Tests of Estrogenicity in Rats Fed Low Levels of o,p'DDT

by T. Randall Wrenn, Joan R. Wood, George F. Fries, and Joel Bitman
Hormone Physiology Laboratory, Animal Husbandry Research Division, U.S. Department of Agriculture, Beltsville, Maryland

The o,p' isomer of DDT, constituting about 15-20 percent of commercial preparation of the insecticide, has recently been found to exert estrogenic effects in a variety of animals when given at relatively high doses (1, 2). Speculation immediately ensues regarding the likelihood of reproductive abnormalities caused by contamination of feed. This experiment was designed to test the effects of dietary o,p'-DDT on reproduction and lactation in the rat. Although feeding studies have been made in the past (3, 4, 5, 6) these apparently considered the effect of technical DDT without regard to its molecular configuration. The present experiment was designed to determine whether the estrogenicity of o,p'-DDT in doses likely to be encountered by environmental pollution or accidental feed adulteration would cause measurable changes in reproductive performance or lactation.

Procedure

The estrogenic effectiveness of o,p'-DDT and p,p'-DDT in reducing the age of vaginal opening was determined in young Wistar rats. Seventy 18-day-old females were randomly assigned to each of 7 groups. They were given the following preparations in daily doses by stomach tube: 0.1 ml olive oil (the injection medium), 10 µg o,p'-DDT, 50 µg o,p'-DDT, 10 µg p,p'-DDT, 50 µg p,p'-DDT, 0.025 µg 17 -estradiol, and 0.1 µg 17 -estradiol, all in 0.1 ml olive oil. Daily observations of vaginal patency were made and the rats were killed when 33 days old after being treated for 15 days. The uteri, ovaries and adrenals were excised, dissected free of fat and weighed on a torsion balance to the nearest 0.1 mg. Whole uteri were used for either water or glycogen determination.

In a longer term feeding experiment, 288 Wistar rats, both male and female were raised from birth on a ground commercial small animal ration containing added o,p'-DDT at levels of 0, 1.0, and 2.5 ppm. DDT was incorporated in the feed of suckling rats from the day of parturition and continued for various intervals through 168 days. Some rats were raised in cohabitation and the reproductive and lactational performance of these were compared. Milk samples for insecticide analysis were obtained on the 22nd day of lactation, 18 hours after removal of the young. The time of vaginal opening was not observed. Representative groups of

Bulletin of Environmental Contamination & Toxicology,
Vol. 5, No. 1, 1970, published by Springer-Verlag New York, Inc.

TABLE 1

Acute Effects of Estradiol and DDT Feeding on Immature Female Rats

	No. of rats	Body weight g	Ovarian weight mg	Adrenal weight mg	Weight mg	Water* %	Glycogen* µg/100 mg DW	Percent vaginal patency at 33 days of age	Rat-days that vaginae were open at 33 days
Olive oil controls, 0.1 ml	10	72±3	20.8±.9	16.8±.6	43±2	78.8±.9	446±29	30	5
Estradiol, 0.025 µg	10	75±2	21.2±.8	17.9±.6	46±2	80.9±.5	321±30	50	10
Estradiol, 0.1 µg	10	74±1	19.4±.9	17.1±.4	46±2	80.6±.5	435±27	100	58
o,p'-DDT, 10 µg	10	74±3	20.8±.7	17.7±.7	47±2	80.6±.5	465±39	10	1
o,p'-DDT, 50 µg	10	72±2	23.0±.9	18.4±.5	47±2	80.5±.6	505±36	90	16
p,p'-DDT, 10 µg	10	67±3	20.6±.6	17.1±.4	42±2	79.6±.7	478±44	10	4
p,p'-DDT, 50 µg	10	68±3	20.8±.6	17.8±.7	42±2	80.3±.5	425±21	30	12

*Five of the uteri were used for water determinations, five for glycogen.

Glycogen concentration is expressed on basis of the mean dry weight (DW) of five uteri.

