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EFFECTS OF 1,1-DICHLORO-2,2-BIS[*p*-CHLOROPHENYL]ETHYLENE (DDE) ON LACTATION IN RATS

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An inverse correlation between the concentration of DDE in human breast milk samples and the duration of breast feeding prompted the present study of the effects of DDE administration on the lactational performance of primiparous rats. Daily doses of 10 mg p,p'-DDE/kg body weight were given to virgin female Sprague-Dawley rats 5 d/wk for 5 wk prior to mating and continued throughout the gestation and lactation periods. Lactation capacity was determined by monitoring neonatal growth and by measuring milk production, milk composition (total protein, total lipid, and lactose), and mammary-gland weight and nucleic acid content on d 9 and 20 postpartum. Gross toxicity was assessed by monitoring clinical signs and body weight of the dams, and by measuring organ weights of the dams on lactation d 9 and 20. Histopathological evaluation of the mammary glands and selected organs in the dams and pups was also performed. The dose level of DDE employed was apparently not toxic to the dams and did not have a pronounced effect on neonatal mortality. No significant differences between DDE-treated and control groups were observed for any of the lactation parameters, even though the concentration of DDE in the milk of treated rats was approximately two orders of magnitude greater than the upper range of the DDE levels measured in human milk samples. These findings indicate that DDE does not adversely affect lactation or neonatal growth in Sprague-Dawley rats at the dose level used in this study.

INTRODUCTION

Although use of DDT [1,1,1-trichloro-2,2-bis(*p*-chlorophenyl) ethane] as an insecticide has been drastically curtailed, its impact on human health remains a source of concern, primarily because of its continued application in certain parts of the world (Snelson, 1975) and also because of the persistence of the chemical and its derivatives in the environment. The persistence of DDT and its major metabolite, DDE [1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene], is due, in part, to their high fat solubility, which retards elimination from exposed organisms and thereby leads to their bioaccumulation in food chains

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(Dustman and Stickel, 1969). Consequently, human exposure to DDT and its metabolites via the diet led to appreciable body burdens, particularly during the period of intensive application of the pesticide (Quimby et al., 1965; Hayes, 1966). Attempts to identify potential adverse effects of this exposure, including numerous studies in laboratory animals, have thus far revealed a relatively low mammalian toxicity (Allen et al., 1979) and little evidence for deleterious effects in humans under ambient exposure conditions (Hayes et al., 1971).

Recently, attention has focused more on DDE. Over the past two decades, production and use of DDT has steadily declined, such that human exposure to DDT has decreased dramatically (for review, see Kimbrough, 1982; Sprindler, 1983). Since DDE is more resistant to metabolic degradation and excretion than DDT in many species, including humans (Fawcett et al., 1981; Morgan and Roan, 1971; Fries et al., 1972; Hayes, 1975), it has become the major DDT-derived residue present in foodstuffs (Szokolay et al., 1977; Spindler, 1983) and is the major storage form in humans, being located primarily in adipose tissue (Wyllie et al., 1972; Barquet et al., 1981). Although the acute toxicity of DDE is less than that of DDT (Murphy, 1980), it is uncertain whether accumulated DDE may cause more subtle toxicities, particularly under conditions where adipose lipids are mobilized, resulting in the release of DDE, such as during starvation (Mitjavila et al., 1981a) or lactation (Vuori et al., 1977; Siddiqui et al., 1981).

The presence of DDT derivatives in human milk was first identified by Laug et al. (1951), and numerous studies have since revealed the ubiquity of this phenomenon (for review, see Spindler, 1983). Adamović et al. (1978) demonstrated that the rate of excretion of DDT derivatives into the milk was much greater than the rate of dietary intake of such derivatives, indicating mobilization of an endogenous reservoir. In this study, DDE concentrations in milk were approximately four times higher than the DDT concentrations. Similarly, in nearly all studies performed after 1970, DDE represented at least 50% and usually as high as 70–80% of the total DDT-derived residues in human milk samples (for review, see Spindler, 1983).

