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Prenatal Exposure to Low Doses of the Estrogenic Chemicals Diethylstilbestrol and o,p′-DDT Alters Aggressive Behavior of Male and Female House Mice

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PALANZA, P., S. PARMIGIANI, H. LIU AND F. S. VOM SAAL. *Prenatal exposure to low doses of the estrogenic chemicals diethylstilbestrol and o,p′-DDT alters aggressive behavior of house mice.* PHARMACOL BIOCHEM BEHAV **64**(4) 665–672, 1999.—Exposure to estrogenic chemicals during critical periods in fetal life can alter the development of reproductive organs, the neuroendocrine system, and subsequent behavior. We examined the effects of prenatal exposure to the estrogenic chemicals, o,p′-DDT (the estrogenic contaminant in commercial DDT) and the drug diethystilbestrol (DES), as a positive control, on different forms of aggressive behavior in both male and female house mice. We also examined effects of these chemicals on male reproductive organs. From gestation days 11–17 female mice were fed an average concentration (dissolved in oil) 0.018 and 0.18 ng/g body weight of DES. Doses of o,p′-DDT were 18 and 180 ng/g body weight, based on the prediction that the in vivo potency of o,p′-DDT would be approximately 1000-times lower than DES. We found that prenatal exposure to DES increased the frequency of both males and females that responded aggressively to a same-sex conspecific. Preputial glands in males exposed to the 0.018 ng/g dose of DES were significantly enlarged relative to controls. Males exposed to the 18 ng/g dose of DDT had smaller testes than controls. The possible implications of perturbing the development of social behaviors, such as aggression, on individuals reproductive success and social structure of the population are discussed. © 1999 Elsevier Science Inc.

Endocrine disruptors DES DDT Environmental estrogens Aggression Reproductive organs House mouse

DURING fetal life, sex steroids, such as estradiol, have marked effects on the development of reproductive organs, the neuroendocrine system, and subsequent behavior (36). A variety of man-made chemicals that are being released into the environments, referred to as endocrine disruptors, are able to alter development of the brain and reproductive organs in animals. Some endocrine disruptors act by binding to estrogen receptors in estrogen responsive cells, and disruption of cell differentiation and, thus, the course of development can occur as a result of the chemical acting as an estrogen agonist or an antagonist (9,37).

Across a wide variety of vertebrate species, including humans, estrogen and other steroid hormones influence sociosexual behaviors in males and females, and the underlying mechanisms of action are similar across species (23). For example, sex steroids play a critical role in regulating the development of the neural areas mediating aggression, as well as the expression of aggression in adulthood, in species that have the genetic predisposition for aggressiveness. Behavior may be particulary sensitive to perturbation of hormonal systems by endocrine disrupting chemicals, because behavior represents the end point of integrated systems, and even subtle alterations in any of the component systems are likely to be reflected in the disruption of behavior. Importantly, disturbances in sociosexual behaviors are no less likely to be of biological significance in humans than in any other animal. Im-

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paired responsiveness to environmental demands could result in reduced social adaptability. We refer to the study of exposure to environmental chemicals on behavioral interactions within the species and between animals and their environments, as ethotoxicology (27).

We use as our model animal for these studies an outbred stock of Swiss house mice (*Mus domesticus*). Previous research has shown that, relative to many other laboratory stocks of mice, CD-1 mice are very similar to wild mice in their behavior and social organization in seminatural environments (26). The house mouse is widely distributed throughout the world, and thus, has been subjected to varied ecological pressures. Although there are reports of feral populations of this species living totally apart from humans, most commonly house mice live associated with human communities as commensals of humans, thus being exposed to many of the same environmental factors, including pollutants, as humans (4,7).

In the studies described here, we examined the effects on the subsequent sociosexual behaviors of both male and female mice of exposure during fetal life to two estrogenic chemicals, o,p′-DDT and diethystilbestrol (DES). The o,p′-isomer of DDT is a contaminant (11–29%) found in DDT (dichlorodiphenyl trichloroethane). o,p′-DDT appears to be the primary estrogenic component of technical-grade DDT, although it is not highly persistent, p,p′-DDE is the highly persistent in vivo metabolite found in animal (including human) tissues (18) that acts primarily as an androgen receptor antagonist (16) but shows little estrogenic activity. Although use of DDT in many developed countries was discontinued in the 1970s, it is still widely used in developing countries, and is being transported via the atmosphere around the world. DES is a potent synthetic estrogen that was examined as a positive control for estrogenic effects of o,p′-DDT.

