Distribution of *p,p'*-DDT Residues in Adipose and Muscle Tissues of Beef Cattle

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Two groups of beef cattle, consisting of two steers and one cow per group, received a total dose of 300 mg. of *p,p'*-DDT per kg. of body weight over either a 3- or 30-day period. One steer from each group was sacrificed before a 7-week depletion period which followed the treatments. The remaining animals were sacrificed at the end of 7 weeks of depletion. Total DDT residues (DDT, DDD, and DDE) appeared on the average to be evenly distributed throughout the extractable fat of beef cattle, based on samples of 13 different tissues. Differences were found in the total DDT residue content of extractable fat due to dose rate ($P < 0.01$) and time after treatment ($P < 0.01$), and the relative amounts of DDT, DDD, and DDE were different ($P < 0.05$) between depot fat, muscle, blood, and milk fat.

Residues of *p,p'* - DDT [1,1,1-trichloro-2,2-bis (*p*-chlorophenyl)ethane] accumulate in the fat of animals exposed to this pesticide (Telford and Guthrie, 1945; Woodard *et al.*, 1945). Little information is available on the distribution of these residues in body fat because in most studies body fat has been sampled from only one or two locations within the animal. It must be assumed that the residues are distributed evenly throughout the animal fat if these samples are to be representative of the residue concentration in the experimental animals. The assumption of even distribution may not be correct. King *et al.* (1966) reported that a higher amount of heptachlor epoxide tended to accumulate in the ether extract from hide and hair and the liver of a lactating dairy cow, although a uniform distribution of this residue was found among other tissues. Harrison and Shanks (1965) found small but consistent differences in the DDT residue content of omental, peri-renal, and back fat of sheep, and Carter *et al.* (1948) reported a possible difference between the DDT residue content of leaf and back fat of swine.

The present study was conducted to investigate the distribution of DDT residues in beef cattle and the effects of sex, dose intensity, and depletion on residue distribution. Four muscle tissues and nine adipose tissues, representing fat deposited at different times during the finishing period of steers, were studied.

EXPERIMENTAL

Treatment of Animals. The animals used in this study were four Shorthorn steers averaging 294-kg. body weight and two lactating Angus cows averaging 322 kg. They were maintained on pasture which had not been treated previously with pesticide. A solution of *p,p'*-DDT dissolved in warm corn oil was injected intraruminally using a hypodermic syringe and a 5-inch, 16-gage needle. Two steers and one cow received a total dose of 300 mg. per kg. of body weight over a 3-day period. Each animal was injected with 1/3 of the total dose once daily for 3 days. The remaining two steers and cow received the same total dose over a 30-day period, each animal receiving its respective dose in eight equal portions during the 30-day period. The largest single daily amount of corn oil injected was 130.4 ml. One steer was sacrificed at the end of each treatment period and fat samples of the caul, cod, kidney, heart, brisket, ruffle, internal rib, external rib, and caudal fat and muscle samples from the diaphragm muscle, psoas major, round steak, and rib eye were collected for analyses. The remaining two steers and cows were sacrificed after a 7-week depletion period and tissue samples were collected. Additional information was obtained by collecting samples of caudal fat, blood, and milk during the experiment.

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ANALYSIS OF SAMPLES

Solvents. Petroleum ether (30° to 60° C.) and hexane were refluxed over dispersed sodium and distilled. Acetonitrile (Matheson Co., Beltsville, Md.) was redistilled. Reagent grade ethyl ether was redistilled.

Sample Extraction. Fat or Muscle Tissue Samples. Ten grams were cut into small pieces, transferred to an Omni mixer (Ivan Sorvall, Inc., New York, N.Y.), and blended with 50 ml. of acetonitrile and 5 grams of anhydrous sodium sulfate for approximately 3 minutes. The extract was filtered with the aid of vacuum, and the filtrate subjected to analysis. Since acetonitrile does not extract the lipids, the amount of total lipid was determined by cutting a 10-gram sample into small pieces, macerating it with sea sand in a mortar, extracting the mixture with petroleum ether, filtering the slurry, evaporating the filtrate to dryness on a steam bath, and weighing the residue.

Milk Samples. Samples were allowed to stand in a refrigerator overnight to separate the cream. The cream was removed from the milk and mixed with enough sodium sulfate to form a friable mass, which was extracted with petroleum ether (30° to 60° C.). The solvent was evaporated on a steam bath and the last traces of petroleum ether were removed by vacuum after the residue was transferred to a small round-bottomed flask. Five grams of butterfat were used for each analysis.

Blood Samples. Fifty milliliters were mixed with enough sodium sulfate to form a friable mass, which was extracted with 200 ml. of acetonitrile.

