14-day sampling resemble a normal frequency curve. The number of animals on each side of the mean, disregarding animals included at the point nearest the mean, represents the most interesting point reference in the curves. For the 14-day samples, 13 animals fall on each side of the mean, whereas for the 7- and 21-day samples greater numbers of animals at the higher levels had greater influence on the mean than the greater number at lower levels.

Data clearly indicate that relatively high residue values do occur. The frequency, however, of excessively high values of dalapon exceeding 2.0 p.p.m. at a feeding of 300 p.p.m. may be extremely small. Only 7% of the samples tested for residue in this experiment exceeded 2.0 p.p.m.

Residue as a Function of Breed. Animals were selected at varied stages of lactation and levels of milk production. The mean values for dalapon residues between and within breeds were nonsignificant. Although they were nonsignificant, a high degree of variability among dairy animals in respect to dalapon residue in milk does occur as shown in Table III. Standard deviations and standard errors for each sampling date are listed by breeds in Table V.

Residue as a Function of Milk Production. It is not unreasonable to assume some direct relation between residue in milk and milk production level. Based on results of this study, there appears to be no correlation between these two factors. Again, this leads to the conclusion that individual animals can vary as to their response to dalapon feeding. To clarify this point, several animals were selected for specific examples of variability (Table VI).

By comparing animals 2 and 5, one could suggest a direct correlation between milk production and dalapon residue. However, the remaining animals tend to disprove this point rather conclusively. Residue values in milk from animals 14 and 17 are completely opposite the values for animals 2 and 5; while animal 19, which about equals animal 14 in milk production, had over twice the dalapon residue.

The feeding of a constant level of dalapon based on dry-matter intake brought about some fluctuations in actual parts per million fed as shown in Table IV. As the experiment progressed, the dosage level approached the 300-p.p.m. feeding level more consistently—anticipated intake approached actual intake. However, variability is still evident in the second and third sampling dates of the feeding period.

Per Cent of Ingested Dalapon Recovered in Milk. Percentages of dalapon recovered in milk based on actual parts per million fed during each weekly period are shown in Table VII. These values vary from a high of 0.747% to a low of 0.105%. The mean value for recovery over all breeds based on 64 samples was 0.3999%. Figure 3 shows this relation clearly.

Rate of Dalapon Disappearance with Time. Data in columns 6 and 7 (Table III) represent the corrected dalapon residue in milk from all animals on the third and seventh days, respectively, after dalapon feeding had been stopped. Data indicate a rapid drop in residue to an average of 0.11 p.p.m. in 3 days and 0.045 p.p.m. in 7 days.

Data in Table VII also suggest that about 0.4% of dalapon ingested by a dairy animal may be in milk as a residue.

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INSECTICIDE RESIDUES

Aldrin and Dieldrin Content of Body Tissues of Livestock Receiving Aldrin in Their Diet

M. C. IVEY, H. V. CLABORN, and H. D. MANN

Entomology Research Division

R. D. RADELEFF and G. T. WOODARD[1]

Animal Disease and Parasite Research Division, U. S. Department of Agriculture, Kerrville, Tex.

Since it was first discovered that DDT and other chlorinated hydrocarbon insecticides (*2, 3*) are stored in the fat of beef cattle and excreted in the milk of dairy cows, practically all of these insecticides have been studied to determine the contamination resulting from the feeding of known levels in the diet.

Claborn *et al.* (*4*) reported on feeding studies of sheep and cattle where aldrin was added to the diet. Residues in the fat were calculated from organic chlorine determinations and reported as aldrin. Present information suggests that these results should have been calculated as dieldrin, because Bann and coworkers (*1*) have shown that aldrin undergoes a rapid epoxidation to dieldrin in the animal's body and that the dieldrin metabolite is stored in the fat. The conversion was shown to take place in pigs, rats, poultry, and dairy cows from oral ingestion, and in beef cattle and sheep following subcutaneous injections.

Since aldrin had been demonstrated as an effective insecticide against forage-feeding insects at low application rates, it was deemed desirable to make further studies with dosages lower than those reported by Claborn (*3, 4*) and to use the more sensitive and specific methods of analysis for determining aldrin (*7*) and dieldrin (*6*). Accordingly, in 1956 a study was carried out to determine these residues in the tissues of cattle, sheep, and hogs fed on normal diets artificially contaminated with aldrin.

Experimental

Two animals of each kind were fed control rations with no aldrin added. On the basis of prior experience and

[1] Present address, Meat Inspection Division, U. S. Department of Agriculture, Texarkana, Tex.

When varying levels of aldrin were given over a period of 12 weeks to steers, sheep, and hogs as a contaminant of the feed, only small amounts of aldrin were found in the body tissues, even at the high level of 10 p.p.m. However, the aldrin was oxidized in the animal body and stored in the tissues as dieldrin. Small amounts of dieldrin were found in the liver, kidney, and muscle tissues, but by far the greatest amounts were found in the fat. The feeding of 0.25 p.p.m. of aldrin caused 0.99 p.p.m. of dieldrin to be stored in the body fat of the steers, and 10 p.p.m. increased the dieldrin content to 39.2 p.p.m.