These findings have raised concern over the possible effects of DDT/DDE on the neonate as well as on the lactation process itself. A study by Deichmann et al. (1975), in which DDT was fed at a low level to mice over seven generations, did not produce any adverse effects on the growth of offspring. Similarly, Ottoboni et al. (1977) fed DDT to beagle dogs in doses as high as 10 mg/kg body weight · d over 3 generations and did not observe any effects on the viability, survival to weaning, and growth of pups. In the latter study, lactation capacity was assessed simply by monitoring the above parameters and by performing a check for the "adequacy" of milk production. The sensitivity of these measurements was evidently compromised by a large variability in the mammary development and mothering behavior of the

dams. In contrast, Del Pup et al. (1978) observed a significant decrease in neonatal survival in stable populations of mice continuously exposed to DDT at a dietary level of 100 ppm. It is uncertain whether this effect was a result of direct toxicity to the neonates by DDT or its derivatives present in the milk or was an indirect consequence of an impairment of lactation.

Recently, Rogan and Gladen (1982) conducted an epidemiological survey of pesticides in breast milk and found a striking negative correlation between DDE levels in human milk samples and the length of time that neonates were breast fed. These data suggested that higher DDE levels in milk produced an adverse effect either on the neonate or the mother (i.e., on lactation), which resulted in an early termination of nursing. Thus, the present study was performed to investigate the effects of DDE on neonatal growth and viability and on various indices of lactational performance on primiparous lactating rats.

MATERIALS AND METHODS

Materials

p,p'-DDE was obtained through the Radian Corporation, Austin, Tex. (Radian inventory no. CF-000453). Purity was greater than 99%, as determined by gas chromatography using a 30-m DB-5 capillary column. Orcinol, diphenylamine, β-galactosidase, and galactose dehydrogenase were obtained from Sigma Chemical Co., St. Louis, Mo.

Animals

Female (110) and male (55) Sprague-Dawley rats [Cr:COBS (CD)BR] were obtained from Charles River Breeding Laboratories, Inc., Kingston, N.Y. Prior to and during the administration of DDE, the rats were housed in humidity- and temperature-controlled rooms with 12-h light–dark cycles. NIH-07 diet (Zeigler Brothers, Gardner, Pa.), containing less than 0.01 ppm DDT and DDE, and deionized (Millipore-filtered) distilled water were supplied ad libitum.

Administration of DDE

At the initiation of the study, female rats approximately 55 d of age (140–160 g) were divided into 2 groups (control and DDE) by a randomization procedure designed to obtain equal average body weights for the two groups. A corn-oil solution of DDE was given by gavage at a dose of 10 mg/kg body weight · d; controls received equivalent volumes of corn oil. DDE was administered 5 d/wk (week days only) for 5 wk prior to mating, throughout gestation and lactation up to the day prior to sacrifice (i.e., either d 8 or d 19 of lactation). The total number of doses was 49–50 and 58–59 for the lactation d 9 and lactation d 20 animals, respectively (see below). Body weights were measured weekly until the animals were mated.

Mating and Normalization of Litters

After 5 wk of administration of DDE or corn-oil vehicle, the females (approximately 80 d of age, 230–265 g) were mated with untreated male rats by cohabitation (1 male per 2 females per cage) for 4 consecutive days. Vaginal smears were performed on all females the morning after each mating night, and those that were sperm-positive were removed and housed one per cage. At parturition (lactation day 0), the dams were assigned to either lactation d 9 or lactation d 20 groups. Most litters were born over a 4-d period, corresponding to the 4 d of cohabitation. Dams littering on the first and fourth of these days were used for lactation d 9 measurements, while those littering on the second and third parturition days were used for lactation day 20 measurements. Thus, the dams assigned to lactation d 9 or 20 groups had received the same average number of doses of DDE (or corn oil) at the time of parturition.

Reproductive parameters (Table 1), body weights (Fig. 1), litter weights (Fig. 2), and pup mortality were monitored for all female rats and litters used in the study. Groups of 10 control and 10 DDE-treated rats were randomly selected at the time of parturition and designated for either lactation d 9 or 20 measurement of the organ weights of the dams (Table 3), milk and serum DDE concentrations (Table 2), and lactation parameters (Table 4). Although subgroups were used (i.e., approximately half of the 10 dams used for either lactation day were derived from two different parturition days), the measurements were made on the appropriate lactation day and the data were pooled. All litters designated for lactation parameter measurements were normalized to 12 pups (6 male and 6 female) on lactation d 2. Pups that died were replaced by age- and size-matched pups from extra litters. Litters were weighed on the day of parturition and on lactation d 2, 8, 14, and 19.