In male rats, exposure to a high dose of o,p′-DDT during early life can lead to marked impairment of fertility and reduced weight of prostate and seminal vesicles (11), as well as neurobehavioral effects, such as a change in locomotor activity associated with a change in muscarinic cholinergic receptors in the cerebral cortex (10). We previously reported that prenatal exposure to a low dose of o,p′-DDT increased the rate of territorial urine marking in male mice; a 1000 times lower dose of DES produced the same effect (37). Because urine marking is correlated with dominance status, in the present studies we examined the hypothesis that prenatal exposure to o,p′-DDT or DES would increase intermale aggressive behavior in males and possibly also interfemale aggression in female mice.

We also examined the behavior of male mice toward unrelated mouse pups. Developmental exposure to supplemental testosterone has opposite effects on aggression toward adult males (which is increased) and unrelated infants (infanticide), which is decreased (32). This finding raised the possibility that developmental exposure to estrogenic chemicals might also result in opposite effects on aggression toward adults vs. aggression toward unrelated infants.

GENERAL METHOD

Animals, Husbandry, and Mating Procedures

CD-1 mice (*Mus domesticus*) used in this experiment were born and reared in laboratories at the University of Parma A breeding stock of males and females was originally purchased from Charles River Laboratories (Curno, Italy). Animals were housed in standard polypropylene mouse cages on sawdust bedding with food (MIL) and water available ad lib. The light:dark cycle was 12 h light and 12 h dark, with lights on at 1000 h. Room temperature was 23 ± 2°C. Adult (3–4-month-old) females were time mated by being placed into the cage of a stud male for 4 h beginning at 0800 h. When a vaginal plug was found (day 0 of pregnancy), females were housed three per cage (40 × 25 × 15 cm).

Maternal Treatment

DES and o,p′-DDT (Sigma) were dissolved in tocopherol-stripped corn oil (Cat# 901415, ICN, Aurora, OH). With the exception of a group of females that were left undisturbed (unhandled group), each pregnant female received daily administration of 30 μl of corn oil (with or without a chemical) from day 11 and day 17 of pregnancy. Two doses of DES and DDT were administered: DES 0.001 and 0.01 μg/30 μl; o,p′-DDT 1 and 10 μg/30 μl.

An electronic micropipetter (Rainin) enabled delivery of an accurate volume of corn oil into the mouth of an animal. Mice were picked up by the skin between the shoulders and held upright while the pipette tip was placed into the mouth, with the pipette tip gently touching the roof of the mouth. Mice readily consume corn oil. This procedure does not appear to result in significant stress to the animals, and thus avoids the stress associated with the more invasive procedure of gavage. Maternal stress can alter the course of fetal development and appears to interact with the effects of estrogens on development (38). The last treatment was on day 17 to reduce the possibility that the higher doses of DES and pesticides would interfere with parturition. On day 17 of pregnancy, females were individually housed and left undisturbed until delivery.

Mean maternal body weights ranged from 44 g on gestation day 11 to 62 g on gestation day 17. Treatment doses of DES and DDT are presented as average maternal doses (in ng/g body weight/day) throughout the 7 days of treatment, based on an average body weight of 53 g and administration of a fixed dose per 30 μl corn oil to the pregnant females.

On day 11 of pregnancy, females were randomly assigned to one of six groups (n = 15 females). Group 1 = unhandled controls: females were left undisturbed; group 2 = vehicle controls: females were administered corn oil alone; groups 3–4 = two DES dose: 0.018 ng/g and 0.18 ng/g body weight; groups 5–6 ± two o,p′DDT doses: 18 ng/g and 180 ng/g body weight.

Within 12 h after delivery litters were culled to eight pups—three to five males and three to five females. Nursing females and their litters were left undisturbed until weaning, when the offspring were housed in same-sex sibling groups. As our intention was not to isolate pre- vs. postnatal periods as effects of these chemicals, but to determine whether maternal ingestion only during pregnancy would have any effect on offspring, we decided not to use a crossfostering procedure, which per se may also exert an effect on maturation and subsequent behavior [e.g., (19)].

