone and cochromatographed with it, while the acetone eluates contained more polar, labeled metabolites which separated completely from [^{14}C]zearalenone on cochromatography.

When the pooled bile samples were extracted with ethyl acetate, only about 4% of the ^{14}C was recovered in the extract (Table IV). Of this, only about 8% could be accounted for as unchanged zearalenone on TLC plates (Table V). However, when the previously extracted pooled bile was hydrolyzed with β-glucuronidase (Dorough et al., 1974) 24 h at 37 °C, 82% of the remaining ^{14}C activity became extractable with ethyl acetate. About 25% of this appeared to be [^{14}C]zearalenone, as determined by TLC, while approximately 50% was in the form of polar metabolites which eluted from silica gel columns with acetone and separated into two major spots on thin-layer chromatograms. Neither of these spots cochromatographed with [^{14}C]zearalenone. Further characterization of these metabolites was not possible without appropriate standard compounds to be used for comparison.

Table VI clearly indicates that most of the ^{14}C residues in egg yolk and clutch are lipophilic metabolites (92–93% remaining in the chloroform layer).

Only 50–60% of the ^{14}C residues in liver samples were extractable with ethyl acetate (Table VI) and it is probable that the unextracted portion reflects a continuing production of glucuronide conjugates. Most of the ^{14}C residues extracted from the liver samples were lipophilic metabolites and are probably the source of the radioactivity found in egg yolk and clutch. Only small amounts (1–6%) of the extractable dpm were eluted from silica gel columns with benzene–acetone (95:5) and there was insufficient radioactivity for detection on thin-layer plates. The elution patterns from the columns, however, would suggest the presence of small quantities of unchanged [^{14}C]zearalenone and polar metabolites.

In summary, the following conclusions appear justified based on the data presented: (1) most (about 94%) of a single dose of zearalenone had been excreted within 72 h after administration, (2) about one-third of the dose was excreted as unchanged zearalenone while another one-third was a polar metabolite, (3) zearalenone was readily conjugated with glucuronic acid, and (4) there appeared to be no major retention sites in edible muscle tissues but persistent levels of lipophilic metabolite(s) of unknown composition and toxicological significance were detected in egg yolks for at least 72 h after administration of the parent compound.

Thus, it would appear that after a single exposure of laying hens to feed contaminated with low levels of zearalenone, the health hazard to the human population would be minimal. However, if the exposure time was prolonged, it is possible that significant levels of the lipophilic metabolite(s) might accumulate in egg yolk. Further work to elucidate the structure and toxicity of such metabolite(s) appears justified.

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Metabolism of *cis*-[^{14}C]Chlordane and *cis*-[^{14}C]Photochlordane in Bluegill Fish

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Bluegill fish rapidly absorbed *cis*-[^{14}C]chlordane or *cis*-[^{14}C]photochlordane during a 48-h exposure to 5 parts per billion of each insecticide. Elimination of chlordane was linear but slow during the 6-week period, while that of photochlordane was biphasic, being rapid in the early phase (first 3 weeks). Less than 7% of the radioactivity retained in the chlordane-treated fish was in the form of two conjugates which on acid hydrolysis yielded at least eight hydroxylated products. In the case of photochlordane, 16% of the radioactivity in fish was in the form of nine apolar and five polar metabolites. Twenty-five percent of the radioactivity excreted in water by photochlordane-treated fish was in the form of two metabolites, the remaining being unchanged photochlordane.

Contamination of aquatic environments with chlorinated cyclodiene insecticides (Brooks, 1974; Matsumura, 1975; Edwards, 1977) and their accumulation and ecological concentration by aquatic food chains (Craig and Rudd, 1974; Metcalf, 1977) can result in high levels of their residues in organisms at higher trophic levels (Rudd, 1964; Woodwell et al., 1967; Sanborn et al., 1976; Blus et al., 1977). The residues of cyclodiene insecticides, because of

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