Effect of Several Dietary Levels of o,p'-DDT on Reproduction and Lactation in the Rat

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The o-p' isomer of DDT, present in commercial preparations of the insecticide in amounts of about 15-20 percent, has been found to exert estrogenic influence on the reproductive tract of rats and birds (1, 2, 3, 4). Further examination of the reproductive consequences of exposure to o,p'-DDT is important in assessing the ecologic consequences involved in choosing insecticides and in making recommendations for their use.

A previous paper (5) from this laboratory reported that long-term, very low level feeding of o,p'-DDT (2.5 ppm) to rats did not interfere with normal reproduction, nor were estrogen-sensitive physiological parameters significantly affected.

The present paper describes the effects of much higher doses of o,p'-DDT on reproductive phenomena in rats. Uterine factors affected by estrogen were examined at intervals during growth of young rats, and reproductive and lactational capabilities were studied in adults. Concentrations of metabolites of the pesticide were determined both in whole rats and as excreted in the milk of lactating rats.

Methods

Virgin female Wistar rats 21 days of age were randomly separated into five groups of 46 rats each and placed on diets of ground laboratory chow containing 0, 1, 10, 20, and 40 ppm o,p'-DDT. The rats were caged in groups of 8 or 10 and housed under controlled conditions of temperature and humidity in an environment of 12 hours of continuous fluorescent illumination and 12 hours of darkness. The diets were fed ad libitum until the time the rats were killed.

The rats were examined daily to determine the time of vaginal patency. At the age of 6, 13, and 17 weeks, 10 rats on each of the five dose levels of DDT were killed for whole body analysis of DDT and DDT metabolites, and for determination of estrogenic effects in reproductive tissues. Comparisons were made of body, ovarian, and uterine weights; and the uterine tissue was analyzed for water, glycogen, and glucose. Whole body pesticide residues were determined in some of the rats of each age and feeding level.

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Sixteen of the rats on each level of DDT feeding were mated and allowed to produce two successive litters. They were caged with males when 40 days old (2 males per cage of 8 females) and examined daily for vaginal copulation plugs. The males were rotated among the various cages twice weekly so as to obviate any effect of a particular dietary level on male fertility. This mating procedure allowed comparison of the various groups with regard to time of sexual maturation, reproductive efficiency, and lactational performance. Two weeks following mating, the pregnant rats were put in individual littering cages, and continued on their dietary regimen. Litters and dams were weighed just after birth and at weekly intervals until weaning at 21 days of age. No adjustments were made in the numbers of young per litter.

The young were killed at 21 days and their reproductive tracts examined for evidence of estrogenic stimulation. The ovaries and uteri of young females were weighed and the water content and glycogen concentration of their uteri determined. Males were compared by body weight, and weights of testes and seminal vesicles. The mother rats were killed at either 21 or 26 weeks of age and their reproductive organs studied in the same manner as the unmated groups killed at younger ages.

In another DDT feeding trial, three groups of sixteen 75-day old female rats were force-fed the following daily amounts of o,p'-DDT in 0.1 ml of olive oil by esophageal intubation: Group A, zero level controls; Group B, 50 μg; and Group C, 200 μg. These groups were mated two weeks following the initiation of the treatments. Observations were made on the growth and survival of young standardized at nine per litter. Dams' milk was collected for DDT analysis on the 22nd day of lactation. Young rats were killed at 22-23 days of age. The adult females were continued on the DDT feeding for varying periods and equal numbers of the same age from each group killed at intervals between 200 and 250 days of age after being fed DDT for 125-175 days. The same reproductive tract parameters of young and adults were measured as in the preceding trial, and in addition livers were weighed and analyzed for glycogen and water content.

Glycogen was determined by the colorimetric anthrone method of Seifter et al. (6) and glucose by the glucose oxidase method as described by the Worthington Biochemical Corporation (Manual No. 11:75, Freehold, New Jersey). Percent water was determined by reweighing after overnight drying in vacuo at 100°C.

For whole body pesticide analysis entire animals, minus reproductive tissue but including the alimentary tract and its contents, were placed with about an equal weight of water and twice the weight of ice, and then homogenized in a Waring blender.

Samples of milk and whole animals were dried by mixing with anhydrous sodium sulfate, then extracted with petroleum ether and further purified by passing through a florisil column (7).