TABLE 1. Effect of Administration of DDE[a] on Various Reproductive Parameters

Parameter	Control	*n*	DDE	*n*
Percent sperm positive[b]	77.8	42/54	88.2	45/51
Percent pregnant	77.8	42/54	78.4	40/51
Length of gestation (d)	21.6 (±0.5)[c]	42	21.2 (±0.7)	40
Litter size	14.1 (±3.5)	42	13.8 (±2.4)	40
Percent female pups	53.3	591	50.7	553

[a] DDE was administered as single daily gavage doses of 10 mg/kg body weight to female rats, 5 d/wk (weekends off), for 5 wk prior to mating, throughout gestation and lactation up to the time of sacrifice.

[b] Percent exhibiting sperm in vaginal smears taken daily during 4 successive days of mating.

[c] Mean (±SD).

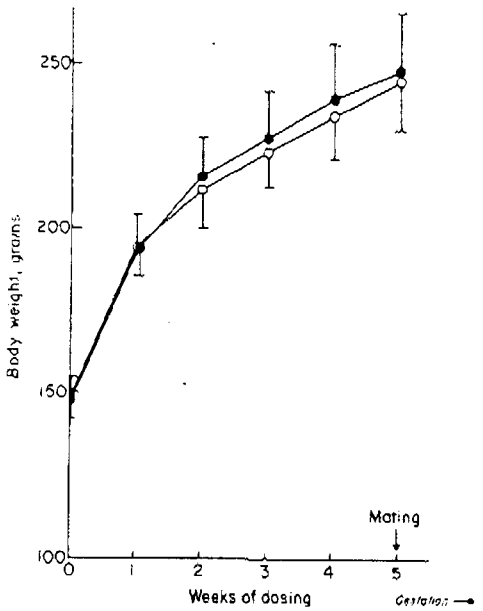

FIGURE 1. Body weight of DDE (●) and control (○) dams monitored over the 5-wk period of administration of DDP prior to mating. Each point represents the mean ± SD from 50 DDE-treated and 53 control rats. The data were not significantly different when compared using Student's *t*-test and an a priori significance level of $p = 0.05$.

Measurement of Milk Production, Collection of Milk, and Tissue Samples

Prior to the milk production measurements, dams and pups were separated for 12 h. Babicky et al. (1976) have shown that removal of pups for up to 12 h does not cause irreversible inhibition of milk production. In order to ensure that the measurement of milk production in DDE-treated dams was independent of an effect on nursing behavior in pups, "surrogate" litters from untreated dams were used for milk production estimates in the DDE group. These surrogate litters were also separated from their respective dams for 12 h prior to being used for milk production estimates in DDE dams. On the morning of lactation d 9 or 20, the dams were anesthetized with 40 mg sodium pentobarbital/kg body weight, ip, and the 6 nipples on the right side of the animals were covered with tape. Oxytocin (1 I.U.) was given ip and then the dams were weighed and placed, right side down, in cages with a randomly chosen surrogate litter ($n = 12$) of 12-h-fasted control pups. The uncovered nipples on the left side of the dams were moistened with warm water to encourage suckling. After 60 min, the dams were removed and reweighed. The decrease in body weight was taken

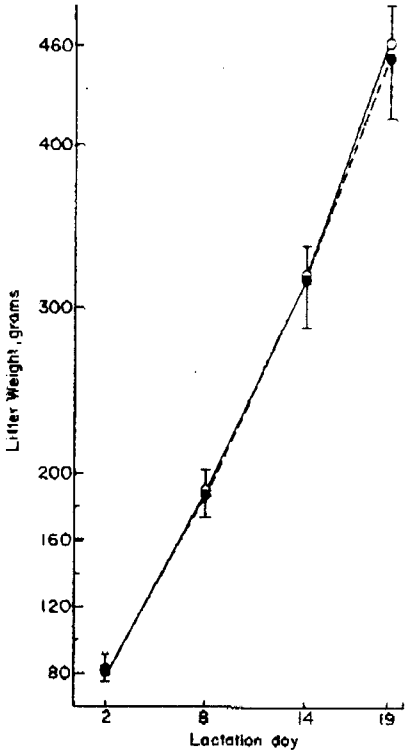

FIGURE 2. The growth of litters from DDE-treated (●) and control (○) dams from lactation d 2 to lactation d 19. Each point represents the mean ± SD from 16 DDE and 13 control litters.

as a measure of milk production. The tape was then removed from the right side of the dams, oxytocin was again administered, and milk was collected by manual expression from the six glands that had not been suckled.

