Metabolism of Low Oral Doses of DDT and DDE by Tame Mule Deer Fawns

by MICHAEL WATSON, BARRY PHARAOH, JOSEPH WYLLIE, and W. W. BENSON
Idaho Community Study on Pesticides
Department of Environmental and Community Services
Statehouse
Boise, Idaho 83720

Numerous investigators have surveyed pesticide levels in wild mule deer, Odocoileus hemionus, and whitetail deer, O. virginianus. To a lesser extent, other big game mammals belonging to the deer family have also been examined for pesticide residues. During a recent survey of organochlorine pesticides in mule deer in Idaho (BENSON and SMITH 1972), we noted that adipose DDT-derived residues in these and various other wild North American ungulates appear to follow a common but a typical storage pattern. Almost without exception, stored levels of 1,1,1-trichloro-2,2-bis (p-chlorophenyl) ethane (DDT) reported in these animals are considerably higher than those of its metabolite 1,1-dichloro-2,2-bis (p-chlorophenyl) ethylene (DDE). This is clearly the reverse of the normal human situation (MORGAN and ROAN 1971), in which p,p'DDE is usually found in much higher quantities than is p,p'DDT.

In mule deer, the pattern of higher DDT levels is apparent in data obtained from pesticide monitoring programs in Idaho (CASEBEER 1965, WALKER et al. 1965, BENSON and SMITH 1972), Washington (WALKER et al. 1965) and South Dakota (GREENWOOD et al. 1967), as well as in Montana, Colorado and New Mexico (PILLMORE and FINLEY 1963). The same trend is seen in whitetail deer surveyed in similar programs in Mississippi (BAETCKE et al. 1972), the Carolinas (BARRIER 1970), Alabama (CAUSEY et al. 1972), and South Dakota (GREENWOOD et al. 1967). Elk, Cervus canadensis, from herds surveyed in Idaho (CASEBEER 1965, WALKER et al. 1965), South Dakota (GREENWOOD et al. 1967), Washington (WALKER et al. 1965), Colorado and New Mexico (PILLMORE and FINLEY 1963) also exhibit this pattern. Although fewer such studies have been made of moose and antelope herds, available data on Idaho moose, Alces americana, WALKER et al. 1965, BENSON et al. 1973) and Idaho (WALKER et al. 1965) and South Dakota pronghorn antelope, Antilocapra americana, (GREENWOOD et al. 1967, MOORE, et al. 1968) also substantiate the tendancy. Because of this apparent difference in pesticide storage among these wild ruminants, we have

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investigated the metabolism of orally administered p,p'-DDT and p,p'-DDE in two captive fawn mule deer, O. heminonus.

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MATERIALS AND METHODS

Two wild female fawn mule deer were obtained from various areas of the Boise National Forest by personnel of the Idaho Fish and Game Department. Both animals were estimated at about three weeks of age and were kept in private quarters at the Boise City Zoo. They were maintained on a diet of "Similac" for a period of approximately two months, until weaned and placed on a diet of alfalfa hay.

Dosing Techniques. Once established on a hay diet, the deer were respectively given daily oral doses of 5 mg of p,p'DDE and p,p'DDT which had been previously dissolved in peanut oil and absorbed onto sugar cubes. To prepare the cubes, a hole 1/8" in diameter was first drilled part-way through each cube. Analytical reagent grade p,p'DDT and p,p'DDE (Perrine Primate Laboratory, Perrine, Florida) were then respectively dissolved in warm peanut oil at a concentration of 25 mg/ml. The solutions were thoroughly mixed on a magnetic stirrer, and 200 microliters of this solution were injected into the cavity in each sugar cube. DDT cubes were marked with red food coloring in order to prevent confusion. Deer #1 was then given one DDE cube (a dose of 5 mg) per day. Deer #2 received a DDT cube (5 mg dose) daily. Doses were maintained for a total of 30 days for both animals.

Sampling Techniques. In order to establish the extent of normal body burden in the fawns, whole bloods, urines and feces were collected and analyzed prior to pesticide dosing. Samples were then obtained weekly during the 30 day dosing period, and at somewhat longer intervals after dosing had ceased. At each sampling, the deer were first weighed and then restrained manually by prone enclosure within a soft, thick mattress held by three men. At this time, a 10 ml whole blood sample was taken from the jugular vein. Urines were obtained from the isolated animals by simply waiting for normal urination to occur and quickly collecting the urine from the scrubbed concrete floor by means of a pipette. Defecation was frequent and fecal samples were easily obtainable. For comparative purposes, a five gram sample of axillary flesh was biopsied from each animal thirty days after all dosing had terminated. This was facilitated by the use of the local anesthetic 2-diethylamino-2', 6'-acetoxylidide (Lidocaine) injected subcutaneously around a large area in order to prevent contamination of the tissue sample.