As adults (60–90 days of age), males and females underwent behavioral tests as described below. For males, the first test conducted at about 3 months of age was for behavior toward pups, followed by the test for intermale aggression about 1 week later. At six months of age males were killed by CO_2 asphyxiation followed by cervical dislocation, and testes and preputial glands were removed and weighed. Unless specifically indicated, only one male and one female from each litter used for each test to control for possible litter effects.

Data Analysis

Frequency data were analyzed by Fisher exact probability test, and ratio data were analyzed by ANOVA using SAS (GLM procedure). The effect of maternal treatment was examined separately for DES and DDT relative to controls, because there was a different dose range for DES from that used for DDT, thus precluding an overall ANOVA for dose. Planned comparisons were made using the LS means test. The null hypothesis was rejected at $p < 0.05$.

Ethical Considerations

The experiments were performed in accordance to the requirements of the Italian Act for The Care and Use of Laboratory Animals (No. 116, 1992) based on the European Communities Council Directive 86/609 as well as ASAB guidelines governing animal behavior research. Taking into account the needs of the studies, care was taken to minimize any stress or suffering imposed on animals (both adults and infants) by stopping the tests as soon as an animal was injured.

EXPERIMENTAL METHODS AND RESULTS

Comparison of Control Groups

Prior to comparing animals from the different treatment groups, unhandled and vehicle-exposed control males were compared. These two groups did not differ significantly on any behavioral or morphological measure, and were combined into one control group for comparison with animals treated with chemical estrogens.

Behavior of Males Towards Unrelated Young

Competitive strategies in mice are not limited to direct interactions with adult conspecifics, but also include interactions with offspring produced by other conspecifics. Infanticide is an adaptive competitive strategy in the house mouse. Infanticide by adult males can increase mating opportunities in a species in which lactation inhibits ovulation (14,28,35). In this experiment we examined the behavior of male mice toward unrelated newborn pups.

Methods. At 3 months of age, two males were randomly selected from each litter and housed individually in Plexiglas cages (23 × 18 × 14 cm), and 24 h later the males were tested for their behavior toward a single 2-day-old pup (n = 24–28/group). This test consisted of placing one pup into a corner of each animal's cage with a minimum of disturbance. The animals were observed for a maximum of 20 min. If the pup was attacked, the test was immediately terminated, and the pup was removed from the cage and killed painlessly by CO_2 asphyxiation. These males were labeled as infanticidal, because an attack on a pup will always end in death of the pup. If the test animal responded to the pup by retrieving it to the nest and then grooming and hovering over the pup, the animal was labeled as parental. If the pup was not attacked or retrieved to the nest within the 20-min test period, the test animal was recorded as having ignored the pup. In both of these latter cases, uninjured pups were returned to their mothers.

Results. Sixty percent of control males exhibited infanticide, while 5.8% behaved parentally towards the pup. Prenatal treatment did not influence males' responses towards pups. However, although not significant, DES 0.18 ng/g and DDT 180 ng/g exposed males tended to show a lower frequency of infanticide ($p = 0.12$, Fisher exact probability test).

Male Territorial Aggression

Male mice compete among themselves for establishing and holding a territory and achieving dominance. Because reproduction is largely confined to dominant, territorial males, a male's capacity to defeat male conspecifics intruding into his territory plays a crucial role in determining his reproductive fitness. Male intrasex aggression is also thought to play an important role in spacing conspecifics, thus resulting in the regulation of the density of animals according to ecological conditions (6). The behavior of rodents shown in a resident–intruder paradigm mimics territorial intermale aggression, and conforms to what is believed to happen in the field (6). Therefore, in this experiment we examined whether fetal exposure to estrogenic chemicals influenced intermale aggressive behavior measured during resident–intruder encounters as an indicator or territorial aggression.

Methods. At 3 months of age one male from each litter (not used in the infanticide study) was selected to be tested for aggression (n = 12–14/group). These males were individually housed for 7 days in Plexiglas cages (40 × 20 × 20 cm) to have this become the established home territory of the resident experimental male. One day before testing for aggression, the bedding in the cage was changed.