TABLE 2

Reproduction and Lactation in Rats Consuming o,p'-DDT

Feed	No. of rats	Mean age of dams at parturition (days)	Mean number young born	Mean weight per pup at birth (g)	Mean number young surviving at weaning	Mean weight per pup at weaning (g)
Control	5	92.3±1.8	8.4±1.9	5.9±0.2	7.0±2.2	37.5±3.5
o,p'-DDT, 1.0 ppm	7	96.9±2.6	8.9±1.0	5.8±0.2	6.6±1.3	38.9±2.6
o,p'-DDT, 2.5 ppm	5	99.2±4.0	9.2±0.9	6.6±0.1*	7.2±1.8	41.0±1.9

*Significantly different from control pup weight, $P<.05$.

animals were killed at 50, 75, 107-112 and 168 days of age. Male rats were observed for differences in body, testes, and seminal vesicle weights. Females were compared by determining the body weight and weights of ovaries and uteri. Samples of each uterus were taken for glycogen, glucose, and water analyses. Percent water was determined by reweighing after overnight drying *in vacuo* at 100°C. Glycogen was determined by the colorimetric anthrone method of Seifter *et al.* (7) and glucose by the glucose oxidase method as described by the Worthington Biochemical Corporation (Manual No. 11:75, Freehold, N. J.).

For analysis of pesticide residues, tissues and milk were ground with sufficient anhydrous sodium sulfate to disintegrate the solids and absorb the moisture. The samples were extracted with petroleum ether and cleaned-up using a florisil column (8). The concentrations of the compounds were determined by electron-capture gas chromatography and the identity of the compounds confirmed by thin-layer chromatography (9). Recovery of compounds added to the samples were 94.1 $\pm$ 1.3%. Results were compared statistically by Student's *t* test with correction for unequal group size.

Results and Discussion

The results of the vaginal patency experiment are shown in Table 1. Distinct differences were observed at 33 days when the young rats were killed. All of the vaginae of rats receiving the high oral dose of estradiol were open, and 90% of those given 50 µg daily of o,p'-DDT were patent. When these openings are expressed as "rat days open" a better estimate of the relative estrogenicity of feeding the various materials is provided. In addition to estradiol both the o,p'-DDT and, to a lesser extent, p,p'-DDT at 50 µg per day were effective in hastening the time of vaginal opening. For the relatively short time that the materials were fed, neither o,p'-DDT, p,p'-DDT nor estradiol caused significant changes from the olive oil control animals in any of the other parameters measured at the time of killing.

The long-term feeding of o,p'-DDT to female rats for four different time periods showed only one parameter mean that differed significantly from controls. Weights of the ovaries of 168 day old animals receiving 2.5 ppm o,p'-DDT were 6 mg heavier than control ovaries (75 $\pm$ 5 mg vs. 59 $\pm$ 2 mg respectively). All other comparisons of ovarian, body or uterine weight, as well as percent uterine water, glucose and glycogen concentrations were without significant difference within the age groupings.

More differences occurred among measurements in male rats, with larger body weights, testes and seminal vesicles at the low level (1.0 ppm) of DDT feeding than at the zero control level in the 112 day old group. Males fed 2.5 ppm of o,p'-DDT, however, did not show larger body or testes weights at this same age. These scattered significant parameters show no consistent patterns of DDT

TABLE 3

DDT Residues in Body Fat of Rats Fed DDT, μg/g of adipose tissue

o,p'-DDT fed	Females			Males		
	Age, days	No. of rats	o,p'-DDT	Age, days	No. of rats	o,p'-DDT
0		5	.07±.01		3	.06±.01
1.0 ppm	50	5	.81±.04	50	4	.47±.09
2.5 ppm		5	1.74±.08		4	1.38±.18
0		14	.05±.00		11	.07±.01
1.0 ppm	75	11	.83±.05	75	17	.51±.02
2.5 ppm		13	2.20±.08		16	1.28±.05
0		4	.07±.00		8	.05±.01
1.0 ppm	107	6	.97±.05	112	16	.49±.03
2.5 ppm		14	2.79±.13		18	1.45±.07
0		7	.12±.01			
1.0 ppm	168	7	.88±.06			
2.5 ppm		5	2.37±.16			

TABLE 4

DDT Metabolites in Whole Rat Milk[a]

o,p'-DDT fed	No. of rats	μg/g whole milk		
		p,p'-DDE	o,p'-DDT	p,p'-DDT
0	5	.020±.003	.013±.003	.047±.006
1.0	4	.046±.016	.238±.049*	.097±.042
2.5	5	.040±.011	.403±.069*	.090±.031

[a]Milk samples were obtained on day 22 of lactation, 18 hours after removing the young.

*Highly significantly different from control level P<.005.

effects in either male or female rats fed o,p'-DDT from infancy to maturity.

Effects of these low levels of o,p'-DDT on reproduction and lactation were examined in some of the rats. Age of dams at parturition, numbers and weight of young born, young surviving at weaning and weight of young at weaning are shown in Table 2. The single significant effect was in the mean weight of the young of 2.5 ppm o,p'-DDT rats at birth (6.6 vs. 5.9 g) for control rats, and this may be related to the age of the dams at parturition which, though different, were not significantly so. Slightly more young were born to the rats that received DDT, and their weights at 21 days were heavier, though neither of these differences from controls were significant.