Cleanup and Gas Chromatography. The extracts were each carried through a hexane–acetonitrile partitioning and Florisil column cleanup as previously described (Mills, 1961), and aliquots were analyzed by gas-liquid chromatography employing two modes of detection. For the electron capture analyses, a Barber-Colman Model 20

instrument with a 180-cm. × 0.64-cm. O.D. aluminum column packed with 10% w./w. DC-200 on 60- to 80-mesh Anakrom ABS (Analabs, Hamden, Conn.) was used. Operating parameters were: column temperature 200° C., injection port 215° C., and carrier gas (nitrogen) flow at outlet, 200 ml. per minute. For the microcoulometric analyses, a Dohrmann Instrument equipped with a T-300-S cell for halogen determinations and a 120-cm. × 0.48-cm. O.D. aluminum column containing the same packing was employed. Operating parameters were: column temperature 200° C., injection port 215° C., coulometer sensitivity 100 ohms, carrier gas (nitrogen) 150 ml. per minute with 15 ml. per minute oxygen and 10 ml. per minute sweep nitrogen being introduced into the combustion chamber.

Recovery of residues using these procedures approximated 94%.

Confirming Identity by Infrared Spectra. Samples that contained DDD and DDT residues were combined and chromatographed on activated Florisil (Barry *et al.*, 1965). The *p,p'*-DDT was eluted from the column in the first 100 ml. of petroleum ether and DDD in the next 200 ml. of this solvent. The eluted fractions were concentrated on the steam bath to 10 ml. and transferred to a 15-ml. centrifuge tube, and the remaining petroleum ether was evaporated to dryness in a 50° C. water bath with the aid of a gentle stream of dry air. Several 2-ml. portions of carbon disulfide were added to samples and evaporated. Infrared spectra of *p, p'* - DDT and DDD were obtained from carbon disulfide solution and KBr wafers.

RESULTS AND DISCUSSION

The residue analyses of the tissues were treated statistically (Snedecor, 1956) on the basis of concentration per unit of extractable fat and per unit of whole or crude tissue. The concentrations of DDT, DDD, DDE, and total DDT residues (total residues representing the sum of DDT, DDD, and DDE) were considered and the statistical analyses of the main effects for total DDT residues are presented in Table I. The two-way interactions were not significant and are not included in Table I. The three-way interactions were combined and used as the error term with 24 degrees of freedom.

When expressed on the basis of extractable fat, there were no statistically significant differences found in the concentration of total DDT residues among the various tissues (Table 1). This agrees with results obtained from similar tissues by King *et al.* (1966), who studied heptachlor, another fat-soluble chlorinated pesticide.

The distribution of total DDT residues on an extractable fat basis among the 13 tissues is shown in Figure 1. Each bar represents the average of all animals, and the four cross-hatched bars represent the four muscle samples. Based on the statistical analyses of these tissue data, one would assume that the residue content of any sampling site is representative if expressed on the basis of extracted fat. However, an examination of the data reveals that the concentration values vary from the mid 400's (p.p.m.) to the mid 600's (p.p.m.) for most of the tissues and considerably more in the case of the diaphragm muscle and psoas major, which contain 283 and 1642 p.p.m., respectively. In each of the latter two tissues, the average was greatly influenced by a single sample which varied considerably from the other samples in residue content, and this variation could not be attributed to analytical error. The fact that the average amount of total residues in these two tissues was not significantly different points out the considerable variation among tissues and between animals encountered in this experiment. In general, the raw data displayed considerable variation between animals with none of the tissues being consistently high or low, and there were no apparent trends in total residue distribution due to dose rate, sex, or time after treatment. However, there was more variation among tissues at the beginning of the 7-week depletion period as compared to the end of this period. More consistent results might have been obtained if lower levels of DDT were administered as in the work of Harrison and Shanks (1965) and Carter *et al.* (1948).

An important point shown by these data is that caudal fat, fat deposited at the base of the tail, was as representative of the total residue concentration as the other sample sites. In beef cattle this is a convenient tissue to sample and can be sampled repeatedly from the same animal throughout an experiment using a rather simple surgical procedure.

A more practical picture of the total residue distribution within the animals was obtained when total residue content was graphed on the basis of crude or whole tissue (Figure 2). The average residue content of the samples when presented in this manner more closely represents the amount of residue consumed in beef products. Adipose tissue con-

Table I. Statistical Analysis of Dose Rate, Sex, Residue Depletion, and Tissue Effects on DDT Residues in Beef Cattle

Source of Variation	D.f.	Mean Squares of Total Residue Concn. Extracted fat	Whole tissue
Dosage of 300 mg./kg. body wt. over a 3-day *vs.* 30-day period	1	3456198.9[a]	684.1
Steers *vs.* cows	1	13533.4	708212.2[a]
Sampling before and after a 7-week depletion period	1	4448339.5[a]	692538.4[a]
Sampling different tissues	12	693185.6	172261.4[a]
Error	24	413321.3	21434.0

[a] Highly significant ($P < 0.01$).

