Study of Residues of Dalapon in Chicken Tissues and Eggs Following Repeated Feeding

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Groups of 12 hens were fed continuously on feed containing 0, 25, and 50 p.p.m. of dalapon. Eggs were collected during this period. At the end of 30 days, five hens from all groups were sacrificed. Tissues collected were lean meat, skin and fat, liver, gizzard, kidney, blood, and feathers. At the end of 60 days, the remaining hens were sacrificed. Residues of dalapon were determined by electron-capture

owpon grass killer, containing the sodium salt of dalapon, 2,2-dichloropropionic acid (Dalapon is a trade-mark of The Dow Chemical Co. outside the U.S.), has been used extensively for several years to control problem grasses in a number of crops. Extensive residue studies have been carried out over several years to determine dalapon acid residues. The first report of these was the finding of no residue in sugar cane treated with Dowpon by Smith et al. (1957). Schreiber (1959) reported on the residues of dalapon in Birdsfoot trefoil from a number of different applications of the herbicide and showed that there is wide variation of residue content depending on the rate and timing of the application of Dowpon. Mac Collom and Flanagan (1967) studied residue disappearance from trefoil related to time. Kutschinski (1961) and Fertig and Schreiber (1961) have reported on dalapon residues in milk, and Getzendaner et al. (1965) studied the residue situation on citrus fruit from a wide variety of application schedules. Archer et al. (1967) have studied dalapon residues in asparagus.

Tolerances have been established on a wide variety of crops by the Food and Drug Administration (*Federal Register*, 1963). With tolerances for residues being established in corn, grain, and the possibility of further tolerances in forage crops, chickens may be exposed occasionally to diets containing traces of dalapon. This experiment was carried out to determine the residue levels of dalapon in chicken tissues and eggs which would result from repeated ingestion of feed containing fixed concentrations of this chemical. This experiment was designed to show the variation of residue in the tissues between birds from normal consumption patterns. The feed eaten gas chromatography. Residue values, determined for all the tissues from each individual hen, were roughly proportional to the level of feeding. Maximum values for eggs at the 50 p.p.m. level of feeding were 2 p.p.m.; for meat, skin and fat, liver, gizzard, and feathers, the maximum was about 15 p.p.m., while blood rose to 40 and kidney to 50 p.p.m.

by each group was all at the same concentration, so the consumption pattern of each chicken was the controlling factor on the intake of chemical, which was reflected in the residue levels in individual tissues.

EXPERIMENTAL

Three groups of 12 mature White Rock laying hens were randomly selected. Feed was prepared starting with Farm Bureau Mermash, 20% protein, manufactured by a local elevator. The calculated amount of dalapon was added to 200-pound batches to make 0, 25, and 50 p.p.m. of the chemical. These were thoroughly mixed and bagged in 25-pound bags which were refrigerated until they were used. Feed was consumed *ad libitum*. Eggs were collected daily and refrigerated until they were analyzed. After 30 days on feed, 5 hens from each group were sacrificed, the remaining hens were left on feed for another 30 days. At sacrifice, samples of white and dark meat, fat and skin, liver, gizzard, kidneys, and blood were removed from each hen and frozen as individual samples. Feathers were collected and stored in a refrigerator.

Until recently, most of the data on dalapon residues have been obtained by the original spectrophotometric method of Smith *et al.* (1957) using suitable modifications to adapt it to various crops. Getzendaner (1963) gives a method for determining dalapon residues by gas chromatography which is considerably faster than the chemical method and is capable of determining residues starting with much smaller samples. Modifications were made as indicated below to adapt the method for use with the two general types of tissues encountered: those which contained appreciable quantities of fat; and those which contained very little fat. For samples which contained little or no fat, an extraction from the solid in an acidified water system was adequate to give good recovery of dalapon, with phosphotungstic acid added as a precipitant to reduce the

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amount of water-soluble materials. The samples containing appreciable amounts of fat gave low recoveries using the acid extraction because of partition of the free propionic acid into the fat phase. Therefore, a borax buffered aqueous extraction was used. An exception to this procedure was followed in the case of eggs which contain fat, where the acidified phosphotungstic acid solution was used, but the solid-aqueous mixture was extracted with ether, rather than removing the solid phase from the water phase. It was not practical to follow this procedure generally, because of emulsion formation with the water and ether phases, or because of the large amount of fat extracted into the ether. The differences in procedure used on the tissues, except for the major one given above, result from physical differences of the material and/or levels of dalapon found, necessitating differing aliquots for final analysis.