The concentrations of the compounds were determined by electron-capture gas chromatography and the identity confirmed by thin-layer chromatography (8). Mean differences were compared statistically by Student's t. test, or by Duncan's multiple range test.

Results and Discussion

The effect of the various dietary levels of o,p -DDT on sexual maturation of rats is shown in Table 1. There were no

TABLE 1

Indices of sexual maturation of rats on different levels of dietary o,p'-DDT

		DDT Feeding Level					
		0	1 ppm	10 ppm	20 ppm	40 ppm	S.E.
Age of vaginal opening	days	41.9	42.1	41.4	41.5	42.0	± 0.60
	N	35	35	36	36	35	
Age at first littering*	days	76.5[a]	75.7[a]	78.9[a]	87.4[b]	76.9[a]	± 2.18
	N	15	15	16	16	16	
Interval between 1st and 2nd litter	days	50.7	46.6	47.8	48.8	49.9	± 1.87
	N	15	15	16	16	16	

* Means not followed by a common superscript are significantly different at the 5% level of probability, based on Duncan's multiple range test.

significant differences among treatments in age at vaginal opening in this experiment. This result contrasts with that of an earlier one (5) which indicated that o,p'-DDT in lower dosage hastened vaginal patency. The former finding was based on the force-feeding of oil solutions of the insecticide and was also begun at an earlier age than in the present trials.

Also shown in Table 1 is the age of the mated rats at their first parturition, and the lapsed time between the 1st and 2nd litter. These parameters were included to provide measures of reproductive efficiency. Only in one instance (20 ppm) was there a significant difference in the age at first

littering. Since there was no similar effect at the lower or higher feeding levels, we are unable to offer an explanation for this isolated difference.

Although elaborate plans to detect developmental differences in estrogen-dependent reproductive phenomena were included in this experiment, only one consistent difference was noted. In rats killed at 6 weeks of age (on DDT diets for 3 weeks) the

TABLE 2

Litter size and body weights of young rats of mothers receiving dietary o,p'-DDT[1/]

Level of DDT in Diet	Litter	Mean No. live pups born	Mean birth wt. of pups, g.	Mean No. pups weaned	Mean weaning wt. of pups, g.	% surviving
Control	1st	9.8 ± .5	6.4 ± .2	8.8 ± .8	36.9 ± 1.9	88.7 ± 6.4
	2nd	11.3 ± 1.0	6.1 ± .1	8.5 ± 1.2	34.6 ± 2.5	72.7 ± 9.8
1 ppm	1st	8.1 ± .7	6.4 ± .2	7.0 ± .8	34.9 ± 1.6	82.7 ± 8.0
	2nd	11.0 ± 1.0	6.0 ± .2	9.9 ± .9	34.7 ± 1.3	85.3 ± 6.7
10 ppm	1st	10.0 ± .5	6.4 ± .1	9.2 ± .5	34.1 ± 1.1	92.9 ± 2.8
	2nd	11.9 ± .6	6.3 ± .1	11.3 ± .5	36.1 ± 1.2	95.6* ± 1.7
20 ppm	1st	8.0* ± .4	6.5 ± .1	7.7 ± .5	38.8 ± 1.6	93.3 ± 2.2
	2nd	10.4 ± .9	6.5* ± .1	8.6 ± .9	37.1 ± 2.0	83.5 ± 6.9
40 ppm	1st	8.6 ± .4	6.3 ± .1	7.7 ± .7	37.3 ± 1.4	89.7 ± 6.1
	2nd	10.2 ± .8	6.3 ± .1	8.3 ± .8	33.5 ± 2.1	84.2 ± 6.0

1/ No. of litters observed in each group was either 15 or 16. Values are means ±S.E.

* Significantly different from control, $P<0.05$.

percent water of the uterus was significantly lower ($p < 0.05$) in the 10, 20, and 40 ppm groups than in the zero level controls. There also was a trend toward lower uterine glycogen in these same groups at this time. A possible explanation for this is that the estrogenic activity supplied by the o,p'-DDT, acting through higher neural centers, delayed ovarian maturation and thereby suppressed endogenous estrogen elaboration. At this time period, prior to sexual maturity, estrogen secretion would be at low endogenous levels. At later time periods, after ovarian maturation, endogenous estrogen secretion appears to override any estrogenic influence of o,p'-DDT and there were no differences in ovarian weight or water and glycogen concentrations in the uterus of the older animals. Other parameters measured -- body weight, ovarian weight, uterine weight, and uterine glucose showed no differences due to treatment at 6, 13, 17, 21, or 26 weeks of age. The data supporting these essentially negative conclusions are not presented.