Table I. Average Pounds of Feed Containing Different Amounts of Aldrin, Consumed per Pound of Weight Gain

Aldrin, P.P.M.	Steers	Sheep	Hogs
0 (control)	6.18	8.48	4.29
0.25	6.85	9.37	3.98
0.75	6.40	7.93	4.05
2	5.86	..	..
10	7.72	9.40	4.01

investigation, the aldrin was portioned at 0.25-, 0.75-, and 10-p.p.m. levels, and also at 2-p.p.m. levels to cattle. Three animals of each kind were fed at the same level, except that two of each kind were used at the 10-p.p.m. level. All animals except the controls were fed the toxicant for 12 weeks. The animals were in good health and condition when the studies were begun. They were grouped as uniformly as possible into the different feeding groups. Each animal occupied an individual pen with feeder and water supply.

The cattle and sheep were fed alfalfa hay and a grain concentrate composed of corn chops, crushed oats, cottonseed meal (41% protein), steamed bone meal, and salt. The hogs were fed a mixture of corn chops, crushed oats, cottonseed meal (41% protein), alfalfa (pea green, medium chop), steamed bone meal, and salt. All animals were given as much as they would readily consume.

Technical aldrin (91%) was formulated by preparing acetone solutions so that 1 ml. was sufficient to provide the appropriate amount of pure aldrin in 1 pound of feed. It was added immediately before the feed was offered to the animals. Feed for each animal was weighed on scales sensitive to 1/4 ounce.

Observations during the feeding period showed no evidence of illness other than occasional diarrhea associated with feeding, and at autopsy no abnormalities were found. The feed consumed per pound gain in weight for each group is shown in Table I. There were no significant differences between groups.

Radeleff (*8*) described the technique which was employed to take biopsy samples of omental fat from the steers and sheep before feeding began, to assure use of animals free of insecticide residue. The biopsy technique does not apply to hogs.

Table II. Per Cent Recoveries of Aldrin and Dieldrin Added to Tissues of Control Animals

Insecticide	Method of Detn.	Fat	Liver	Kidney	Muscle
Aldrin	Colorimetric	96	83	87	85
Dieldrin	Colorimetric	90	78	89	100
Dieldrin	Organic chlorine	91	..	..	..

Table III. Aldrin and Dieldrin in Tissues of Animals Given Different Dosages of Aldrin in Diet

(P.p.m.)

Dosage, P.P.M.	No. of Animals[a]	Renal Fat	Body Fat	Liver	Kidney	Muscle
Aldrin				Steers		
0	1	0	..	0	0	0
0	1[b]	0	..	0	0	0
0.25	2	0.03	..	0.02	0	..
0.75	2	0.07	..	0.03	0	..
2	2	0.08	..	0.13	0	..
10	1	0.12	0.08	0.04	0	0
				Sheep		
0	1	0	..	0	0	0
0.25	2	0	..	0	..	..
0.75	2	0	..	0	0	..
10	1	0.35	0.30	0	0	0
				Hogs		
0	1	0	..	0	0	0
0.25	2	0	..	0	..	..
0.75	2	0	..	0	0	..
10	1	0.10	0.08	0	0	0
Dieldrin				Steers		
0	1	0	0	0	0	0
0	1[c]	0	0	0	0	0
0.25	2	0.88	0.99	0.05	0.02	0
0.25	1[c]	0.57	0.68	0.05	0	0
0.75	2	2.90	3.40	0.25	0.38	0.07
0.75	1[c]	1.80	2.10	0.10	0.26	0
2	2	7.80	8.50	0.66	0.65	0.13
2	1[c]	4.20	5.10	0.33	0.28	0.12
10	1	44.5	39.2	3.84	3.50	0.72
10	1[c]	19.2	17.8	0.93	1.32	0.17
				Sheep		
0	1	0	0	0	0	0
0	1[c]	0	0	0	0	0
0.25	2	0.65	0.62	0.09	0.06	0.02
0.25	1[c]	0.33	0.28	0.04	0	0
0.75	2	1.90	2.03	0.14	0.16	0.03
0.75	1[c]	1.50	1.64	0.13	0.08	0.03
10	1	41.5	42.3	3.14	1.36	0.73
10	1[c]	16.4	19.0	1.29	0.60	0.26
				Hogs		
0	1	0	0	0	0	0
0	1[c]	0	0	0	0	0
0.25	2	0.53	0.55	0.03	0	0.05
0.25	1[c]	0.10	0.12	0	0	..
0.75	2	1.40	1.66	0.06	0.05	0.10
0.75	1[c]	0.55	0.50	0	0	0.05
10	1	19.8	17.4	0.77	0.69	0.49
10	1[c]	4.0	4.20	0.19	0.48	0.18

[a] Composite samples analyzed when more than one animal appears in a group.
[b] Control animal, 6 weeks after feeding ceased.
[c] Animals slaughtered 6 weeks after feeding ceased.