Reagents and Apparatus. Phosphotungstic acid solution, 1%. Dissolve 5.0 grams of phosphotungstic acid, reagent grade, Mallinckrodt, in 100 ml. of H₂O, add 0.2 ml. of 75% H₃PO₄, and mix.

Borax solution, salt saturated. Dissolve 20 grams of $Na_2B_4O_7 \cdot 10H_2O$, reagent grade, in 1 liter of H_2O . Saturate this solution with NaCl.

Multi-Mixer, Lourdes Instrument Corp., adapted for 8-ounce bottle by drilling a hole through the middle of a 48 mm. polyethylene bottle cap with a No. 12 cork borer. This cap was forced over the threads and against the shoulder of a spindle assembly 50ATT. A washer was placed between coupling holder and the top bearing, so that the drive shaft would make good contact with the coupling. Lubrication with International lubricant (Cat. No. 1709, International Equipment Co.) or chicken fat was effective in extending the life of the bearings, and caused no problem with contaminants. The spindle assembly was removed, cleaned, and lubricated after blending each pair of duplicate samples.

Extraction Procedures. EGG. Mix egg thoroughly. Add 5.0 ml. to 30-ml. screw-cap centrifuge tube, then add 10 ml. of 1% phosphotungstic acid solution and 6 grams of NaCl. Shake vigorously. Add 5.0 ml. of ether and shake for 1 minute. Centrifuge to separate the layers and pack the solid at the interface (20 minutes at 2500 r.p.m. in a refrigerated centrifuge was adequate), and decant the ether layer into small screw-capped vials (13 \times 100 mm., with aluminum-lined caps) if analysis is not to be completed immediately.

KIDNEY. Blend a 2.5-gram sample for 5 minutes in a plastic test tube with 22.5 ml. of 5% phosphotungstic acid. For recoveries, add dalapon to the test tube before the aqueous extraction. Filter the extract through pleated filter paper and add 4.0 ml. by pipet to a test tube, plus an excess of NaCl. 1 drop of 85% H₃PO₄, and 4.0 ml. of absolute diethyl ether (analytical reagent grade). Cap and shake vigorously. One milliliter of ether is equivalent to 0.1 gram of tissue.

LIVER. Same as kidney except that 5.0 ml. of extract are extracted with 1.0 or 2.0 ml. of ether.

BLOOD. Add 0.5 ml. to a screw-cap test tube with 2.0 ml. of phosphotungstic acid solution. For recovery experiments add dalapon directly to the blood. Saturate with NaCl and add 3.0 ml. of ether. After capping and

shaking vigorously, centrifuge at low speed to separate the phases.

MEAT. Take approximately equal portions of light and dark meat. Blend 10 grams with 30 grams of NaCl and 50 ml. of 5% phosphotungstic acid solution. Weigh 30 grams of this mixture into a 35-ml. screw-capped centrifuge tube and add 5.0 ml. of ether. For dalapon recovery runs, add the dalapon in a small amount of acetone solution to the sample in the centrifuge tube. Shake thoroughly before extraction. After adding ether, cap the tubes and shake 2 minutes on a mechanical shaker, then centrifuge 20 minutes at 2500 r.p.m. Decant the ether into small vials as with the eggs.

FEATHERS. Extract by the procedure used for fatcontaining samples. Cut large feathers into small pieces with scissors and weigh 2.5 grams into a 4-ounce screw-cap bottle. For recovery experiments add dalapon directly to the feathers, then add 50 ml. of NaCl-saturated 2% borax, and cap the bottle. Put the bottles into a shaker on their sides and shake vigorously for 1/2 hour, then allow to stand for 16 hours, followed by 1/2 hour of shaking. Some sampleswere filtered through Reeve Angel 804 paper, and others were decanted. Pipet a 5.0-ml. aliquot into a 18×100 mm. screw-cap test tube with 5 drops of H_3PO_4 , then add 1.0 ml. of ether and cap the tube. Shake for about 1 minute, then centrifuge at low speed if necessary to separate the phases.