Table 2 compares the litter size and pup weight of rats on the various DDT levels both at birth and at weaning (21 days old). Scattered statistical significance was attained, but no consistent patterns of DDT level on number born, weight, or survival were seen.

Our results are consistent with those of Ottoboni (9) who found no adverse effect on fertility, fecundity, or viability of young in rats fed technical DDT. Her highest dose rate was 200 ppm of the commercial grade, which by analysis contained 17% of the o,p'- isomer or a daily dose of 34 μg o,p'-DDT/g of feed.

Extensive measurements of reproductive organs of the young rats revealed no influence of DDT level. Body weight, ovarian weight, uterine weight, uterine water, uterine glycogen, and testes and seminal vesicle weights were not related to the treatments. These data are not included in this report in order to achieve brevity.

The concentration of o,p'-DDT in the whole body of the rats is presented in Figure 1. In general, the concentration increased in unmated rats at all feeding periods through 14 weeks. There are two apparent exceptions to this. The 1 μg/g group reached a plateau after 3 weeks of feeding and the 10 μg/g group was highest after 10 weeks of feeding and then declined at 14 weeks. However, the variability within this group, particularly at 10 weeks, was great and the high point may not be a true representation of the situation at 10 weeks for the 10 μg/g group. The average whole body concentration at any given time, considering the variability within the groups, is proportional to the level of o,p'-DDT in the diet.

The average body weight of the rats, which did not differ significantly among the treatments, was 115 g at 3 weeks, 206 g at 10 weeks, and 228 g at 14 weeks. Thus, the total amount of

o,p'-DDT retained by the rats increased at a rate more marked than is indicated by the whole body concentration, particularly in the first 10 weeks of o,p'-DDT feeding.

One of the most interesting aspects of this work is the effect of pregnancy and lactation on the whole body concentration of o,p'-DDT. The rats which were killed at the average age of 20 weeks had littered twice and had lactated for two 3-week periods. These rats had been fed the o,p'-DDT diet for about 5 weeks before they first became pregnant. Their concentrations of o,p'-DDT, at all levels of feeding except the 1 μg/g, were lower than the concentrations of the o,p'-DDT for unmated rats at both 10 and 14 weeks feeding. These rats had an average weight of 279 g when they were killed, and thus the difference for whole body content is not as marked as the difference in concentrations.