After milking, the dams were sacrificed by an overdose of sodium

TABLE 2. Concentration of DDE[a] in Milk and Maternal Serum

Liquid	Lactation d 9	Lactation d 20
Milk[b]	24.4 (±7.8)[c]	21.9 (±12.4)
Serum[b]	5.66(±2.32)	1.11(± 0.34)
Milk/serum	5.19	19.73

[a] DDE was administered as single daily gavage doses of 10 mg/kg body weight to female rats, 5 d/wk, for 5 wk prior to mating, and throughout gestation and lactation periods up to the time of sacrifice.

[b] In μg/ml (ppm).

[c] Mean (±SD) from 10 rats.

TABLE 3. Effect of Administration of DDE on Maternal Body and Organ Weights

Parameter	Lactation d 9		Lactation d 20	
	Control	DDE	Control	DDE
Liver weight[a]	4.85 (± 0.29)[b]	4.84 (± 0.44)	5.34 (± 0.56)	5.39 (± 0.58)
Kidney weight[a]	0.68 (± 0.05)	0.68 (± 0.08)	0.75 (± 0.06)	0.77 (± 0.06)
Thymus weight[a]	0.068(± 0.016)	0.071(± 0.017)	0.039(± 0.014)	0.042(± 0.021)
Ovaries weight[a]	0.032(± 0.004)	0.035(± 0.007)	0.039(± 0.007)	0.040(± 0.004)
Uterus weight[a]	0.098(± 0.013)	0.105(± 0.009)	0.140(± 0.050)	0.119(± 0.026)
Body weight[c]	329.5 (±13.9)	330.3 (±21.4)	314.3 (±14.8)	320.0 (±23.9)

[a] Grams/100 g body weight.
[b] Mean (±SD) from 10 rats.
[c] Taken before milk production estimate.

pentobarbital. Before death, a blood sample was taken from the inferior vena cava. The "nursed" mammary glands (i.e., three pectoral and three abdominal–inguinal glands on the left side of the dams) were carefully excised, weighed, and fixed in buffered neutral 10% formalin for histomorphological evaluation. The "milked" glands on the right side of the dams were excised and frozen at −80°C for later determination of RNA and DNA content. The liver, kidney, thymus, ovaries, and uterus of the dams were removed, trimmed, and weighed. The

TABLE 4. Effect of Administration of DDE on Various Lactation Parameters

Parameter	Lactation d 9		Lactation d 20	
	Control	DDE	Control	DDE
Mammary gland weight[a]	3.73 (± 1.35)[g]	4.00 (± 0.54)	3.86 (± 1.16)	4.41 (± 1.31)
Milk production[b]	3.21 (± 1.47)	2.39 (± 0.84)	3.61 (± 1.54)	3.45 (± 1.47)
RNA[c]	21.21 (± 2.20)	20.20 (± 3.04)	30.21 (± 1.85)	30.90 (± 2.55)
DNA[c]	13.93 (± 2.05)	15.16 (± 2.00)	14.87 (± 1.42)	14.52 (± 1.52)
RNA/DNA	1.52	1.33	2.03	2.13
Milk composition				
Lactose[d]	27.6 (± 6.5)	20.3 (±10.7)	28.5 (±20.4)	38.2 (±25.7)
Protein[d] A[e]	83.8 (± 8.7)	89.2 (±11.3)	91.1 (±28.1)	88.8 (±19.6)
Protein[d] B[e]	77.3 (± 9.8)	80.4 (± 5.3)	73.9 (±14.1)	74.8 (±19.3)
Lipid[d] A[f]	33 (±15)	50 (±17)	50 (±25)	67 (±31)
Lipid[d] B[f]	38 (±15)	42 (±16)	68 (±23)	66 (±33)