SKIN AND FAT. Grind skin and fat while frozen in a meat grinder. Blend 10 grams in a square 8-ounce bottle on a Lourdes Multi-Mixer with 50 ml. of 2% borax solution for 5 minutes after chilling the mixture in an ice bath or freezer to nearly 0°C. Maintain an ice bath around the jar during blending. Remove the ice bath after 5 minutes and continue blending for 5 minutes to bring the temperature to about 25°C. Hold the mixture at room temperature for 1/2 hour with occasional shaking and filter through fluted paper (Reeve Angel 804 12.5 cm.). For recovery experiments, add dalapon in acetone solution before blending.

Pipet 5 ml. of the filtrate into 13×100 mm. screwcapped test tubes containing 5 drops of 85% H₃PO₄. Add enough NaCl to provide an excess, and 1 ml. of ether. Shake vigorously for 1 minute after capping with aluminum-lined caps, and allow the phases to separate. Use low centrifugation in some cases to break the emulsion.

The above cold extraction procedure for skin and fat was verified by extracting samples with "grown-in" dalapon on a soxhlet extractor. Ten grams of tissue were mixed with 10 grams of anhydrous Na₂SO₄ and 10 drops of 85% H₃PO₄ in a beaker. This was transferred to a Soxhlet thimble and extracted 8 hours. The ether extract was extracted three times with 10-ml. portions of borate buffer saturated with NaCl. A 5-ml. aliquot was removed from the combined aqueous extracts, made to 50 ml. with borate buffer in a volumetric flask, acidified with 5 drops of H₃PO₄, saturated with NaCl, and extracted with ether. The dalapon content was then determined as given above. Values obtained on one sample by the cold extraction averaged 4.8 p.p.m. and by the Soxhlet extraction 4.3 p.p.m., on the other sample 2.8 p.p.m. (cold) vs. 2.5 (Soxhlet) with no correction factors included. This demonstration that the "cold" procedure was as effective as a more exhaustive extraction in removing all the dalapon from the skin and fat justifies the use of the shorter cold extraction to obtain the data reported.

GIZZARD. Grind samples of gizzard in a meat grinder prior to extraction. Extractions by the method used for kidney and liver were only partially successful. Recovery of dalapon was low in some cases. Consequently the procedure for samples containing fat was used. Weigh 2.5 grams into a 4-ounce screw-cap bottle, and add 22.5 ml. of NaCl-saturated 2% borax. Blend on the Lourdes Multi-Mixer 5 minutes and filter. Pipet 5.0 ml. into the 13×100 mm. screw-cap test tubes, add 5 drops of H₃PO₄, a small amount of NaCl, and 1.0 or 2.0 ml. of diethyl ether, depending on the dalapon level. Shake vigorously and allow the phases to separate.

Analyze aliquots of the ether extracts of the samples by the method described by Getzendaner (1963). In all samples except for feathers from the 25 p.p.m. feeding group, $1.0 \ \mu$ l. or smaller aliquots of the ether extracts were injected. The aliquot was increased to 2.0 $\ \mu$ l. for the extract from feathers from the low-level birds.