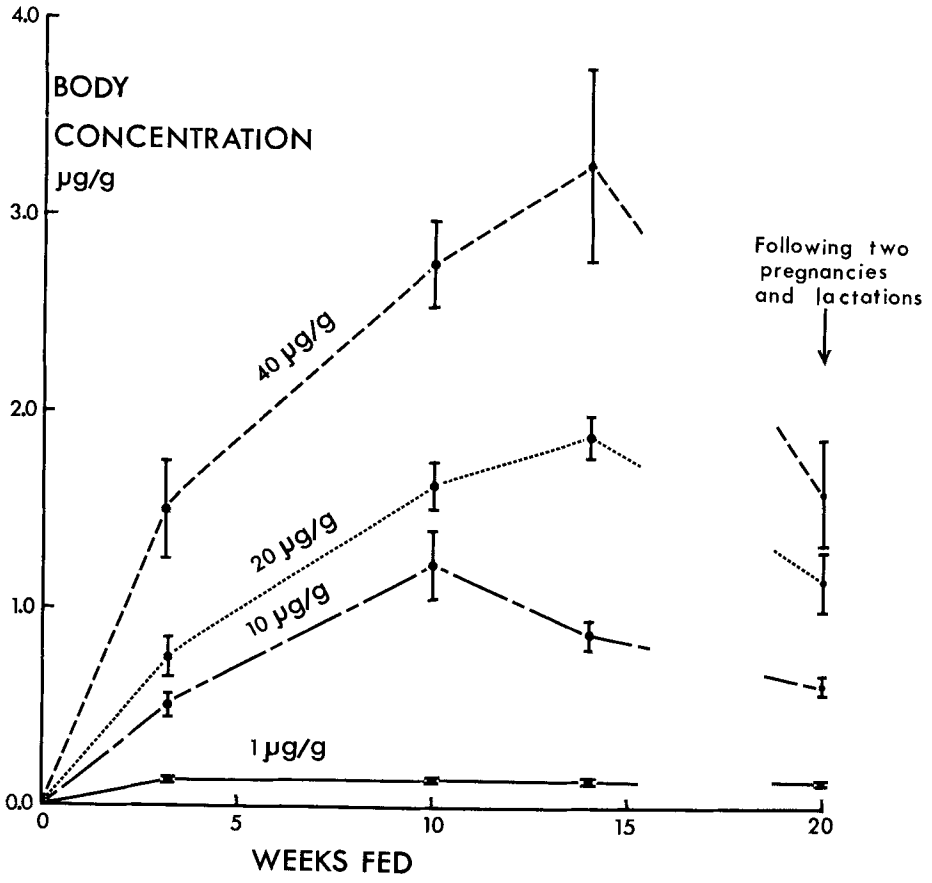

Figure 1. Whole body concentration of o,p'-DDT following feeding to rats for various periods. Values are means ± S.E.

There are two factors which could have caused the lower whole body concentrations with the rats that have gone through pregnancy and lactation. Pregnancy could stimulate the metabolic processes which degrade o,p'-DDT. More likely, the fetus and milk would be such significant routes of excretion that an equilibrium value for the whole body concentration would be established at a level lower in these rats than the rats which have not been subjected to these physiological factors.

In addition to the o,p'-DDT, there were also increases in the whole body content of p,p'-DDE and p,p'-DDT, both with length of time of feeding and with concentration of o,p -DDT in the diet. The values of the whole body concentration of these two analogs at the 14-week feeding period are presented in Figure 2. The concentrations of both of these analogs were linearly related to the concentration of o,p'-DDT in the diet.

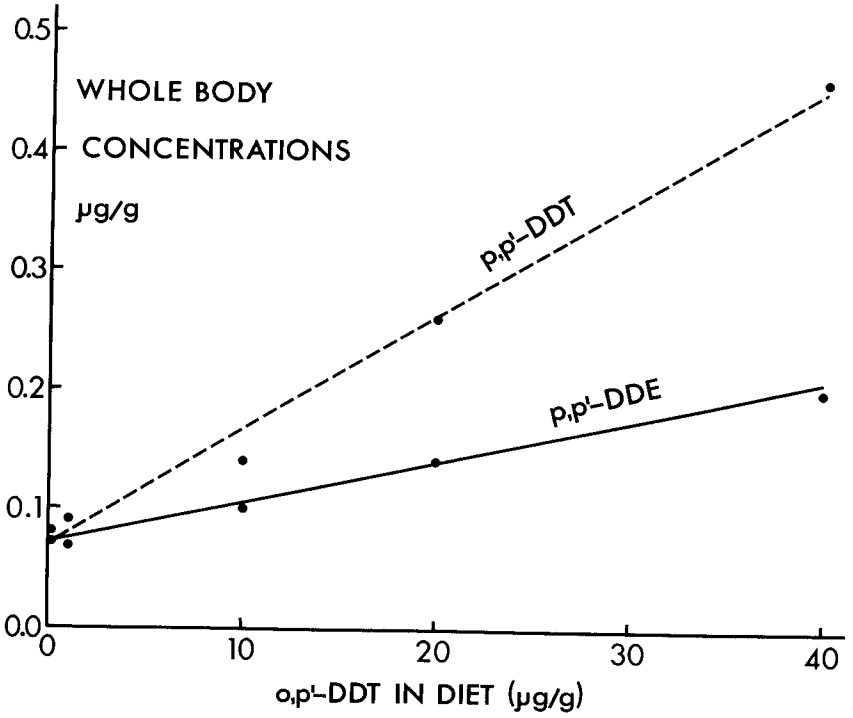

Figure 2. Regression lines for concentration of p,p'-DDT and p,p'-DDE in the whole bodies of rats fed o,p'-DDT for 14 weeks. Each point is the mean of 4 rats.