Extraction and Analysis. Whole bloods were centrifuged immediately to separate the serums. Serums were extracted for pesticides by a revised method of (DALE et al. 1966). Two ml serum was combined in a round bottom tube with six ml nanograde hexane. This mixture was gently agitated at 50-60 rpm by a rotating mixer. Five ml of the hexane layer were then pipetted to a 10 ml concentrator tube and evaporated on a steam bath to a final volume of 1 ml by means of a Snyder two-ball column.

Tissue samples were extracted with petroleum ether and partitioned against acetonitrile using a modified procedure of (DE FAUBERT MAUNDER et al. 1964). The petroleum ether extract was then fractionated on a Florisil column according to the procedure of (MILLS and MILLS et al. 1961, 1963). Urines were extracted for bis (p-chlorophenylacetic) acid (DDA) using the method recommended by the Environmental Protection Agency Pesticide Research Laboratories, Perrine, Florida (THOMPSON 1971). Feces were extracted for pesticides as follows: five grams of fecal material were combined in a stoppered 250 ml Erlenmeyer flask with 100 ml acetonitrile and shaken thoroughly for two minutes. The acetonitrile was then filtered off into a 1000 ml separatory funnel. The residue was then washed twice with 25 ml acetonitrile and the filtered washings added to the funnel. Three hundred ml of pre-extracted distilled water were then added to the combined acetonitrile phases. After thorough mixing, the solution was extracted three times with 100 ml portions of petroleum ether. The combined ethereal fractions were then concentrated to five ml and fractionated on a Florisil column after the methods of (MILLS et al. 1961, 1963).

Analysis was by tritium foil electron capture gas chromatography, using a Micro-Tek 220 instrument equipped with two differing columns for confirmatory analysis. The following parameters were observed: Columns - 4% SE-30, 6% QF-1 on Chromosorb W, DCMS, 80-100 mesh and 1.5% OV-17, 1.95% QF-1 on Chromosorb W, DCMS, 100-120 mesh; Temperatures - Columns 200°C, Injection Chamber 220°C and Detector 205°C; and Carrier Gas Flow (nitrogen) - SE-30, QF-1, 90 ml/min. and OV-17, QF-1 70 ml/min.

Prior to extraction, all samples were spiked with 100 ng of Aldrin (Octalene), which was used as the internal standard. All qualitative retention times were based on the retention time of aldrin. Per cent recovery was also computed from the aldrin standard, and ranged from 78 to 100 per cent. Quantitation of residues was based on relative peak heights.

RESULTS AND DISCUSSION

Because of the relatively low oral doses of pesticides administered and the small urine volumes obtained, urinary levels of DDA were not detectable in any of the samples. Discussion will thus be confined to pesticide residues in serum and feces. Theoretically, fecal pesticide levels should heavily reflect metabolic events

mediated by rumen microorganisms, while serum levels should be more indicative of metabolic conversion due to microsomal enzyme activity of the deer per se. With this in mind, fecal pesticides and serum pesticides will be considered separately.

Figure 1 shows changes in the amounts of DDE, DDT and 1,1-dichloro-2,2-bis (p-chlorophenyl) ethane (DDD) in the serum of Deer #1, which received a daily oral dose of 5 mg p,p'DDE throughout the 30-day period. In this animal, serum DDE levels increased rapidly as the dosing period progressed, reaching a plateau of about 14 parts per billion (ppb) midway through the period and falling to below 5 ppb within eight days after doses had ceased. Levels of p,p'DDT increased briefly early in the dosing period, after which no DDT was detected until well after pesticide doses had stopped. No p,p'DDD was found in the serum of Deer #1 until after the doses had stopped, and then only in negligible quantities (Figure 1). No o,p'DDT was detected at any time.

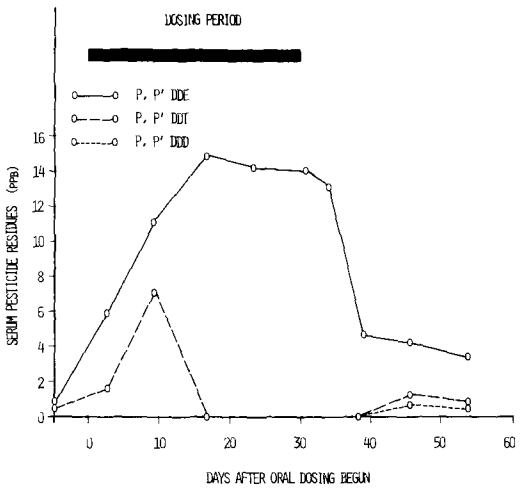

FIGURE 1 - Serum pesticides detected in Deer #1 (fed 5 mg p,p'DDE daily for 30 days).