An untreated sexually naive male, matched for age and weight with the resident test animal, was introduced into the cage for 10 min. The following were recorded: 1) number of males attacking an intruder (i.e., delivering at least one bite to the opponent); 2) latency to attack (i.e., time interval from the first contact to the first attack in second; 3) number of attacks; 4) total time spent attacking the intruder (in seconds); 5) social investigation (in seconds); 6) tail rattling (a behavior typically seen prior to an attack); 7) self-grooming (in seconds); and 8) defense (upright submissive posture, immobility or freezing behavior).

The first attack was scored when the resident male attempted to bite the intruder. In only a few cases the resident did not attack first and, instead, was attacked by the intruder. Attacks also consisted of chasing and circling, in addition to biting, and the time (in seconds) spent exhibiting these behaviors was included in the total attack time.

Results. Figure 1 shows the proportion of resident males attacking an intruder within the 10-min test period. A significantly higher proportion of males exposed to each dose of DES attacked a conspecific intruder relative to control males ($p < 0.05$ for the 0.018 ng/g dose and $p < 0.01$ for the 0.18 ng/g dose of DES). There was a tendency for DDT 18 ng/g resident males to show a higher proportion that attacked relative to control males ($p = 0.08$).

Table 1 shows the results for aggression and other parameters recorded during the 10-min test.

There was a significant effect of maternal treatment with DES, $F2 = 3.94$, $p < 0.05$, on latency to attack. Males exposed to the 0.018 ng/g dose of DES ($p < 0.05$) and the 0.18 ng/g dose of DES ($p < 0.01$) showed a significantly shorter latency to attack the intruder than control males. Neither doses of DDT affected the latency to attack. There were no significant differences between control and prenatally treated males on intensity of aggression measures (total attack time and number of attacks), although for DES, as dose increased, intensity of aggression appeared to increase. In contrast, for DDT, intensity of attack appeared to decrease as dose increased.

We conducted a separate analysis on data for intensity of aggression measures (bite frequency, total attack, and tail rattling) for only the males that attacked the intruder during the

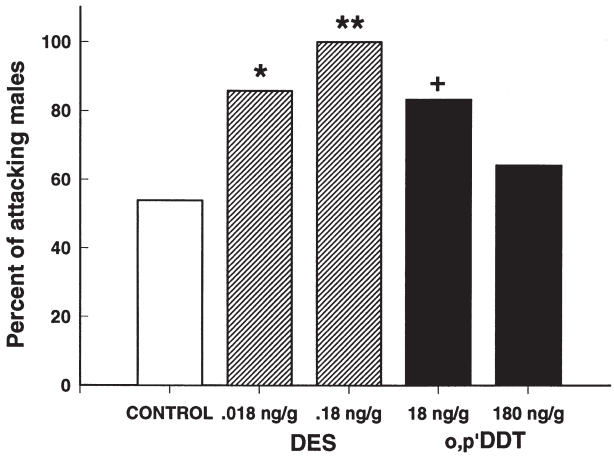

FIG. 1. Percent of individually housed resident males prenatally exposed to different doses of DES, o,p′DDT, or no chemical (combined oil exposed and unhandled controls), attacking a male intruder in a 10-min test. *$p < 0.05$; **$p < 0.01$; †$p < 0.08$, Fisher exact probability test.

10-min test (14 of 26 controls; 12 of 14 DES 0.018 ng/g; 13 of 13 DES 0.18 ng/g; 10 of 12 DDT 18 ng/g; 9 of 14 DDT 180 ng/g). Neither dose of DES had a significant effect on these measures. In contrast, males exposed to DDT showed reduced bite frequency ($F = 3.31$, $p < 0.05$), total attack time ($F = 4.61$, $p < 0.02$) and, partially, tail rattling ($F = 2.88$, $p < 0.08$). Specifically, males exposed to 18 ng/g DDT showed lower bite frequency (23.3 ± 6.2; $p \leqslant 0.05$) and total attack time (26.4 ± 5.9; $p < 0.01$) than control males (45.0 ± 8.5 and 51.1 ± 9.1, respectively); males exposed to the 180 ng/g dose of DDT also showed significant lower bite frequency (20.0 ± 6.6; $p < 0.05$), total attack time (20.5 ± 5.1; $p < 0.05$) relative to controls. In addition, 180 ng/g DDT males showed significantly less tail rattling (0.88 ± 0.45; $p < 0.05$) relative to control males (107 ± 4.4). Prenatal treatment did not significantly affect any other behavioral measures recorded.