[a] Grams/100 g body weight for 3 pectoral and 3 abdominal–inguinal glands on left side of animal.
[b] Milliliters/100 g body weight from 3 pectoral and 3 abdominal–inguinal left mammary glands in 1 h (see methods).
[c] Milligrams/100 g body weight in 3 pectoral and 3 abdominal–inguinal right mammary glands.
[d] Milligrams/ml milk.
[e] Method A: Lowry et al. (1951). Method B: Bio-Rad kit (Bradford, 1976).
[f] Method A: modification of Bligh and Dyer (1959). Method B: Roese-Gottlieb (Biance et al., 1978).
[g] Mean (±SD) from 10 rats.

organs of the dams and whole pups (four per litter) were fixed in formalin and processed for histomorphological evaluation, using hematoxylin and eosin staining. Milk and blood (i.e., serum) samples were frozen at −80°C until analyses were performed.

Measurement of DDE

Milk and serum samples (100 μl) were extracted with hexane/acetone, as described by Brevik (1978). The resulting extract was delipidated with sulfuric acid according to the method of Veierov and Aharonson (1980). The final residue was dissolved in 10 ml hexane, and 40 μg mirex was added as an internal standard. Separation was performed on a Perkin Elmer Sigma 2000 gas chromatograph equipped with a ^{63}Ni electron-capture detector and fitted with a 6 ft × 2 mm ID glass column packed with 1.5% OV-17/1.95% QF-1 on 100–120 mesh Chromosorb W-HP. Argon/methane was used as the carrier gas at a flow rate of 60 ml/min; injector, oven, and detector temperatures were, respectively, 250°C, 210°C, and 300°C. Recovery of *p,p'*-DDE from the extraction and cleanup procedure was 101 ± 4.6%. DDE was quantified by comparing the sample peak height ratio of DDE/mirex to a standard curve of DDE/mirex peak height ratios versus DDE concentration.

Mammary-Gland DNA and RNA

RNA and DNA were isolated from mammary glands by "Method I" of Kornbrust et al. (1982). In the respective isolates, DNA was quantified by the diphenylamine assay of Burton (1956) and RNA by the orcinol method of Ceriotti (1955). Recovery of calf thymus DNA and yeast RNA was determined, in separate experiments, to be 85 and 96%, respectively.

Milk Composition

Milk lactose was measured in a 100-μl aliquot by the method of Kurz and Wallenfels (1974). Total protein was measured by the method of Lowry et al. (1951) (method A) and by the dye-binding method of Bradford (1976) using a Bio-Rad Kit (method B). Total lipid was determined using the Bligh and Dyer (1959) (method A) and the Roese-Gottlieb (Biance et al., 1978) (method B) extraction procedures, both of which were scaled down to 4.8- and 6.25-ml total volumes, respectively, and applied to 100-μl aliquots of milk. The weight of the lipid residue after complete evaporation of solvents yielded the total lipid values.

RESULTS

Administration to female rats of 10 mg DDE/kg body weight · d, 5 d/wk, for 5 wk prior to mating and throughout gestation apparently had no effect on a variety of reproductive functions, including coital be-

havior (percent sperm positive), fertility (percent pregnant and litter size), length of gestation, and sex distribution of offspring (Table 1). The body weights of the DDE-treated rats appeared to be slightly higher than the controls from wk 2 through wk 5 of the study (Fig. 1), but this difference was not significant.

All litters were normalized on lactation d 2 in order to establish a basis for comparison of growth rates. A litter size of 12 was chosen (1) to ensure uniform suckling-induced development of all 12 mammary glands (Bruce, 1961), thereby reducing intergland variability in size, RNA and DNA content, etc. (Tucker, 1966), and (2) to promote a higher overall lactation capacity in terms of milk production, mammary-gland nucleic acid content, etc., which has been shown to vary with the intensity of the suckling stimulus (i.e., number of pups) (Kumaresan et al., 1967; Tucker, 1966; Moon, 1969). Thus, it was assumed that relatively large litters would stimulate a nearly maximal lactational response in the dams, which might make DDE-induced impairments of the lactation process more apparent. However, growth of litters from DDE-treated dams was virtually identical to control litters (Fig. 2).

