A Comparison of DDT and Methoxychlor Accumulation and Depletion in Sheep

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Notwithstanding their similarities in chemical structure, DDT and methoxychlor differ appreciably in the extent to which they accumulate in animal tissues. While this difference has been recognized since at least 1950 (LEHMAN 1950), no direct comparison has ever been reported of DDT and methoxychlor residues in a meat animal species. To make such a comparison in the sheep was the objective of this study.

Experimental

Eighteen crossbred yearling ewes, weighing initially 38.4 ± 2.4 kg, were randomized to three groups of six head. The three groups were fed a basal ration consisting of 30% cottonseed hulls, 67% ground barley, 0.6% urea and the balance minerals, vitamin A and insecticide. In the accumulation phase, the ration of group 1 was supplemented with technical DDT to supply 250 ppm of p,p'-DDT; the rations of groups 2 and 3 were supplemented with technical methoxychlor to supply 250 and 2500 ppm, respectively, of p,p'-methoxychlor.

The technical DDT[1/] contained by gas chromatographic analysis 61.3% of p,p'-DDT, 27.2% of o,p-DDT, no detectable DDD and 0.7% of p,p'-DDE. The technical methoxychlor[1/] contained by analysis 62% of p,p' methoxychlor and several unidentified electron capturing components.

The sheep were confined to individual pens with raised screened floors and offered their respective rations and tap water *ad libitum*. The accumulation phase, during which the insecticide containing rations were fed, was 18 weeks in duration, and was followed by a 16 week depletion period, during which the unsupplemented basal was fed to all sheep.

[1/] The technical DDT [1,1,1-trichloro-2,2-bis(p-chlorophenyl) ethane was a product of Ulin-Matheson and the technical methoxychlor [1,1,1-triochloro-2,2-bis(p-methoxyphenyl) ethane] was obtained from Sigma (Class II). Mention of a trade name or source of supply is for identification only and does not constitute a warrenty or guarantee of the product by the government nor imply superiority over other similar products not mentioned.

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Biopsies of subcutaneous fat were taken biweekly from the rump of all sheep throughout the study. These samples, generally from 100 to 400 mg, were trimmed free of connective tissue, and extracted with hexane in a high speed blender. Aliquots of the hexane extract were evaporated to dryness for the gravimetric determination of total fat. Other aliquots of the hexane extract were cleaned up and chromatographed as described by FRIES et al. (1973). Residue concentrations are expressed on the basis of extracted fat. Statistical procedures were those outlined by STEEL and TORRIE (1960).

Results and Discussion

In the accumulation phase, the average ingestion of insecticide was 240 mg per head per day of p,p'-DDT, 220 mg per head per day of methoxychlor offered at 250 ppm in the feed and 1870 mg per head per day of methoxychlor offered at 2500 ppm. The intakes of p,p'-DDT and methoxychlor offered at 250 ppm in the ration were not significantly different ($P>.05$), but the feed consumption at 2500 ppm of methoxychlor, 0.75 kg per head per day, was less ($P<.05$) than that at the lower level, 0.88 kg per head per day. No other adverse signs attributable to insecticide ingestion were observed. WELCH (1948) administered 4500 mg of technical methoxychlor per day to sheep for 60 days without signs of toxicity; 4500 mg per day of technical DDT however, was toxic within 10 days, but recovery subsequently occurred. Thus, the levels of DDT or methoxychlor ingestion in the present experiment were considerably less than those required to produce signs of acute toxicity in sheep.

The accumulation of p,p'-DDT and its metabolites, p,p'-DDD and p,p'-DDE is shown in table 1. From week 2 through

TABLE 1

Accumulation of DDT, DDD and DDE in the Subcutaneous Fat of Sheep

Weeks of Exposure	PPM in Extracted Fat		
	p,p'-DDT	p,p'-DDD	p,p'-DDE
2	118	25.6	2.5
4	286	57.3	7.9
6	383	65.6	9.6
8	560	75.6	21.8
Plateau:			
Weeks	8-18	8-18	8-18
Mean ± SEM	524±28	75±5.4	25±2.1

8 of DDT feeding, a clear increase in residue level was observed with time of exposure. The values shown for week 8 are the beginning of the plateau period; the plateau evidently resulted from the rate of insecticide accumulation being essentially equal to the rates of removal. The plateau periods were located graphically for each compound and were confirmed by computing the least square linear regression of residue level on weeks of exposure over the interval chosen graphically as the plateau period. The slopes of these regressions were not different ($P>.05$) from zero. The plateau values observed, 524 ppm of DDT, 75.5 ppm of DDD and 25.0 ppm of DDE, are the averages of the biweekly values observed during the plateau period. No attempt has been made to calculate a net rate of accumulation for these compounds; no doubt such rates would be quite different, because they proceed from essentially zero to quite different plateau levels. It is more notable that the time required to reach a plateau level was similar for all three compounds.