Collection of Male Reproductive Organs

Differentiation of reproductive organs from embryonic tissues occurs in mice during the last third of gestation and continues for different organs for varying periods of time after birth. Specifically, sexual differentiation is initiated by the secretion of testosterone from the testes in male fetuses on day 12 of gestation (5). Morphological organization of the testes (the formation of the spermatogenic cord) also begins at this time, while development of the accessory reproductive organs in males begins on gestation days 15–16.

We examined the effects of prenatal exposure to estrogenic chemicals on the subsequent weight of testes and preputial gland in adulthood. These organs were examined because they both play a role in the regulation of sociosexual behaviors in mice. Testosterone is secreted by the testes, and influences male aggressive and sexual behaviors (36). Preputial glands produce pheromones involved in social communication (15).

Methods. At 6 months of age, 12 control males and 8 males from each estrogenic chemical dose group were individually housed. In this experiment we selected one male from each litter that had not been used in the prior behavioral tests. One week later the males were killed, body weights were recorded, and the testes and preputial glands were removed and weighed on a scale accurate to 0.01 mg.

Results. Neither dose of DES or DDT had a significant effect on body weight (control: 47.1 ± 0.8 g; DES 0.018 ng/g: 47.8 ± 1.4 g; DES 0.18 ng/g: 48.3 ± 2.1 g; DDT 18 ng/g: 44.9 ± 2.1 g; DDT 180 ng/g: 47.4 ± 1.2 g). Based on ANCOVA, body weight did not account for a significant component of the variance in the weight of the testes or preputial glands. The effects of prenatal exposure to DES or DDT on the preputial glands vescicles and testes were thus compared by ANOVA.

Figure 2 shows that prenatal treatment with DES ($F = 3.40$, $p < 0.05$) but not with DDT influenced the weight of preputial glands in males. Specifically, males exposed to the 0.018 ng/g dose of DES tended to have larger preputial glands relative to control males ($p < 0.06$).

Neither dose of DES significantly affected testes weight (Fig. 3). In contrast, testes weight was influenced by prenatal exposition to DDT ($F = 4.86$, $p < 0.02$): males exposed to a 18 ng/g, but not 180 ng/g, dose of DDT had smaller testes than controls ($p < 0.05$).

Female Intrasexual Aggression

Based on studies of some domesticated stocks of mice, females that were not lactating had been considered to be nonaggressive towards conspecifics. However, it is now clear that wild female mice exhibit aggression in a variety of situations. Aggression by females can play an important role in the regulation of reproductive potential and population dynamics of house mice social units (25,34,40). Female mice become ag-

TABLE 1

AGGRESSION BY INDIVIDUALLY HOUSED MALES PRENATALLY EXPOSED TO DIFFERENT DOSES OF DES, o,p'DDT, OR NO CHEMICAL (COMBINED OIL AND UNHANDLED CONTROLS) CONFRONTING MALE INTRUDERS IN A 10-MIN TEST

Prenatal Treatment	Latency to Attack (s)	Number of Bites	Total Attack Time (s)	Tail Rattling (s)	Social Behavior (s)	Defensive Behavior (s)
Control	360 ± 48.4	24.2 ± 6.3	27.5 ± 7	5.8 ± 2	84.8 ± 12.2	1.7 ± 1
DES 0.018 ng/g	216.8 ± 40.8*	28.3 ± 8.5	37 ± 8.8	8.5 ± 2.7	92.8 ± 14.6	2 ± 1.3
DES 0.18 ng/g	160 ± 34.8†	39.3 ± 8.8	38 ± 7.4	9.3 ± 2.9	53.8 ± 7	0.3 ± 0.1
DDT 18 ng/g	268 ± 63.7	19.4 ± 5.7	22 ± 5.7	5 ± 2.2	77.5 ± 15.2	0.5 ± 0.3
DDT 180 ng/g	376.8 ± 56	12.8 ± 4.9	13.2 ± 4.2	0.5 ± 0.3	93.2 ± 10.8	5 ± 4.7

Mean and SEM are given.
*$p < 0.05$; † $p < 0.01$ vs. control.