Pup mortality during the first 8 d postpartum was 3.8% for DDE litters and 2.2% for the control litters. Most of the deaths occurred on the day of parturition, and there were no more than three deaths in any one litter (data not shown). Although the incidence of mortality was slightly higher in the DDE-treated litters, it was well within the range of historical controls (unpublished observations) and was, therefore, not considered to indicate a treatment-related effect.

The concentration of DDE in milk and serum samples is shown in Table 2. These levels are approximately 100 times higher than the upper range of DDE concentrations in the human milk samples collected by Rogan and Gladen (1982). DDE levels in control rat milk and serum were less than the limits of detection of the method employed. Between lactation d 9 and 20, the DDE concentration in milk declined approximately 25%, while the DDE concentration in serum decreased more than 80%, such that the milk/plasma ratio increased almost fourfold. No 1,1-bis(*p*-chlorophenyl)-2,2-dichloroethane (DDD) was detected in the milk or serum samples.

DDE treatment had no effects on maternal body weight or on liver, kidney, thymus, ovaries, or uterus weights, expressed per 100 g body weight, when measured on lactation d 9 or 20 (Table 3). Likewise, the various indices of lactation capacity in the dams did not appear to be affected by DDE exposure (Table 4). Milk yield was somewhat less than that reported by Moon (1969) for Holtzman rats nursing 12 14-d-old pups via 6 abdominal–inguinal glands; however, the values in Table 4 were comparable, on a per gland basis, to those reported by Kumaresan et al. (1967) for Sprague-Dawley-Rolfsmeyer rats nursing litters of 12 pups. The RNA and DNA content of mammary gland was similar to

previous reports (Tucker, 1966; Thibodeau and Thayer, 1967), particularly with respect to the increase in RNA, which has been shown to peak around d 19–20 postpartum (Thibodeau and Thayer, 1967). The lactose and protein concentrations in milk were also similar to previous reports of rat milk composition (Dymsza et al., 1964; Bussman et al., 1984); however, the total lipid levels were lower than in these reports. This discrepancy is probably due to the effects of repeated freeze-thawing of the milk samples (since total lipid was the last analysis performed), which may have caused an undetected separation of milk fat and a disproportionate removal during previous aliquoting. However, subsequent experiments have shown that this loss of lipid is a reproducible and predictable phenomenon that would probably occur to a similar extent in both DDE-treated and control milk samples. Therefore, it is likely that the relative total lipid measurements still constitute a valid comparison between DDE and control groups.

No gross lesions were observed during necropsy of the dams. However, histomorphological evaluation of the livers of DDE-treated dams revealed a variety of hepatocellular changes, including swelling, inflammation, increased eosinophilic appearance, increased mitoses, and, occasionally, focal necrosis and hemorrhage. The hepatic lesions were mild and not considered to be life-threatening. Treatment-related lesions were not observed in other organs of dams or pups.

DISCUSSION

The observation of Rogan and Gladen (1982) that DDE levels in human breast milk samples were inversely correlated with the duration of breast feeding was suggestive of a deleterious effect of DDE, either on the lactation process or on the neonate, which culminated in an early termination of nursing. Although the intake of DDT by the general population has been quite low in recent years (Kimbrough, 1982; Spindler, 1983), tissue depots of the lipophilic metabolite, DDE, apparently persist for a considerable length of time. These depots may be supplemented by a low level of preformed DDE present in the diet (Szokolay et al., 1977; Spindler, 1983). During lactation, adipose lipids are mobilized for the production of milk fat, which induces the comigration of DDE from adipose stores through the general circulation and ultimately into the mammary gland. Consequently, the liberation of DDE from relatively innocuous depots may perturb the delicate processes of lactation or induce neonatal toxicity when imparted via the milk.

In order to test this hypothesis, rats were administered DDE at a dosage and duration that produced concentrations of DDE in milk approximately 100 times greater than the highest levels measured in human samples collected by Rogan and Gladen (1982). Under these exposure conditions, no impairment of lactation was detected when

assessed on lactation d 9 and 20. The growth of litters was nearly identical for the DDE-treated and control groups, which indicated that the quality and quantity of milk from DDE dams was sufficient for normal growth of pups. However, litter weight gain is not always a good indicator of milk yield (Kumaresan et al., 1967). Hence, the similarity in the milk production estimates provides an important confirmation of the lack of effect of DDE on milk yield. Likewise, DDE treatment did not alter the composition of milk with respect to lactose, total protein, and total lipid content.