Figure #2 shows serum pesticides for Deer #2, which was given only DDT. Serum DDE was not detected in this animal at any time during the study. Although p,p'DDT was the most common metabolite found during the feeding period, p,p'DDD was also present in nearly equal quantities. Levels of p,p'DDT were found to plateau rather quickly at about 3.5 ppb, while DDD levels were slightly lower. Serum levels of both residues were found to have decreased abruptly within 4 days after doses had been stopped, but both substances and especially p,p'DDD experienced a brief increase again in a sample taken approximately two weeks subsequently (Figure 2). As with Deer #1, o,p'DDT was not found.

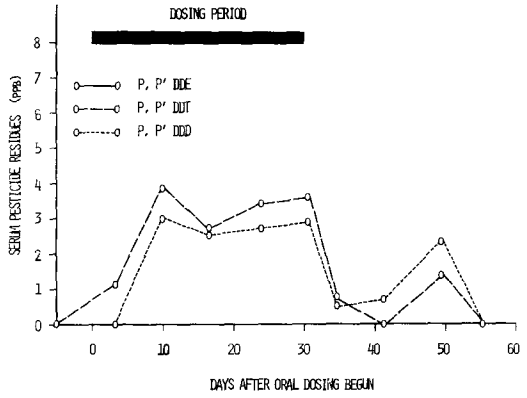

FIGURE 2 - Serum pesticides detected in Deer #2 (fed 5 mg p,p'DDT daily for 30 days)

Fecal pesticide residues for Deer #1 are shown in Figure 3. As in the case of serum, o,p'DDT was not found. Although overall residue levels were higher than for serums (Figure 1) by a factor of approximately one hundred, fecal pesticides in this animal showed a trend similar to that noted previously for serum. DDE levels appeared to be directly related to dose, and its conversion to DDT appeared most unlikely. Levels of p,p'DDT and p,p'DDD were both relatively low and were usually below 11 ppb (Figure 3).

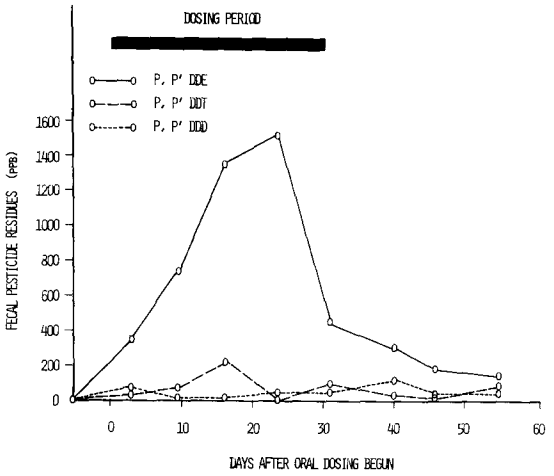

FIGURE 3 - Fecal pesticides detected in Deer #1 (fed 5 mg p,p'DDE daily for 30 days).

In contrast to the serums from Deer #2, fecal pesticides from this animal (Figure 4) contained much greater levels of p,p'DDD than of p,p'DDT. Although some p,p'DDT was present in each sample, levels were usually below 50 ppb. Some fecal p,p'DDE was present at each analysis, but such levels in this animal were seldom in excess of 10 ppb. As was consistent with all other samples analyzed, no o,p'DDT was found. A dramatic decrease in fecal p,p'DDD

was noted after the oral DDT regimen had ceased. These findings are consistent with those in domestic cattle (MCCULLY et al. 1966) in which ingested p,p'DDT was found to be readily converted in the rumen to DDD.

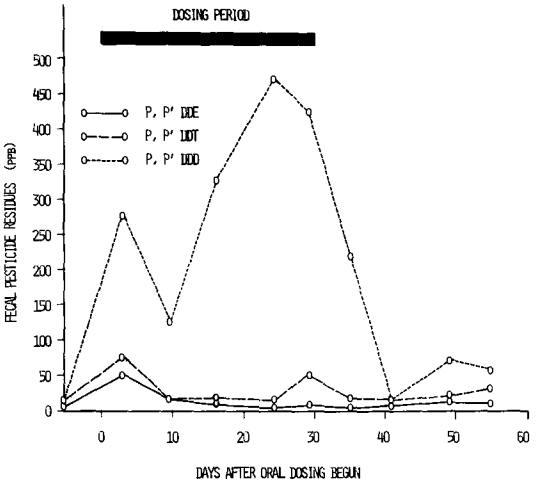

FIGURE 4 - Fecal pesticides detected in Deer #2 (fed 5 mg p,p'DDT daily for 30 days).