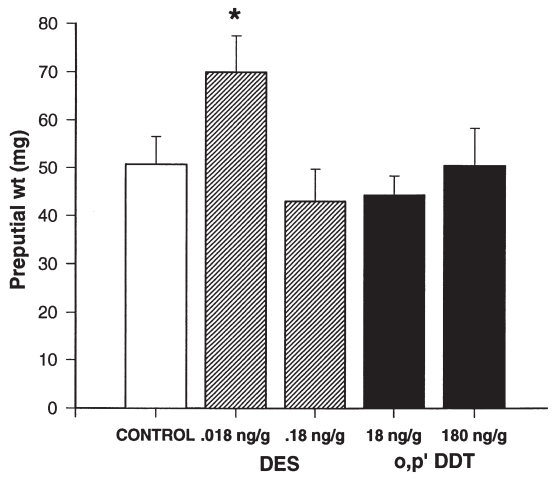

FIG. 2. Weight of paired preputial glands (in mg) in adult male mice produced by females fed DES, o,p′DDT, or no chemical (combined oil exposed and unhandled controls) from gestation days 11–17. *$p <$ 0.06 vs. control.

gressive towards other females after short periods of cohabitation with a male or in response to male urinary cues, suggesting that interfemale aggression is related to sexual competition (24,26).

This preliminary study was conducted to examine the possibility that exposure during fetal life to the 0.18 ng/g dose of DES might influence female aggressive behavior towards another female. Females exposed prenatally to this dose of DES were examined, because aggression in males was increased by this treatment. Based on our previous findings, the test for aggression was conducted after exposure of the experimental females to male olfactory cues.

Methods. We examined females exposed to the 0.18 ng/g body weight/day dose of DES (n = 13) and control females (n = 12) selected equally from both oil-treated and untreated litters. Only one female within a litter was randomly selected and individually housed for 24 h in a cage previously inhabited by a control male for 48 h; the soiled bedding from the male remained in the cage. An untreated virgin female matched for weight was introduced into this cage for 20 min. An attack was considered to have occurred if the resident experimental female attacked and attempted to bite the intruder, which often occurred while the resident was chasing the intruder. Rough grooming (which often preceeds an attack) and mounting attempts were not scored as attacks, but these behaviors were recorded.

The following variables were recorded: 1) proportion of intruders attacked; 2) latency to attack; 3) number of attacks; 4) social investigation; 5) frequency of mounting behavior and rough grooming.

Results. Figure 4 shows that a significantly higher proportion of females exposed in utero to the 0.18 ng/g dose of DES exhibited biting attacks toward same-sex intruders relative to control females ($p < 0.05$). Because so few control females (2 of 10) exhibited attacks, all of the measures of intensity of aggression differed significantly ($p < 0.01$) when the data for all females tested were analyzed (including zeros for the nonattacking females). However, frequency of rough grooming and mounting behavior (control: 1.8 ± 0.8 vs DES: 3.2 ± 1.1) and social investigation (5.9 ± 0.7 vs. 7.0 ± 0.5) did not differ.

DISCUSSION

The results of this study show that exposure during fetal life to low doses of the estrogenic chemicals DES and o,p′-DDT influence adult sociosexual behaviors in male and female mice. Specifically, prenatal exposure to low, 18 or 180 part per trillion (ppt), doses of DES increased the proportion of both males and females responding aggressively to a same-sex intruder into an animal's home territory; the lower dose of o,p′ DDT, a 180 part per billion (ppb) dose, also tended to increase the proportion of males that exhibited aggression toward a male intruder. The increase in the proportion of DES-exposed animals that responded aggressively to a same-sex intruder was not related to an increase in intensity of attack (total number or duration of attacks), but rather to a reduced time interval from first contact with the conspecific intruder to the onset of attack (latency to attack). This suggests that animals exposed in utero to this estrogenic compound may differ in their reactivity to aggression-inducing stimuli.