RUMSEY et al. (1967) found that total DDT residues, i.e. the sum of p,p'-DDT, DDD and DDE, were distributed throughout the extractable fat obtained at 13 locations in beef cattle, with perianal fat representative of the average residue load in fat tissue. Thus, the data from subcutaneous biopsy presented here are considered to apply to extractable fat generally.

The rapid accumulation and persistance of DDT residue in mammalian tissue is well documented; HAYES (1959) has reviewed the older literature and MATSUMURA (1975) has summarized the more recent metabolic studies of DDT including distribution and depletion kinetics.

The accumulation of methoxychlor is shown in table 2.

TABLE 2

Accumulation of DDT and Methoxychlor in the Subcutaneous Fat of Sheep[1/]

Weeks of Exposure	Dietary Level of Methoxychlor	
	250 ppm	2500 ppm
2	1.7	0.6
4	2.0	5.9
6	3.4	21.8
8	6.8	(plateau)
Plateau:		
Weeks	(No plateau)	6-18
Mean±SEM		24±4.6

[1/] ppm in extracted fat

At the lower level of methoxychlor ingestion, residue increased through week 10 of exposure but thereafter did not plateau; rather, a linear decline ($P<.05$) of residue of 0.6 ppm per week was observed through week 18. This decline cannot be attributed to a reduced feed consumption and thus to a concomitant reduced rate of insecticide ingestion, for feed consumption was not decreased over the interval of 10 to 18 weeks of methoxychlor feeding. Rather, the decline of residue level suggests some metabolic adaptation to methoxychlor ingestion. In a companion study to the present one, CECIL et al. (1975) found that liver microsomal aminopyrine demethylase and aniline hydroxylase activities of sheep fed 250 ppm of DDT or 250, 1000 or 2500 ppm of methoxychlor were appreciably increased in yearling ewes relative to unsupplemented controls; these observations were made after 10 weeks of insecticide exposure in one study and 17 weeks in another. Clearly, therefore, some adaptation of the liver microsomal mixed function oxidases does occur in sheep in response to DDT or methoxychlor exposure, and the activities of this enzymatic complex is no doubt important in the excretion of these compounds and their metabolites (METCALF 1973), but the time course of that adaptation in sheep, which might be useful in rationalizing the decline of methoxychlor residues observed here, has not been investigated.

The absence of a plateau of methoxychlor residue has also been observed in the rat by KUNZE et al. (1950). Methoxychlor levels in the of 25,100 and 500 ppm were fed for 18 weeks. At 25 ppm, no residues were found in perirenal fat. At 100 ppm, none was observed until 9 weeks, after which the levels of 4 and 7 ppm in males and females, respectively, declined in both sexes to 1 ppm at 18 weeks. At 500 ppm, the levels in males were 36, 26, 19 and 16 ppm at 4, 9, 13 and 18 weeks, respectively; residues in females were generally lower, but declined with continued ingestion in a manner similar to that of males.

At the higher level of methoxychlor supplementation, a plateau was reached after 6 weeks of exposure at 24.4 ppm (table 2). This result suggests that whether a plateau of methoxychlor residue is observed or not following continuous exposure depends on the level of ingestion.

Table 3 shows residue levels remaining at selected times during the 16 week depletion period on the unsupplemented basal ration, and the whole of the biweekly observations are summarized in the first order rate constants, which were fitted by least squares, presented in the last line of the table. HUNNEGO and HARRISON (1971) have noted that how recently exposure to DDT has occurred in sheep can be estimated from the relative proportions of residual DDT, DDD and DDE; generally, the smaller the proportion of the more rapidly removed DDD, the more distant the exposure in time.

TABLE 3

Depletion of DDT and its Metabolites and of Methoxychlor from the Subcutaneous Fat of Sheep[1/]

Weeks of Depletion	Previous Insecticide and Dietary Level				
	p,p'-DDT, 250 ppm			Methoxychlor	
	p,p'-DDT	p,p'-DDD	p,p'-DDE	250 ppm	2500 ppm
2	444	32.1	26.5	0.3	1.2
6	238	17.5	20.2	0.1	0.3
10	241	11.8	25.6	0.9	1.7
12	223	4.7	17.7	0.2	0.0
14	246	5.4	22.4	0.0	0.0
16	172	1.8	15.8	0.0	0.1
First order rate Constant, days^{-1} ±SD	-.00768 ±.0016	-.026 ±.0029	-.0031 ±.0013	-.0549 ±.017	-.109 ±.032

[1/] ppm in extracted fat.