Column and conditions used for the gas chromatographic

Dalar						ry of Known Amounts of Dalapon A				
	on Added	Dalapon Found, P.P.M.	07 D	· · · · · · · · · · · · · · · · · · ·	alapon Added	Dalapon Found,	0-7 D			
μ g .	P.P.M.		% Recovery	μ g.	P.P.M.	P.P.M.	% Recovery			
		Egg			Skin and	Fat (Continued)				
1.0	0.20	0.22	108	50	5	3.9	78			
1.25	0.25	0.25	100			4.2	84			
_						4.5	90			
2.5	0.5	0.44	88			4.4	88			
		0.48	96			Aver	age 88			
		0.50	100			Liver				
		0.38	76	1.0	1.0	1.0	100			
5.0	0.1	0.80	80			1.0	100			
5.0	1.0	1.02	102							
		0.84	84	2.0	2.0	1.84	92			
		0.88	88			2.0	100			
		0100	00							
10.0	2.0	1.7	85	3.0	3.0	2.4	80			
		2.0	100			2.8	93			
		1.7	85							
		1.9	95	5,0	5.0	5.4	109			
						4.6	92			
20.0	4.0	3.2	80	10.0	10.0	0.0	00			
		2.8	70	10.0	10.0	8.8 9.4	88 94			
		3.4	85			9.4	94			
		Avera	ige 90	20.0	20.0	17.0	85			
		ght and		20.0	20.0	Avera				
		rk Meat				Gizzard				
8.3	0.83	0.68	82	5.0	2.0	1.84	92			
		0.70	84	5.0	2.0	1.84	92			
17	1.7	1.5	88			1.04	2			
17	1.7	1.7	100	12.5	5.0	5.2	104			
		1.7	100			5.4	108			
42	4.2	4.5	107							
		4.3	102	25,0	10.0	10.0	100			
						10.0	100			
84	8.4	9.1	108			14.0	107			
		8.6	102	37.5	15.0	16.0	107			
				50.0	20.0	19.6	98			
170	17.0	16.0	94	20.0	20.0	18.4	92			
		16.0 Avera	94 1ge 96			Avera				
	5 1 ·		ige 90			Kidney	C			
		and Fat		1.0	1.0	0.75	75			
10	1	0.96	96	1.0	1.0	0.75	75			
		0.91	91			0.75	78			
20	2	1.8	90			0170				
20	2	1.5	75	2.0	2.0	2.0	100			
		1.8	90			2.0				
				4.0	4.0	3.35	84			
30	3	2.5	84							
		2.8	93	5.0	5.0	4.5	90			
						4.5	90 190			
40	4	3.6	90			5.0	100			
		3.2	80 105							
		4.2	105							
·										

determination of dalapon residues were not uniform throughout the entire experiment. However, the changes were minor. Typical conditions employed were Barber-Colman Model 10 gas chromatograph; N₂ flow, 85 ml. per minute; Sr detector Model A-4148, 13.5 to 18 volts; or tritium detector Model A-4168, 45 volts; column, 40 inches \times 3-mm. I.D. glass U-shaped column packed with 3.8% LAC-2R-446 + 1% H₃PO₄ on Chromosorb WAW, 80- to 100-mesh, conditioned at 175° C.; column temp., 100° to 110° C.; cell bath temp., 150° to 165° C.; flash heater, temp., 175° C. Column prepared with no plug at

to Untreated Chicken Tis	ssues and Eggs
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	on Added	Delenen Ferr	د
μg.	P.P.M.	Dalapon Foun P.P.M.	a, % Recovery
		Kidney	
10.0	10.0	9.0	90
		9.5	95
		9.3	93
20.0	20.0	17.5	88
		16.5	82
30.0	30.0	28.0	93
40.0	40.0	35.5	89
(0, 0	(0, 0	37.5	94
60.0	60.0	54.0	90 Average 86
		Blood	Weinge 00
0.5	0.5	0.45	90
0,5	0.0	0.50	100
1.0	1.0	1.0	100
3.0	3.0	2.5	84
		3.0	100
5.0	5.0	5.4	108
		5.75	115
10.0	10.0	11.0	110
10.0	1010	9.3	93
20.0	20.0	21.9	109
20.0	20.0	21.9	109
30.0	30.0	29.4	98
50.0	50,0	28.6	95
		А	verage 101
		Feathers	
0.5	0.5	0.54	108
1.0	1.0	1.12 1.00	100 100
		1.00	100
2.0	2.0	1.76	88
		2.0	100
5.0	5.0	4.0	80
		3.7	73
		4.8	96
25.0	10.0	8.4	84
		8.4	84
50.0	20.0	16.4	82
		21.6	108
		17.0 A	verage 91

entrance end, and a fine stainless steel cloth plug at exit end; chaser, 0.2% phosphoric acid in acetone or ethyl acetate.

RESULTS AND DISCUSSION

Results of experiments to determine recovery of dalapon from tissues are given in Table I. These data show a good level of recovery in all cases. In the case of gizzard, recoveries were run first by the method of acid extraction. Although most of the recoveries were good, a few were as low as 60% so it was concluded that this tissue contained enough fat so that the borax buffered extractant should be used. The recoveries given in Table I for gizzard are from the borax buffered extraction, and all the residue values obtained were by this method.

