Feeding Rats Tissues from Lambs and Butterfat from Cows that Consumed DDT-Dusted Alfalfa Hay.* (20385)

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Investigations relating to the toxicity of DDT have been reviewed elsewhere(1-3). Previous studies by this station have been concerned with effects of feeding DDT-dusted alfalfa hay to fattening lambs(4,5), dairy cows(6), chickens(7,8), and swine(9).

The purpose of this paper is to report studies on the effects of feeding rats tissues from lambs and butterfat from cows that consumed DDT-dusted alfalfa hay.

Materials and methods. Alfalfa was dusted with technical DDT for control of insects at the following levels: no DDT, 0.5, 1, 2, and 4 lb. per acre as described previously(4). The hay thus treated was harvested and fed to 32 lambs for 120 days(5) and to 16 dairy cows for 113 days(6). An additional 32 lambs were fed DDT in capsules at levels of 0, 50, 100, and 200 ppm. of hay consumed(5). Following slaughter, the kidney and mesenteric fat and the boneless leg of each lamb were incorporated separately into diets (Table I). Food consumption was recorded. Milk obtained from cows that had consumed hay dusted with different levels of DDT(6) was separated, the cream churned, and the butterfat incorporated in the experimental diets as shown in Table I. The lamb fat and lamb leg diets were each fed to 4 weanling male and 4 weanling female rats (Tables II, III, and IV). The rats were housed in individual cages and weighed at 2-week intervals. The diets containing the lamb fat were fed for 16 weeks, those containing lamb leg for 12 weeks. The number of rats fed butterfat containing different levels of DDT is given in Table IV. They were fed for 16 weeks and then sacrificed and an autopsy made on each animal. Samples of liver and kidney were taken for histological study, and DDT determinations were made on the abdominal fat. The DDT content of the tissues and butterfat was determined by the colorimetric method of Schechter *et al.*(10,11) as modified by Harris *et al.*(4). The amount of DDT residue on the hay was determined by the total chloride method of Umhoefer(12).

Results and discussion. There were no significant differences in the growth of rats fed lamb fat and lamb leg containing the lowest and highest levels of DDT, or between control and experimental rats. Food consumption was essentially the same for all rats.

The average DDT residue on hay, lamb fat and leg, and the storage in rats fed lamb fat and leg are summarized in Tables II and III. The values for DDT in the rat fat varied from 10 to 144 ppm. Rats fed lamb leg stored higher levels of DDT than those fed lamb fat, even though the lamb leg contained less DDT and was fed for 12 weeks, whereas the diets containing lamb fat were fed for 16 weeks. The greater storage of DDT following intake of a smaller amount of insecticide in the diet is in essential agreement with the data previously reported(4) in which storage of DDT was greater in lambs fed DDT-dusted hay than in lambs fed a greater amount of DDT in capsules.

The storage of DDT in the fat of the rats fed butterfat is shown in Table IV. There was no statistically significant difference in the growth of rats fed butterfat containing the lowest and highest levels of DDT or between control and experimental rats. All of the rats in the above experiments gained at the same rate as rats fed the stock diet.

It should be noted that the fat of rats fed the control diets and undusted hay contained DDT. The exact cause of this is not known. A possible explanation for the accumulation of DDT in the fat of control animals is that DDT contamination occurred by way of dust

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TABLE I. Composition of Rat Diets.

Ingredients	Dry matter of ingredients, %	Lamb fat as fed, %	Lamb leg as fed, %	Butterfat as fed, %
Lamb kidney and mesenteric fat	86.9	22	—	—
Lamb leg	38	—	65	—
Butterfat	83.5	—	—	22
Casein	93	22	—	22
Salt mixture*	95.7	4	2.4	4
Sodium chloride	100	1	—	1
Wheat germ oil	100	0.5	—	0.5
Liver concentration 1-20†	97.3	2	—	2
Brewers yeast	94.7	5	4	5
Dextrinized starch	92.6	43.5	28.1	43.5
Cod-liver oil‡	100	—	.5	—
Total		100	100	100
Dry matter		91.5	59.9	90
Ether extract		19.6	10.7	16.9
Protein		23.1	13.7	21.9

* Ingredients in salt mixture in %: NaCl 27.5, K_2HPO_4 25.85, $Ca(H_2PO_4)_2 \cdot H_2O$ 21.65, $CaCO_3$ 18.515, $MgCO_3$ 4.6, $FeC_6H_5O_7 \cdot 3H_2O$ 1.8, $CuSO_4 \cdot 5H_2O$ .02, $MnSO_4$ .035, $K_2Al_2(SO_4)_4$ .24, H_2O .01, KI .005, $CoCl_2 \cdot 6H_2O$ .01, $ZnCO_3$ .005, NaF .0001.

† Obtained from Wilson Laboratories, Chicago, Ill.

‡ Contained 3000 I. U. vit. A and 400 A. O. A. C. chick units vit. D/g.

TABLE II. DDT Content of Rats Fed Lamb Fat and Leg from Lambs That Had Consumed DDT-Dusted Alfalfa Hay (8 Rats in Each Series).