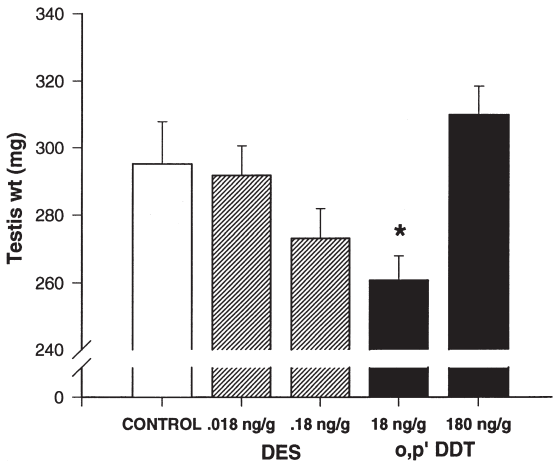

FIG. 3. Weight of paired testes (in mg) in adult male mice produced by females fed DES, o,p′DDT, or no chemical (combined oil exposed and unhandled controls) from gestation day 11. *$p < 0.05$ vs. control.

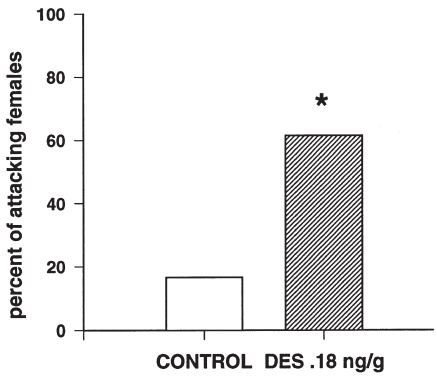

FIG. 4. Percent of attack on a female intruder by virgin females prenatally exposed to DES or no chemical (combined oil exposed and unhandled controls) isolated in cages with male-soiled sawdust. *$p <$ 0.05, Fisher exact probability test.

Males exposed to the 0.018 ng/g dose of DES also had larger preputial glands than did control males. This organ plays a role in social interactions, and it is likely that the change in organ weight reflects changes in preputial gland function. In mice, preputial gland pheromones are involved in social communication between males and females (8), and preputial gland secretions influence aggressiveness between males (15,22). Preputial glands secretions pass through ducts that empty into the prepuce, which is especially adapted in mice for depositing urine marks (21). The placing of these pheromones into a male mouse's environment is thus via urine-marking behavior, which is influenced by dominance status; dominant males mark at high rates, and subordination inhibits this behavior (7). We previously showed that the rate of depositing urine marks in a novel environment was increased by prenatal exposure to the same doses of DES and o,p′-DDT used in the present study (37). Based on our current findings regarding the decrease in latency to attack an intruder in prenatally treated males, it is also possible that this previously observed increase in urine marking behavior reflects an increased reactivity to novel environments, in addition to being an index of heightened territoriality. Taken together, our findings suggest that exposure to a low doses of man-made estrogenic chemicals during fetal life in mice can increase the propensity of males to attack a same-sex conspecific and change the functioning of the preputial glands that produce pheromonal signals deposited into the urine as well as increase the rate of urine marking.

A slightly different set of effects on behavioral and organ development resulted from exposure to o,p′-DDT than occurred with DES. Although the lowest DDT dose examined (18 ng/g) tended to increase the proportion of males that attacked a male intruder, analysis of the behavior displayed by aggressive males (utilizing data only from those males that attacked the intruder) revealed that DDT-exposed males showed a lower intensity of attack than controls. Prenatal exposure to this low dose of o,p′-DDT thus appeared to result in a quantitative change in the aggressive behavior of males by reducing the intensity of attack (i.e., number of attacks and time spent in agonistic behaviors). Because there is a correlation between intensity of aggression and social status (6,29) these animals may be less effective at achieving and/or maintaining dominance. However, whether the difference between control and DDT-exposed individuals is only quantitative will require further investigation.

The present finding that males exposed to the lower o,p′-DDT dose had smaller testes suggests the possibility that exposure to o,p′-DDT during fetal life may have impaired normal testicular function, resulting in lower levels of circulating testosterone. This, in turn, could have also affected the intensity of attack. This remains to be examined as do possible effects on testicular sperm production. Fetal exposure to a low (20 ng/g body weight/day) dose of bisphenol A (an estrogenic chemical used to make polycarbonate plastic) during fetal life (using the same procedures reported here) resulted in a decrease in daily sperm production in adulthood (33).