Similar increases in p,p'-DDT were ascribed by Klein et al. (10, 11) as being due to the conversion of o,p'-DDT to p,p'-DDT. However, a different explanation has been provided by Bitman et al. (12, 13) who find that p,p'-DDT arises from minor contamination of the o,p'-DDT fed. After long feeding periods, when the o,p'-DDT is largely metabolized, this minor contamination becomes a major remaining component. The o,p'-DDT fed in this experiment contained 1.196% p,p'-DDT as a contaminant. Therefore the results from this experiment are consistent with the explanations of Bitman et al. (13) in which o,p'-DDT from the same source was used.

The force-feeding of 0, 50, and 200 μg per day of o,p'-DDT starting at 75 days of age also was without significant effect on reproduction or estrogen-dependent phenomena. The groups did not differ in their time of littering, number of young born, litter weight, dam weight, pup weaning weight, or pup survival. Similarly, the adult rats, when killed between 202 and 250 days of age, showed no differences in body weight, ovarian weight, uterine weight, uterine hydration, uterine glycogen, uterine glucose, liver weight, or liver glycogen.

Analysis of milk samples of the rats force fed known quantities of o,p'-DDT are shown in Table 3. Highly significant increases over control levels were found for both o,p'- and p,p'-DDT at both of the dose levels.

TABLE 3

DDT Metabolites in milk of rats

o,p'-DDT fed per day, μg	No. of rats	Mean length of feeding interval, days	μg/g Whole Milk 1/		
			p,p'-DDE	o,p'-DDT	p,p'-DDT
0	9	--	.012±.001	.005±.000	.018±.001
50	9	62	.014±.001	.340±.027**	.023±.002**
200	7	58	.017±.002*	1.35 ±.111**	.028±.002**

1/ Values are means ± SE
* Significantly different from control level, $P<.050$.
** Highly significantly different from control level, $P<.005$.

Contamination of milk by transfer of DDT and its metabolic products has been studied extensively in cows (8, 14, 15, 16). The cow work generally considered either the p,p'-isomer of DDT or technical DDT, which is a combination of the o,p'- and p,p'-isomers. Ottoboni and Ferguson (17) have determined various analogs of DDT in the stomach milk of young rats suckling dams fed technical DDT. Both their work with the commercial product and ours with the o,p'-isomer show certain similarities to the

results in cattle. In both rats and cows the levels of DDT in milk appear in proportion to dietary levels and/or body stores, and in both animals the secretion in milk appears to be a major pathway for the reduction of body stores.

Since it is difficult to get a large sample of the rat milk, it was necessary to analyze this material and express results on a whole milk basis. The concentration in whole milk might be affected by the total quantity of milk produced and the fat concentrations in the milk as well as the dietary intake. Recognizing these possible sources of variation, however, it is possible to compare our rat work with the levels that are excreted into milk by dairy cows in mid lactation (Fries, unpublished data). Cows fed 100 mg per day o,p'-DDT and producing 16.7 kg of milk per day excreted 1.74 mg per day of o,p'-DDT and o,p'-DDD into the milk. If one assumes that the rats produce approximately 7.5 g of milk per day, then the equivalent excretion of o,p'-DDT in the milk is approximately 5% of the intake, or about three times as great as the value for cows. In addition to the higher amount of total residue excreted into milk by the rats, the other major difference is that in the cow approximately 80% of the residue occurred as o,p'-DDD, apparently from conversion in the rumen (8), while in the rat all of the residue occurred as o,p'-DDT. This excretory route for DDT stores is in accord with the diminution of whole body concentrations of rats following lactation (Fig. 1). The relative contribution of lactation, as compared to the fetal tissue growth, in reducing maternal stores could not be assessed.

Conclusions

The levels of o,p'-DDT fed in this experiment were considerably above those likely to be encountered in accidental feed adulteration or through careless use of the insecticide. Even though these experiments included the critical periods of reproductive maturation and were extended through two pregnancies, no adverse effect on female reproduction could be determined. Since o,p'-DDT is the most estrogenically potent of the isomers or metabolic analogs of DDT, these experiments serve to indicate levels of exposure that can be safely tolerated throughout growth, pregnancy, and lactation. Further, since technical DDT contains only 15-20% of the o,p'-isomer, by extrapolation it would be expected that daily amounts as high as 1 gram of the technical insecticide preparation (80-85%, p,p'-DDT) could be ingested without deleterious effects on reproduction, except for the physiological effects of so large a dose.

As judged from these experiments on a small laboratory rodent, our results indicate that o,p'-DDT, an estrogenically active pesticide, does not adversely affect reproduction in mammals.

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