The eggs were analyzed on about a 3-day schedule. All eggs from each pen on each third day were divided into two groups. The individual eggs in each group were combined and mixed well and an aliquot was taken for analysis. As indicated in Table II, on the 29th day of feeding, each egg from the two treated pens was analyzed separately to determine the range of variation within each group for a single day. The maximum residue found from the 25p.p.m. diet was 1.1 p.p.m., while 2.0 p.p.m. was the maximum from the hens receiving 50 p.p.m.

Residues found in the other tissues analyzed are given in Table III. Part A of this table shows results of analysis

Table II. Residues in Eggs from Continuous Ingestion of Dalapon-Containing Feed

Rate of Feeding of Dalapon										
Days on Feed	25 p.p.m.	50 p.p.m.								
Containing	Residues of Dal	• •								
Dalapon	,									
	0, 0	0, 0								
	0, 0	0, 0								
	0, 0	0, 0								
1	0, 0	0, 0								
4		0.1, 0.1								
7	0.5, 0.2	0.7, 0.4								
10	0.6, 0.6	0.9, 1.1								
13	0.6, 0.7	1.1, 1.3								
16	0.6, 0.9	0.8, 1.2								
19	0.5, 0.6	1.0, 1.1								
22	0.7, 0.8	1.1, 1.2								
25	0.8, 0.8	0.7, 1.3								
28	0.7.0.7	1.0, 1.6								
29 ^b	0.7	1.5								
	0.8	1.5								
	0.7	1.6								
	0.6	1.6								
	0.7	0.9								
	1.1	1.1								
	0.5	1.2								
	0.4	1.5								
31	0.6, 0.6	1.3, 1.7								
33	0.9, 1.0	2.0, 1.7								
34	0.6, 0.6	1.6, 1.5								
37	0.2, 0.3	0.9								
40	0.4, 0.9	1.2, 1.0								
43	0.7, 0.5	1.2								
47	0.7, 0.5	1.2								
49	0.7, 0.7	1.2, 1.1								
52	0.5	1.5, 1.2								
55	0.8, 0.8	1.3, 1.7								
58	0.4	1.8, 1.2								
^{<i>a</i>} Values not corre ^{<i>b</i>} Values from indi	cted for recovery whic vidual eggs laid on 29t	h averaged 90%. h day.								

Chicken	Days on					Ap	parent Re	esidueGro	ss P.P.N	1. Dalapo	n		
No. Feed		Lean	meat	Ski	n, fat		Liver	Gizzard	Ki	dney	Blood	Fea	thers
						A-Pen	1- Contro	1					
711	30	(0	0		0 0	0	0	1
703		()					0		0	0	()
710							0				0 0	0	1
702					0		0			0	0	0	. 1
,01					Ő		Ŭ			ŏ	ŏ		
701					-		0			0	0		
										0	0	0	
712	60	()				0	0		0	0		. 1
709		()		0		0	0		0			. 2
708		()		0 0		0			0	0	0	. 1
705		()		0		0	0			0	0	. 1
704))		0		0				0	0	. 1
706)		0		0	0		0	0	0	. 1
						Appar	ent Resid	ueP.P.M.					
		Lean	Meat	Skin	i, Fat	Li	ver			lney			thers
		Gross	Corr. ^a	Gross	Corr. ^a	Gross	Corr. ^a	Gizzard	Gross	Corr. ^a	Blood	Gross	Corr. ^a
					B	Pen 2, 25	p.p.m. in	Feed					
713	30	2.8	3	5.2	6	6.4	7	4.8	18	21	15	3.0	3
				4.5	5	7.4	8	5.2	18	21	13	3.2	4
722		2.9	3	4.4	5	7.2	8	5.6	22	26	12	3.0	3
				• •	2	7.2	8	5.8	20	23	11	2.4	3
718		3.3	4	2.8	3	6.0	6	4.6	14	16	14 13	1.2 1.2	1
715		1 0	2	2.9	3	6.2 4.4	7 5	5.4 3.4	12 12.5	14 15	6.6	1.2	1 2
715		1.9	2			4.4	4	3.4 3.4	12.5	15	6.3	2.0	2
716		2.0	2			4.2	5	3.4	13.5	16	10	2.0	4
/10		2.0	2			4.7	5	2.9	13.5	15	11		
720	60	4.5	5	2.3	3	6.0	6	5.0	13	15	13	1.2	1
	00	4.8	5	2.3	3	6.2	7	4.4			14	0.9	1
714		4.7	5	1.6	2	6.2	7	4.2	12.5	15	13	1.0	1
		4.3	5	2.0	2	6.8	7	4.4	13	15	13	0.9	1