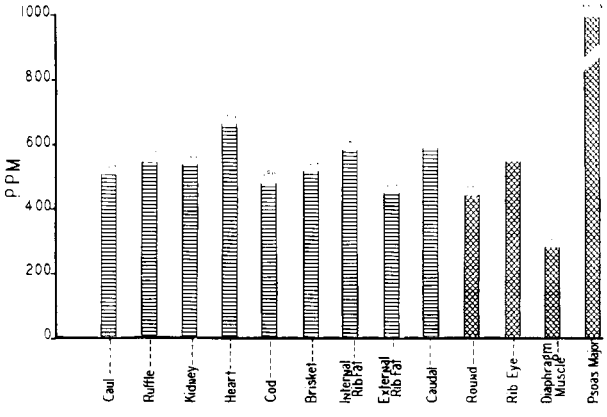

Figure 1. Total DDT residue content of extracted fat from body tissues of beef cattle

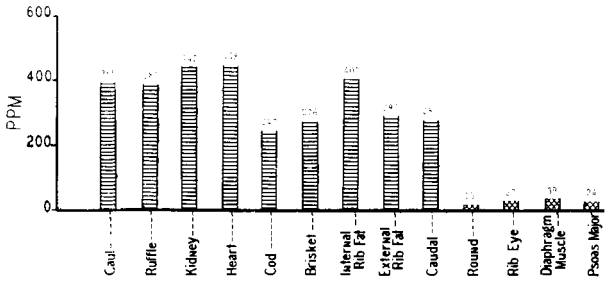

Figure 2. Total DDT residue content in body tissues of beef cattle, expressed on a whole tissue basis

tained a relatively high level of residue, which corresponds to the established fact that chlorinated pesticide residues tend to accumulate in the body fat of animals. Concurrently, the average residue content of the four muscle tissues was significantly lower ($P < 0.01$) than the adipose tissue and accounts for the significant tissue effect shown in Table I. The actual residue concentration in the muscle was only 7% that of the fat. Also, the organ fat, which was represented in this study by the fat accumulating around the heart, kidney, and gut (ruffle and caul) averaged 418 p.p.m. total residue compared to 300 p.p.m. for the remaining adipose tissues.

As shown in Table I, there was a highly significant difference ($P < 0.01$) in total residue content of the extractable fat due to the rate of dosing. For the animals receiving the 300 mg. of DDT per kg. of body weight for the 3- and 30-day periods, the average concentrations were, respectively, 1187 and 666 p.p.m. in the animals sacrificed before the depletion period, and 571 *vs.* 321 p.p.m. in the animals sacrificed after the depletion period.

The increase in residue level due to a higher dose rate was not apparent when residues were expressed on a whole tissue basis. This may be explained by the variation in the extractable fat content of the samples and the fact that the chlorinated residues of DDT accumulate in the fat rather than the nonfat portion of the tissue. On the average, the samples from animals treated for 3 days contained less extractable fat than samples from the 30-day treated animals (44.3 and 52.1%, respectively). Although one might not expect this difference, it occurred in this experiment probably because of the small number of animals involved. Thus, the difference in residue concentration decreased when changed from the extractable fat basis to the whole tissue basis. The same thing was applicable when comparing steers and cows—that is, the fat tissue of steers contained a higher proportion of extractable fat than the cows (55.9 and 32.8%, respectively). Although both steers and cows contained an equal level of total residue on an extractable basis, they contained significantly different levels on a whole tissue basis. This points out the fact that one should define the basis on which residue concentrations are expressed, and since tissue fat is the major site of residue accumulation, one should probably use extractable fat as a common denominator when comparing samples.

There was no difference in residue content of the 13 tissues between cows and steers when expressed on an extractable fat basis. However, this was a comparison of animals after the 7-week depletion period when all animals had a more equal residue level. Although the cows were lactating, one would not expect a large loss of residues via the milk because the experiment was conducted during the latter part of their lactation and milk production was low. Using an average milk fat per cent of 3.5, the total amount of DDT residues eliminated via the milk during the 7-week depletion period was less than 5% of the total dose administered.