Table IV. Dieldrin in Renal Fat

(P.p.m.)

Colorimetric	Organic Chlorine
Steers	
2.90	2.87
7.80	7.90
4.20	2.97
44.5	38.7
Sheep	
1.90	1.96
1.50	1.83
41.5	47.7
16.4	20.3
Hogs	
1.40	2.05
19.8	20.50

Table V. Dieldrin in Fat from Beef Roast Compared with That of Uncooked Fat from Same Animal

(P.p.m.)

Fat from Roast	Body Fat	Renal Fat
1.40	0.99	0.88
3.23	3.38	2.90
7.80	8.50	7.84
33.30	39.2	44.5

Samples of liver, kidney, heart, renal fat, body fat, steak, and roast were taken at slaughter. Two animals from each group were slaughtered on the day after the last feeding of insecticide, and a third animal after a 6-week feed-off period. Slaughter was carried out by a commercial establishment under the supervision of the veterinarians conducting the studies.

As the analytical work progressed, it became apparent that the residues of aldrin and dieldrin were present primarily in the fat. It did not seem expedient to analyze the steaks and roasts with variable fat content, since the residue would be directly proportional to the amount of fat in each sample. Samples of muscle tissue taken from the steak cuts were analyzed instead of a uniform sample of the whole cut.

To determine whether other metabolites of aldrin and dieldrin were present, some of the fat samples were also analyzed for total organically bound chlorine. The combustion method described by Hudy and Dunn (*5*) was used for the analyses. The samples were saponified and extracted with *n*-hexane. The solvent was then evaporated and the residue dissolved in peanut oil and burned in the furnace. The chlorine was absorbed in a sodium carbonate solution and titrated amperometrically with silver nitrate.

To determine the effects of the cooking process on dieldrin residues, four samples of beef roast were baked in open pans for $3^1/_2$ hours at 350° F. A sample of melted fat was taken from each roast for analysis.

Colorimetric Analyses. The colorimetric methods for aldrin and dieldrin described by O'Donnell *et al.* (*6, 7*) and modified by the Shell Chemical Co. were followed, with the following exceptions:

Low recoveries of aldrin were caused by losses resulting from evaporation of the solvent with a jet of air prior to addition of phenyl azide. Therefore, 1 ml. of this reagent was added before evaporation. The solvent was reduced to 0.5 ml. by heating in an oil bath at 73° ± 2° C., and then the temperature of the bath was raised to 77° ± 2° for 30 minutes.

In the dieldrin analysis, by using a 20-cm. column of adsorbent mixture loosely packed, instead of a 10-cm. column, and adjusting the solvent flow to 200 ml. per hour, a forecut of 150 ml. could be discarded, with consequent reduction in interference and constant recoveries of 89 to 100% for fat, muscle, and heart tissue. In the liver, only 35 to 40% of added dieldrin could be extracted when the tissue was saponified with alcoholic potassium hydroxide, but by extracting with ethyl alcohol and saponifying the ethanolic extract with potassium hydroxide, recoveries were increased to 78%.

The methods used were first tested for satisfactory recoveries from each tissue under study before the samples were analyzed. Also, check recoveries were run concurrently with the analysis of the samples. The recovery data are shown in Table II.

Results

Aldrin Determination. The apparent aldrin content of the fat from the control steers, sheep, and hogs was 0.04, 0.01, and 0.08 p.p.m., respectively. The blanks on the other tissues were of the same order as in the fat. The results are shown in Table III, where the control samples were given a theoretical value of zero. The values shown for the test samples are net values from which the readings of the control samples have been subtracted. When the net reading of a test sample was equivalent to less than 0.02 p.p.m. of aldrin, it was not considered significant and the result was recorded as zero. The results were not corrected for recovery.

Dieldrin Determination. The apparent dieldrin content of the fat from control steers, sheep, and hogs was 0.07, 0.12, and 0.05 p.p.m., respectively (Table III). The blanks of other tissues were not significantly different from those of the fat. The procedure for calculating results was the same as that described for aldrin.

In general, the dieldrin residues were slightly lower in the sheep than in the steers. About half as much was stored in the fat of hogs as in that of steers.

Chlorine Determination. Results of these analyses were calculated as dieldrin. There was some aldrin present in the samples, but the amounts were so small they were not considered in the calculations. Table IV compares the dieldrin content of the renal fat by the colorimetric method with that from the chlorine determinations.

Practically all the chlorine found in the fat from steers could be accounted for by the dieldrin and aldrin present. Chlorine-containing metabolites, other than dieldrin and aldrin, were not present in significant amounts.

Effect of Cooking on Dieldrin Residues. Four samples of fat from beef roasts were analyzed for dieldrin. Table V shows these results, together with the analyses of raw body and renal fat.

The dieldrin in beef roast was not reduced significantly by cooking.

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