Table III. Residues of Dalapon in Chicken Tissues after Continuous Ingestion of Dalapon-Fortified Feed

of tissue from untreated hens. In only one tissue, the feathers, was any blank found. In the case of the feathers, a material was eluted which had the same retention time as dalapon, although the peak height indicated a level of less than 0.2 p.p.m. in all cases. This might have been dalapon contamination by dust from the other pens. Because of this low level, no effort was made to identify it, and a correction was made for its presence in calculation of the final corrected residue values. The greatest normal variation of residue content in the tissues was found in the feathers. This could have resulted from gross contamination of the feathers by feed during the course of the feeding period.

When hen No. 735 was sacrificed, she had a ruptured crop. There was no other obvious gross difference between her and the other hens from the same pen. However, the dalapon content of the tissues was extremely low when compared to her pen mates. A possible explanation for this could be that she stopped eating a few days prior to termination of the experiment. However, this seems unlikely in view of the weight comparisons.

The data on the tissues are summarized in Table IV. The lowest level of residue was in the eggs, while kidneys have the highest level. The usual edible tissues, lean

Table IV. Summary of Dalapon Residues in Chicken Tissues

	P.P.M. of Dalapon								
	Conta	n Ration aining . Dalapon		aining Dalapor					
	Min.	Max.	Min. ^b	Max.					
Egg^a	0.2	1.0	0.9	2.0					
Lean meat	2	7	3	13					
Skin, fat	2	7	6	15					
Liver	4	9	9	16					
Gizzard	3	6	7	12					
Kidney	14	28	12	50					
Blood	6	19	12	40					
Feathers	1	4	1	11					

 a 31 or more days from start of datapoin feeding b Hen 735 with ruptured crop not included.

meat, skin and fat, liver, and gizzard all have about the same dalapon content. In general, the level in the tissue is proportional tc the level in the feed, but the maximum in the major edible tissues reaches only about 1/3 of the level fed.

Chicken						Арра	rent Resid	lue—P.P.M	. Dalapo	n					
Chicken	Days on	Lean meat		Skin fat		Li	ver		Kid	ney		Feathers			
No.	Feed	Gross	Corr."	Gross	Corr. ^a	Gross	Corr. ^a	Gizzard	Gross	Corr. ^a	Blood	Gross	Corr.ª		
					B-Pen 2,	25 p.p.m	. in Feed	(Continued)							
721		5.1	5	2.7	3	6.8	7	5.2	17	20	12	1.3	1		
		5.8	6	2.2	3	6.4	7	5.6	14	16	13	1.4	2		
725		6.3	7	3.7	4	5.4	6	5.4	16	19	14	1.2	1		
						4.8	5	5.2	19	22	12	1.2	1		
724		2.8	3	1.4	2	4.8	5	4.8	16	19		1.0	1		
		4.0	4	1.7	2	5.2	6	4.1	12	14		1.0	1		
719		5.5	6	1.4	2	8.4	9	5.2	16	19	17	1.6	2		
		5.5	6	2.1	2	7.6	8	5.4	17	20	16	1.2	1		
723		6.2	7	5.8	7	7.8	8	5.7	24	28	15	0.8	1		
		6.2	7	5.2	6	8.0	9	6.0	23	27	19	1.0	1		
						Appare	ent Residu	e—P.P.M. I	Dalapon						
		Lean	Meat	Skin	, Fat	Liv	ver		Kid	ney			thers		
		Gross	Corr."	Gross	Corr."	Gross	Corr. ^a	Gizzard	Gross	Corr. ^a	Blood	Gross	Corr. ^a		
					C-	Pen 3, 50	p.p.m. in	Feed							
727	30	4.0	4	6.7	8	8.4	9	8.2	14	16	20	2.4	3		
				7.5	9	9.6	10	8.6	10	12	18	3.4	4		
729		7.3	8	13	15	13	14	10.8	19	22	26	6.0	7		
				11	13	14	15	10.4	22	26	26	5.2	6		
737		3.5	4	4.9	6	10	11	8.7	16	19	12	1.7			
				5.7	7	11	12	8.2	14	16	16	1.6	2 2 5		
736		4.1	4	9.6	11	14	15	10	25	29	27	4.3	5		
		4.9	5			14	15	10	27	31	27	4.6	5 3		
733		3.0	3	5.9	7	9	10	7.4	19	22		2.8			
				6.0	7			7.6	21	25		3.0	3		
726	60	10	10	8.5	10	9.6	10	9.6	32	37	25	5.6	6		
		8.8	9			10	11	9.2	34	40	23	6.4	7		
730		8.5	9	7.3	8	12	13	8.2	22	26	26	10	11		
		7.3	8	7.4	9	11	12	7.2			26	8.0	9		
731		10	10			14	15	12	29	34	28	4.0	4		
		8.8	9			14	15	12	30	35	29	1.3	1		
735 ^b		1.7	2	1.2	1	2.8	3	1.8	4.0	5	6.8	2.0	2		
		1.7	2	1.7	2	2.1	2	1.8	4.0	5	5.4	2.4	3		
728		12	13	10	12	14	15	13	41	48	40	9.8	10		
		12	13			15	16	12	43	50	40	8.4	9		
734		7.2	8	6.8	8	15	16	11.4	25	29	28	4.8	5		
		5.0	5	7.1	8	13	14	10.4	26	30		4.4	5		
732		7.7	8	6.3	7	13	14	11	25	29	23	4.4	5		
		7.3	8	5.6	6	14	15	7.4	22	26		4.0	4		