The data show a highly significant loss of residues from the tissues during the 7-week depletion period. This difference was apparent when tissue residue content was expressed on both the extractable fat and whole tissue bases. The animals dosed over the 3-day period lost an average of 59% of their total residues and the 30-day treated animals lost an average of 41%. Unfortunately, the loss of total residues during the depletion period is confounded with animal differences because the samples collected at the beginning and end of depletion came from different animals. Therefore, a more correct picture of depletion is obtained if we look at the residue concentrations in the caudal fat samples collected from the same animals at various times during the depletion period (Figures 3 and 4). The 3-day treated animals started at a much higher concentration but after 7 weeks the residue levels were similar. The interaction between dose rate and time after treatment approached significance at the 0.05 level of probability. A similar pattern was found with milk fat.

The caudal fat curves in Figures 3 and 4 indicate that a considerable quantity of residues moved out of the body fat during the depletion period, but there was no apparent effect on the distribution of total residues among the 13 tissues during this time. However, the relative amounts of DDT, DDD, and DDE changed between different groups of samples. The changes in these residues with time may be important in explaining the pathway DDT follows within the animal. Table II contains the average relative amounts of these three compounds in adipose tissue, muscle, blood, and milk. The proportional amounts in the adipose tissues were significantly different from muscle and

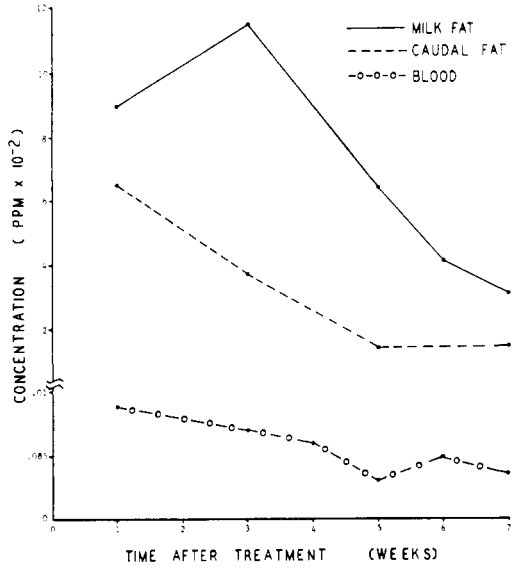

Figure 3. Effect of time after treatment on the level of total DDT residues in milk fat, caudal fat, and blood of beef cattle treated with a total dose of 300 mg. DDT per kg. body weight over a 3-day period

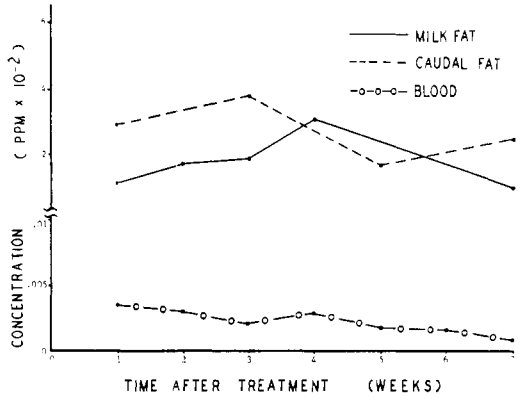

Figure 4. Effect of time after treatment on the level of total DDT residues in milk fat, caudal fat, and blood of beef cattle treated with a total dose of 300 mg. DDT per kg. body weight over a 30-day period

Table II. DDT, DDD, and DDE as Per Cent of Total Residue in Adipose, Muscle, Blood, and Milk of Beef Cattle

Sample	Residue, %		
	DDT	DDD	DDE
Adipose	66.9	25.4	7.7
Muscle	54.1	30.3	15.6
Blood	56.9	28.7	14.3
Milk	81.4	10.8	7.8

blood ($P < 0.05$) and from milk ($P < 0.05$). The amounts found in blood and muscle were similar.

The reason for the differences between these samples is not clear, since there is conflicting information on the metabolism of DDT to its dechlorinated analogs in vertebrate animals. The conversion may be related to the amount of O_2 supplied or available to the tissues, since Morrello (1965) has shown that rat liver microsomal enzymes, which require O_2, catalyze the conversion of DDT to DDD in the liver of rats. On the other hand, Bunyan *et al.* (1966) demonstrated with pigeon liver preparations that O_2 is not required.

DDT is dechlorinated by microorganisms in the gut (Mendel and Walton, 1966; Miskus *et al.*, 1965). Possibly a selective absorption or movement of the compounds occurs, causing different proportions of DDT, DDD, and DDE in the various tissues. Although these data are limited and were not collected in a manner which would permit answering the question, they indicated that a selective movement of these compounds might have occurred during the depletion period when a significant amount of total residue moved out of the animal body. In general, there tended to be a higher proportion of DDE and a lower proportion of DDD in the tissues of animals after depletion, while the proportion of DDT was unchanged. However, metabolism of these compounds could have been occurring at the same time. Further work is needed which will describe the pathway these residues follow prior to and after their distribution in the body fat.

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