DDT		DDT			DDT		
App./acre, lb	Residue on hay, ppm.	In lamb fat, ppm.	In rat diet (dry basis), ppm.	In rat abdominal fat, ppm.	In lamb leg, ppm.	In rat diet (dry basis), ppm.	In rat abdominal fat, ppm.
0	0	2	.5	14	.0	.1	12
1	15	16	3.8	49	.35	.5	81
2	22	18	5.3	60	.64	.7	83
4	42	44	10.1	95	1.15	1.0	101

TABLE III. DDT Content of Rats Fed Lamb Fat and Leg from Lambs That Had Consumed DDT in Capsules (8 Rats in Each Series).

DDT fed in capsules to sheep, ppm. of hay	DDT			DDT		
	In lamb fat, ppm.	In rat diet (dry basis), ppm.	In rat abdominal fat, ppm.	In lamb leg fed to rats, ppm.	In rat diet (dry basis), ppm.	In rat abdominal fat, ppm.
0	.0	.1	10	.17	.3	38
50	19.6	4.7	67	.61	.7	90
100	32.1	7.7	95	.60	.7	93
200	73.4	17.6	100	1.56	1.8	144

from experimental diets containing DDT. Laug *et al.*(3) also found that the fat of control rats housed in the same and different rooms contained DDT, demonstrating the avidity with which adipose tissue stores DDT.

No symptoms of DDT poisoning or histological changes in liver or kidney were observed in any of the rats under the conditions of these experiments.

Laug *et al.*(3) reported hepatic cell alterations after feeding diets containing as little as 5 ppm. DDT but did not indicate the exact length of DDT feeding required to produce these changes. Although these experiments differ in many details from those conducted on rats by Deichmann *et al.*(2), the results obtained in the phases of the two studies that can be compared are in substantial agreement.

TABLE IV. DDT Content of Rats Fed Butter from Cows That Had Consumed DDT-Dusted Alfalfa Hay.

No. of rats*	No. of cows	DDT				
		Applied/ acre,† lb	Residue on hay, ppm.	In butter-fat, ppm.	In rat diet (dry basis), ppm.	In rat abdominal fat, ppm.
16	4	.0	0	0	.1	12
8	2	.5	9	13	3	46
16	4	1.0	13	42	10	69
16	4	2.0	26	70	17	107
8	2	4.0	42	123‡	30	188

* Equal number of males and females.

† The .0, 1 and 2 lb levels of DDT applied in 1948 and 1949. The 0.5 lb level in 1949, and 4 lb in 1948.

‡ Estimated value.

There is a need for additional chronic studies on the toxicity of DDT and other insecticides in diets containing varying levels of fat and protein.

Summary. Growing rats were fed tissues from lambs or butterfat from cows that had consumed DDT-dusted alfalfa hay as part of their diets for 12 to 16 weeks. The concentration of DDT varied from .1 to 30 ppm. on a dry basis. No significant differences in food consumption, rate of growth, or tissue changes could be detected by gross and microscopic examination in the rats fed the highest and lowest levels of DDT. High concentrations of DDT in the diet of rats gave the greatest storage of it in their body fat.

1. Leary, J. C., Fishbein, W. I., and Salter, L. C., *DDT and the Insect Problem,* McGraw-Hill Co., New York, 1946.

2. Deichmann, W. B., Witherup, S., Kitzmiller, K. V., and Heyroth, F. F., *Toxicity of DDT,* Univ. of Cincinnati, F. F. Kettering Laboratory, 1950.

3. Laug, E. P., Nelson, A. A., Fitzhugh, O. G., and Kunze, F. M., *J. Pharm. Exp. Therap.,* 1950, v98, 268.

4. Harris, J. R., Biddulph, C., Greenwood, D. A., Harris, L. E., Bryson, M. J., Binns, W., Miner, M. L., and Madsen, L. L., *Arch. Biochem.,* 1949, v21, 370.

5. Harris, L. E., Myint, T., Biddulph, C., Greenwood, D. A., Binns, W., Miner, M. L., and Madsen, L. L., *J. Animal Sci.,* 1951, v10, 581.

6. Biddulph, C., Bateman, G. Q., Bryson, M. J., Harris, J. R., Greenwood, D. A., Binns, W., Miner, M. L., Harris, L. E., and Madsen, L. L., *Advances in Chemistry,* Series I, 1950, 237.

7. Bryson, M. J., Draper, C. I., Harris, J. R., Biddulph, C., Greenwood, D. A., Harris, L. E., Binns, W., Miner, M. L., and Madsen, L. L., *Advances in Chemistry,* Series I, 1950, 232.

8. Draper, C. I., Harris, J. R., Greenwood, D. A., Biddulph, C., Harris, L. E., Mangelson, F., Binns, W., and Miner, M. L., *Poultry Sci.,* 1952, v31, 388.

9. Harris, L. E., Harris, J. R., Mangelson, F. L., Greenwood, D. A., Biddulph, C., Binns, W., and Miner, M. L., Unpublished data.

10. Schecter, M. S., Porgorelskin, M. A., and Haller, H. L., *Anal. Chem.,* 1947, v19, 51.

11. Soloway, S. B., Hayes, R. A., and Haller, H. L., *Ind. Eng. Chem.,* Anal. Ed., 1945, v17, 704.

12. Umhoefer, R. R., *Ind. Eng. Chem.,* Anal. Ed., 1943, v15, 383.

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