 a Corr. = corrected for average recovery, in Table I. b Ruptured crop.

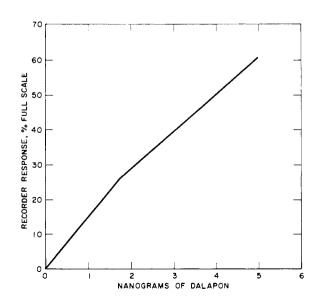


Figure 1. Typical standard curve used to determine residues of dalapon in chicken tissues

Chromatographic conditions: Column, 40 inches × 3 mm., 3.8% LAC-446 + 0.9% H₃PO₄ on Chromosorb WAW, 80- to 100-mesh Column temperature, 120° C. Injector temperature, 175° C. Detector, cell A-4148 N₂ flow, 80 ml. per minute Voltage, 10.5 Electrometer sensitivity. 2 × 10⁻⁹ amp. full scale 5-my.

Electrometer sensitivity, 2×10^{-9} amp. full scale 5-mv. recorder

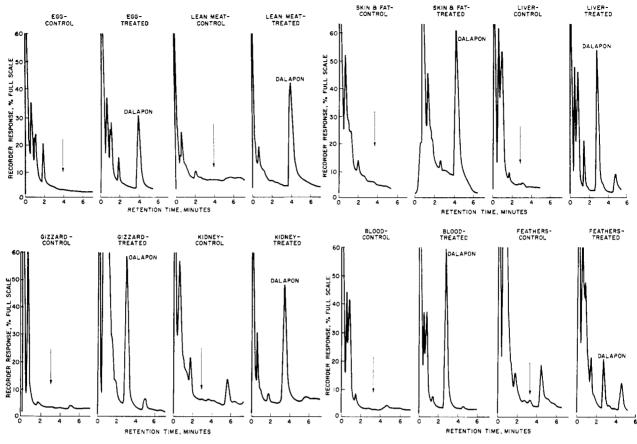


Figure 2. Typical chromatograms of chicken tissue extracts

Typical Chromatograms. A typical standard curve used for determination of residue of dalapon in chicken tissues is given in Figure 1. Because of changing conditions of chromatography, a standard curve was run every morning and every afternoon. Standards were also interspersed with samples. Any one curve could be used only at the time it was shown to be applicable.

Tracings of typical chromatograms of untreated tissue, and tissue from treated birds, are found for the eight tissues in Figure 2. These graphs show the generally good resolution of dalapon from interfering peaks. Operating conditions for obtaining these chromatograms are as given in the text.

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