Table 1 shows the results of pesticide analysis of samples of axillary flesh biopsied from each animal one month after oral dosing had ceased. It is unfortunate that we were unable to obtain biopsy samples prior to the dosing period in order to establish baseline levels. Curiously, in the flesh sample biopsied from the animal receiving only DDE (Deer #1, Table 1), nearly equal quantities of DDT were also present. This plus the initial brief appearance of DDT levels in the serum of this animal (Figure 1) invite speculation as to whether the deer is indeed capable of metabolizing DDE to DDT. However, the failure of DDT to persist or increase in subsequent serum samples would make this possibility unlikely, as would the relative absence of DDT in the feces of the animal (Figure 3). In contrast, Deer #2 (receiving DDT), had flesh concentrations of DDT that were more than 13 times in excess of those for DDE. This would suggest that the animal may not be able to easily mobilize stored DDT, but this would also seem unlikely because of the predominance of DDD in both serum (Figure 2) and feces (Figure 4) of this animal during the dosing period.

TABLE 1

DDE and DDT Residues in Biopsied Samples of Axillary Flesh Obtained 30 Days After Terminating Oral Doses

		FLESH RESIDUES, PARTS PER BILLION	
	ORAL REGIMEN	p,p'DDE	p,p'DDT
Deer #1	p,p'DDE	25.3	20.6
Deer #2	p,p'DDT	8.7	114.1

Because it has the greatest insecticidal properties of the series (MELNIKOV 1971), the p,p' isomer of DDT has been by far the DDT congener most commonly dispersed into the environment as a pesticide. Over a period of time, however, environmental p,p'DDT undergoes a rather facile dehydrochlorination to form p,p'DDE. DDE is quite stable and does not easily degrade further. Because of this, the member of the series most commonly found in environment is usually DDE. When ingested by humans (MORGAN and ROAN 1971) and by the rat (PETERSON and ROBISON 1964), DDE is stored in adipose tissue for extended periods and presumably is excreted rather slowly. Moreover, neither ingested nor free DDE is believed to form DDT. On the other hand, ingested DDT is easily dechlorinated in most mammals to DDD, which is then ultimately converted to the more polar DDA and excreted (MORGAN and ROAN 1971 and 1972, ROAN et al. 1971).

From the foregoing, it is obvious that in the numberous monitoring studies of wild ungulates cited in the introductory section of this report, the residue most commonly ingested should usually be DDE rather than DDT. That these animals tend to sequester more DDT than DDE suggests the possibility that they are somehow able to convert DDE to the more degradable DDT. Our use of only two animals precludes the use of absolute terms with respect to metabolism. However, our data from Deer #1 (Figure 1 and 3) do not appear in any way to support the possibility that the tame animal is able to easily metabolize DDE. Although some serum DDT was detected early in the feeding study, it would seem unlikely that these levels are of metabolic significance. That some DDT and DDD were present in the feces of Deer #1 are also probably of little consequence, since there could most probably reflect a dietary source.

Review of the data from the deer given only DDT (Deer #2, Figure 2 and 4), indicates that the animal is easily able to degrade DDT to DDD and presumably on to DDA). That no DDE is found in the serum of the animal is also consistent with expected in vivo metabolism. The great amount of DDD noted in the fecal samples of Deer #2 (Figure 4) is also indicative of the ability to metabolize DDT easily.

From these results, we tentatively conclude that the tendency of wild mule deer (and presumably various of the other large wild ungulates) to store DDT in apparent preference to DDE is most likely a result of enteric changes in the rumen prior to absorption rather than a common microsomal phenomenon residing in the biochemistry of the animal per se. Because of their lack of fully developed microsomal enzyme activity, newborn and very young animals are often noted for their relative inability to metabolize drugs and other foreign compounds (CONNEY 1967). It may thus be possible that fawn deer do not yet share the complete enzymatic status of adult animals, and are thus not fully comparable in terms of metabolic abilities. However, this would seem most unlikely in the case of weanling fawns. Certainly, the tame deer utilized here would in no way resemble their wild counterparts in terms of rumen

microflora, and it is quite possible that the dietary habits of wild cervids enables them to acquire the organism(s) responsible for the proposed difference in DDT metabolism and storage. We are currently exploring this possibility by investigating the metabolism of the DDT series by rumen fluid obtained from both tame and wild animals.

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