Another well-recognized component of the lactational process is the increase in the size of the mammary gland, which, in the rat, begins early in pregnancy and peaks at about d 10–12 postpartum. This growth (i.e., increase in cellularity) is reflected by the mammary-gland DNA content (Tucker and Reece, 1963a,b). In addition to a hyperplastic change, a pronounced increase in mammary-gland RNA content occurs (Thibodeau and Thayer, 1967; Tucker and Reece, 1963a,b), which is indicative of a hypertrophic response (i.e., increased protein synthesis activity). Neither the DNA or RNA content of the mammary glands, nor mammary-gland weight, was affected by DDE treatment, which further attests to the lack of effect of DDE on the lactation capacity. Histomorphological evaluation also did not reveal any effect of DDE on mammary-gland structure.

The data of Rogan and Gladen (1982) suggested that DDE exposure might induce an early termination of lactation or nursing. Therefore, in the present study, measurements of lactation capacity were performed on both d 9 and 20 of lactation, the latter timepoint being close to the time of spontaneous weaning. No evidence for a premature suppression of lactation or an early onset of weaning (e.g., mammary involution) was obtained, however.

The mortality of pups from DDE-treated dams was slightly higher than control pups. These findings are similar to the results of a study by Del Pup et al. (1978), in which exposure of stable populations of mice to 100 ppm DDT in the diet produced a decrease in the survival of neonates to weaning followed by an increase in postweaning survival. This effect was attributed to the early death of DDT-susceptible neonates and an improved survival of DDT-resistant animals compared to the control population. However, the increase in neonatal mortality observed by Del Pup et al. (1978) occurred between lactation d 4 and 30, whereas in the present study, most pup deaths occurred prior to lactation d 3 (data not shown). In fact, mortality after lactation d 3 was greater for controls (1.0%) than the DDE group (0.4%). Therefore, it is unlikely that the early deaths observed in the present study were due to DDE toxicity, particularly since transplacental transfer of DDE to the fetus is considerably less than the transfer via the milk to the neonate (Wooley and Talens, 1971), such that the body burden of DDE would be larger in older neonates than in newborn rats.

The present investigation provides convincing evidence that DDE does not adversely affect lactation, at least in Sprague-Dawley rats. Thus, the findings do not corroborate the epidemiological data of Rogan and Gladen (1982). It is possible that impairment of lactation in humans is caused by another environmental contaminant, perhaps DDT itself or a minor isomer or metabolite, exposure to which is coincidental with the presence of DDE in breast milk samples. However, retention of DDE in humans is much greater than DDT itself or any other DDT metabolite (Morgan and Roan, 1971), such that it is the major species present at the time of lactation and, therefore, the most likely candidate to perturb this process or the neonate. Since conversion of DDT to DDE is low in humans (Morgan and Roan, 1971), the predominance of DDE in both adipose and milk samples (Wyllie et al., 1972; Barquet et al., 1981; Spindler, 1983) may be, in part, attributable to ingestion of preformed DDE in the diet, as suggested by Morgan and Roan (1971). Considering the negative findings obtained with DDE in the present study, however, a similar study with *p,p'*-DDT or a technical grade mixture may be warranted. Unfortunately, the rat is not a good model for simulating human disposition of DDT, particularly with respect to metabolism. Rats metabolize DDT primarily to water-soluble products and accumulate more DDT than DDE in adipose stores (Mitjavila et al., 1981b). Of the DDE that is formed, its excretion is at least 12 times faster than in humans [compare Morgan and Roan, (1971) to Datta and Nelson (1968)]. In the study by Wooley and Talens (1971) in which DDT was administered in the diet to female rats during gestation and lactation, the milk concentration of DDT was 10 times greater than the DDE concentration, which is approximately the converse of the relative presence of these 2 species in most human milk samples (except in countries where DDT is still actively sprayed). Consequently, high dosage levels of DDT must be administered to produce DDE concentrations in milk as high as those achieved in the present study, which may elicit systemic toxicity in the dams, and thus confound interpretation of the results. Therefore, the DDE exposure conditions employed in the present study have considerable relevance to the human situation, and the lack of any adverse effects on the parameters examined suggests that DDE does not pose a threat to the lactational process.

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