Based on the present findings, it is clear that not all effects of prenatal exposure to DES or o,p′DDT (aggression, testes, and preputial gland size) follow the same dose–response curves. It is well recognized in toxicology that different organs, and different systems, respond to different doses of hormones. So, to get an effect on behavior at a lower dose than on an organ, or to get an effect at one dose and not another, is, in fact, becoming common for endocrine disrupting chemicals [e.g., (33,37,39)]. Why some doses are active and others appear to not cause the same effect, requires further study. Also, testis weight is a crude measure of organ functionality; Hess and coworkers (13) showed that testes weight can increase while sperm count decreases due to interference with epididymidal resorption of fluid from seminiferous tubules, leading to back pressure and swelling of testes. The system is complicated, and complex combinations of effects are seen at different doses; dose–response curves are not monotonic as once thought, so it is not simply a matter of more chemical leading to a greater response.

Males exposed to the 0.18 ng/g dose of DES and to the 180 ng/g dose of o,p′-DDT tended to exhibit lower rates of infanticide than control males. It is known that perinatal testosterone has opposite effects on adult intermale aggression and infanticide in male CF-1 mice (32). Our finding here suggests that during fetal life, estrogenic chemicals may also have opposite effects on intermale aggression and infanticide. However, it appears that the effects of estrogenic chemicals on intermale aggression may occur at lower doses than do effects on behavior toward pups.

In the house mouse competition for resources and mates, and ultimately for reproductive opportunities, is often intense. Male mice compete to establish and hold a territory and to establish high social rank to mate with females. Because reproduction is largely confined to dominant or highly territorial males, intermale aggression is a primary determinant of reproductive success in this species (7). Reproductive competition in female mice has been observed in several studies (12,20,25,31). Females can be exclusively territorial (aggressively excluding other females) or form a dominance hierarchy that determines reproductive success (12,25). In social species such as the house mouse, intrasex aggression regulates the density of animals, leading to an appropriate spacing in relation to socio-ecological conditions. Thus, our finding that the low (0.18 ng/g) dose of DES significantly increased the proportion of female mice that attacked a same-sex intruder into her territory suggests that exposure to estrogenic chemicals during fetal life could influence population dynamics by changing the sociosexual behaviors of females as well as males.

It is generally accepted that natural selection operates on developmental processes such that fitness is maximized; that is, animals have evolved an optimum phenotype for the environment that they inhabit. Perturbation of systems that differentiate under endocrine control will result in disruption of the normal course of development, and the consequence will be that the fitness of affected individuals may be reduced. There are many factors that give rise to individual differences in social behaviors, such as aggressiveness. Consequently, there is an evolved range of social behaviors that occurs among animals within any population due to variation in genotype, hormone levels, experience, etc. In natural populations of mice, aggression seems to have undergone diversifying selection in which both extremes on an aggression scale are favored, i.e., both high aggressive (fast attackers) and nonaggressive (or slow attackers) males are abundant (30). Aggressive and nonaggressive males not only differ in their response to social interaction, but may also differ more fundamentally in their general relation with the environment and in the way they respond to threatening situations, i.e., in their coping strategy (1–3). Aggressive males appear to be more successful under stable conditions (e.g., within a family group or deme), whereas nonaggressive males function better under changing conditions (i.e., migratory circumstances) (29,31).

This range of phenotypic variation of a particular species is adapted to specific environmental (ecological) conditions

(17). If exposure to endocrine disruptors changes that phenotype, leading to a different (not selected) range of traits, such as an altered distribution of aggressive animals or functioning of the testes and preputial glands, for that environment, an impact on the individuals in the population is likely to occur, and changes in population dynamics could follow. Alteration of intraspecific competitive strategies and the modification in sociosexual behaviors may disrupt the genetically based individual variation in coping strategies, thus resulting in disruption of social systems, with profound impact on the behavioral ecology of the species. Effects due to developmental exposure to endocrine disruptors that are detectible at the population level, which have been described in wildlife (9), are thus of particular concern. Population effects need to be considered when assessing the potential for man-made chemicals to adversely affect development. The broad approach used in ethotoxicology provides information that is relevant with regard to effects of chemicals on individuals, and also provides insights regarding potential consequences for population dynamics.

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