The analyses of body fat taken from the dorsal area just posterior to the kidneys are perhaps the most interesting data of this experiment. The concentrations of o,p'-DDT for both males and females for the different ages are shown in Table 3. Both p,p'-DDT were found and both increased in concentration with age in females, but this was less apparent in males. Concentrations of p,p'-DDE ranged between .22 and .68 μg/g, and p,p'-DDT between .28 and .83. Neither seemed affected by the level of dietary o,p'-DDT. This result is contrary to the finding of Klein et al. (10) who noted transformation to p,p'-DDT in fat of rats fed 50 ppm of o,p'-DDT.

Residual concentrations of o,p'-DDT in body fat of both sexes increased with higher feeding levels. The o,p'-DDT was stored at a rate approximately proportional to the dosage level. The tendency for the fat to have similar concentrations of o,p'-DDT regardless of the length of time of feeding indicates that this isomer probably does not accumulate rapidly at the levels we fed. This supports observations previously made in a number of species (2, 11, 12) which indicate less storage of o,p'-DDT and more of p,p'-DDT. A summary (13) of fat storage data following feeding of DDT (presumed to be primarily the p,p'analog) to rats indicates concentrations between 10 to 60 ppm for the levels that we have fed.

The concentration of DDT analogs was 98 and 92 percent greater respectively in females fed 1.0 and 2.5 ppm than in similar males at 107 days. We can only speculate regarding these observed differences in DDT storage in body fat (Table 3). The lower concentrations in the males may be more apparent than real, and could be caused by differences in feed intake, total body fat or sex differences in rate of metabolism. The sex difference in storage of DDT analogs has not been observed to occur in all species studied (13).

Analyses of milk samples taken at the 22nd day of lactation are shown in Table 4. Significant differences were found in o,p'-DDT, which contained 18 and 31 times control amounts in the milk of groups fed 1.0 and 2.5 ppm o,p'. The rats had been fed o,p'-DDT for 110-120 days at the time they were milked.

The presence of o,p'-DDT in the body fat and milk of the rats was not unexpected. Observations (13) have previously demonstrated rather large residues in the milk of rats, goats, dogs and cows fed DDT.

The short-term experiments reported here have demonstrated that o,p'-DDT in high dosage to immature rats exerted clear estrogenic action in causing early vaginal opening. However, long-term, low level, chronic feeding of o,p'-DDT exerted no detrimental effect. Feed adulterated to the extent of 2.5 ppm does not interfere with normal reproduction in young rats, nor sufficiently alter estrogen dependent physiological events to cause it to be considered dangerous. It is noteworthy that no significant changes occurred in uterine weight, water, glucose and glycogen, the parameters most sensitive to estrogen stimulation.

References

1. LEVIN, W., WELCH, R.M. and CONNEY, A.C. Fed. Proc. 27, 649 (1968).

2. BITMAN, J., CECIL, H.C., HARRIS, S.J. and FRIES, G.F. Science 162, 371 (1968).

3. BERNARD, R.F. and GAERTNER, R.A. J. Mammalogy 45, 272 (1964).

4. TREON, J. F. and CLEVELAND, F.P. J. Agr. Food Chem. 3, 402 (1955).

5. DEICHMANN, W.B. and KEPLINGER, M.L. Tox & Appl. Pharm. 8, 337 (1966).

6. WARE, G. W. and GOOD, E.E. Tox & Appl. Pharm. 10, 54 (1967).

7. SEIFTER, S., DAYTON, S., NOVIC, B. and MUNTWYLER, E. Arch. Biochem. 25, 191 (1950).

8. Association of Official Agricultural Chemists, "Changes in Official Methods of Analysis," J. Assoc. Offic. Agr. Chem. 49, 222 (1966).

9. FRIES, G.F., MARROW, G.S. and GORDON, C.H. J. Agr. Food Chem. (in press).

10. KLEIN, A.K., LAUG, E.P., DATTA, P.R., WATTS, J.O. and CHEN, J.T. J. Assoc. Offic. Agr. Chem. 47, 1129 (1964).

11. DURHAM, W.F. Residue Rev. 18, 21 (1967).

12. FRIES, G.F., HARTMAN, A.M. and DRYDEN, L.P. Bull. Environ. Contam. and Toxicol. (in press).

13. HAYES, W.J.,JR. In DDT Insecticides Vol. II Human and Veterinary Medicine. P. Müller, ed. Birkhauser, Basel, 1959.