That suggestion is confirmed here. Moreover, these results suggest that tissue residue of methoxychlor can be depleted in time by simply removing the source of ingestion; but such is not likely ever to be achieved for DDT during the useful life span of sheep.

Half for the depletion of DDT, DDD and DDE obtained in the present study (computed from the first order rate constants in table 3) are compared in table 4 with other values obtained in cattle and sheep.

In the study of HUNNEGO and HARRISON (1971), grazing sheep were treated by capsule for 30 days with DDT, DDD, DDE or a combination of the three to supply 1 mg of compound per head per day. The sheep were followed by biopsy for more than a year after the treatment period. The half time of DDD was similar to that observed in the present study, but those of DDT and DDE, which have the slower rates of removal, were greater than those observed here.

In the study of MCCULLY et al. (1967), forage was sprayed with 1.3 kg of DDT per ha, harvested as chopped forage and fed to steers for 83 days; thereafter the cattle were followed by omental biopsy for more than a year. Half times were computed by least squares from the data in table 1 of MCCULLY et al. (1967). The values for DDT and DDE are very similar to those of the present study, but that for the more rapidly removed DDD is about twice as great.

In the work of FRIES et al. (1969), lactating cows were given 25 mg of DDT, DDD or DDE per day for 60 days; tissue residues were followed in tail head biopsies at 20 day intervals for 60 days after insecticide ingestion was terminated. Half lives for the removal of DDT, DDD and DDE during the post-ingestion period, estimated from Figures 1, 2 and 3 of FRIES et al. (1969), were 140, 37 and 70 days, respectively. Taking the data of table 4 as a whole, it is clear that in both cattle and sheep removal from extractable fat is in the order of DDD>DDT>DDE and that the rate may vary by as much as a factor of two depending on unidentified circumstances.

Half times for the removal of methoxychlor, computed from the rate constants of table 3, were 13 and 6 days from previously fed levels of 250 and 2500 ppm, respectively. These values did not differ significantly ($P>.05$), and the average estimate is thus 10 days. Apparently no literature values are available with which to compare these.

To summarize: Technical DDT, added to the rations of pen-fed sheep to supply 250 ppm of p,p'-isomer, yielded plateau levels at 8 weeks exposure of 524 ppm of p,p'-DDT,

TABLE 4

Half Times for the Depletion of DDT, DDD and DDE from Body Fat of Cattle and Sheep

Source	Species	Time Units	$T_{1/2}$ DDT	DDD	DDE
This study	Sheep	Days	90	26	223
Hunnego and Harrison (1971)	Sheep	Weeks	9	4	14
McCully et al. (1966)	Beef cattle	Days	103	50	213
Fries et al (1969)	Lactating cows	Days	140	37	70

75 ppm of p,p'-DDD and 25 ppm of p,p'-DDE in subcutaneous fat; technical methoxychlor, added to supply 250 ppm of methoxychlor, yielded no plateau, but rather a maximum value of 7.8 ppm of the insecticide after 10 weeks exposure, which was followed by a linear decline of 0.6 ppm per week through the remainder of the accumulation period. At 2500 ppm of methoxychlor in the ration, a plateau was reached in 6 weeks at 24.4 ppm. Thus, residues of methoxychlor evidently cannot be made to approach those of DDT, and at similar levels of ingestion may differ at a near plateau level by an order of 100:1.

Half times for the depletion of DDT, DDD and DDE, computed from first order rate constants following the removal of the insecticide from the ration were, respectively, 90, 26 and 223 days; methoxychlor yielded a value of 10 days, independent of previous level of ingestion.

KAPOOR et al. (1970) have compared the elimination of radio labeled DDT and methoxychlor in the mouse. Approximately 1% of the DDT and 98% of the methoxychlor was eliminated in the first 24 hours after a single dose. The sheep reacts to these two compounds in a manner generally similar to the mouse.

METCALF (1973) has put forward the concept of the degradophore, by which he means, as applied to DDT-like compounds, the introduction into the molecule of functional groups which promote its degradation and excretion. Methoxychlor has such degradophores - its p-methoxyl groups - (METCALF 1973), and the data presented demonstrate the merit of that concept in a meat animal species.

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