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 end o,p'-DDT alters aggressive behavior of house mice. PHARMA COL. BIOCHEM. BEHAV. 46(1): 605-612, 1996. — 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 (a estrogenic contaminant in commercial DDT) and the drug diethylstilbestrol (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 0.01% and 0.15% body weight of DES. Doses of o,p'-DDT were 14 and 140 nglg body weight, based on the preclinical data that the in vivo potency of o,p'-DDT would be approximately 100-times lower than DES. We found that prenatal exposure to DES increased the frequency of both male and females that responded aggressively to a same-sex conspecific. Prepubertal gands in males exposed to the 0.01% dose of DES were significantly enlarged relative to controls. Males exposed to the 18 ng 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. © 1996 Elsevier Science Inc.

<table>
<thead>
<tr>
<th>Endocrine disruptors</th>
<th>DES</th>
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<td>House mouse</td>
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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 environment, 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 particularly 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 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 expo-
sure to environmental chemicals on behavioral interactions
within the species and between animals and their environ-
ments, as ethology (27).

We use as our model animal for these studies an outbred
stock of Swiss house mice (Mus domesticus). Previous re-
search has shown that, relative to many other laboratory
stocks of mice, CD-1 mice are similar to wild mice in
their behavior and social organization in seminatural environ-
ments (26). The house mouse is widely distributed through-
out the world, and thus, has been subjected to variable 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 civilizations as com-
ments 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 social and behavioral development of both male and fe-
male mice of exposure during fetal life to two estrogenic
chemicals, o-β-DT and diethylstilbestrol (DES). The o-β-
Dimer of DD is a CONTAMINANT (11-29%) found in DD
(dichlorophenyl trifluoroethane). o-β-DT appears to be
the primary estrogenic component of technical-grade DD,
although it is not highly persistent, o-β-DT is the highly
persistent in vivo metabolite found in animal (including hu-
man) tissues (18) that acts primarily as an androgen receptor
agonist (48) but shows little estrogenic activity. Although
use of DD 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-β-DT.

Male rats, exposed to a high dose of o-β-DT during early
life can lead to marked impairment of fertility and re-
duced weight of prostate and seminal vesicles (11), as well as
neurobehavioral effects, such as a change in locomotor activ-
ity associated with a change in neurexin chemoreceptors
in the cerebellum cortex (10). We previously reported that
proenzylo conversion to a low dose of o-β-DT increased the
ratio of immunoreactive synapses 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 ex-
poure to o-β-DT or DES would increase intermale aggres-
sive behavior in males and possibly also interfere aggres-
sion in female mice.

We also examined the behavior of male mice toward unre-
lated mouse pups. Developmental exposure to supplemental
testosterone has opposite effects on aggression toward adult
males which is increased and unrelated to age (infanility),
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. ag-
ger toward unrelated infants.

GENERAL METHOD

Animals, Housing, and Making Procedures
CD-1 mice (Mus domesticus) used in this experiment were
be and reared in laboratories at the University of Parma A
breed transmission of males and females were purchased from
Charles River Laboratories (Curno, Italy). Animals were
housed in standard polystyrene mouse cages on saw-
dust 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 (16-4 month-
old) females were timed mated by being placed into caging 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
cage (40 x 25 x 15 cm).

Materials and Methods

DEs and o-β-DT (Sigma) were dissolved in tocopherol
-stripped corn oil (Carl 90415, ICN, Aurora, OH). With
the exception of a group of females that were left undisturbed
(unhandled group), each pregnant female received daily ad-
imistration 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/mu o-β-
DDT 1 and 10 µg/mu.

An electronic microprocessor (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 ap-
pear 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 deval-
opment and appears to interact with the effects of estrogen
on development (38). The last treatment was on day 17 to re-
duce the possibility that the higher doses of DES and pesti-
cides would interfere with parturition. On day 17 of preg-
nancy, females were individually housed and left undisturbed
until delivery.

Maternal body weights were gained from 44 g on gesta-
tion day 11 to 62 g on gestation day 17. Treatment doses of
DES and DDT are presented as average maternal body weight
in g per body weight/day throughout the 7 days of treatment,
basically, as 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; female were left undisturbed; group 2 = vehicle
controls: females were administered corn oil alone; groups
3-4 = two DDT doses: 0.018 ng and 0.18 ng body weight/day;
groups 5-6 = two o-β-DT doses: 18 ng and 180 ng body
weight/day.

Within 12 h after delivery litter were culled to eight pups—three to five males and three to five 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- or postnatal periods
as effects of these chemicals, but to determine whether mater-
nal care/digestion only during pregnancy would have any efe
on offspring, we decided not to use a crossfostering procedure,
which per se may also exert an effect on maturation and sub-
sequent behavior (e.g., 19).

As adults (60-90 days of age), males and females under-
went behavioral tests as described below. For males, the first
test conducted at about 3 months of age was for behavior to-
toward pups, followed by the test for intermale aggression
about 1 week later. At six months of age males were killed by
CO2 asphyxiation followed by cervical dislocation, and testes
and preputial glands were removed and weighed. For females,
specifically indicated, only one male and one female from each lit-
ter used for each test to control for possible litter effects.
ESTROGENIC PESTICIDES AND AGGRESSION

Data Analysis
Frequency data were analyzed by Fisher exact probability test, and ratio data were analyzed by ANOVA using SAS (OLM procedure). The effect of maternal treatment was ex- amined separately for DES and DDT relative to controls; be- cause there was a different dose range for DES from that used for DDT, thus precluding an overall ANOVA fit done. Planned comparisons were made using the 1.3 Levene test. The null hypothesis was rejected at $p < 0.05$.

Ethical Considerations
The experiments were performed in accordance to the re- quirements of the Italian Act for The Care and Use of Labo- ratory Animals (No. 116, 1992) based on the European Com- munities Council Directive 86/609 as well as ASAB guidelines governing animal behavior research. Taking into account the needs of the study, 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 com- bined 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 in- teractions with adult conspecifics, but also include interactions with offspring produced by other conspecifics. Infantile- cide 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 (14,28,29). In this experiment we examined the behavior of male mice to- ward unrelated newborn pups.

Methods
At 3 months of age, two males were randomly selected from each litter and housed individually in Plexiglas cages (23 X 11 X 14 cm), and 24 hours 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 male’s cage with a maximum of disturbance. The ani- mals 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 CO2 an- phixiation. 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 rearing 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 retained 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, unhandled pups were returned to their mothers.

Results Sixty percent of control males exhibited infanti- cide, while 5.8% behaved parentally towards the pup. Prena- tal treatment did not influence males’ responses towards pups. However, although not significant, DES 0.18 ng/d and DDT 180 ng/d 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 repro- duction 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 intruder aggression is also thought to play an im- portant role in spacing conspecifics, thus resulting in the regu- lation of the density of animals according to ecological condi- tions (6). The behavior of residents shown in a resident-intruder paradigm mimics territorial intermale aggression, and con- forms to what is believed to happen in the field (8). There- fore, in this experiment we examined whether female exposure to estrogenic chemicals influenced intermale aggressive be- havior measured during resident-intruder encounters as an indicator of 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 individu- ally housed for 7 days in Plexiglas cages (40 X 20 X 28 cm) to have this become the established home territory of the resi- dent experimental male. One day before testing for aggres- sion, the bedding in the cage was changed.

An unrestrained 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 seconds); 3) number of at- tacks; 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 sec- onds); and 8) defense (opponent-substrate posture, immobility or freezing behavior).

The first attack was scored when the resident male at- tempted 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 behav- iors was included in the analysis.

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

Table 1 shows the results for aggression and other parame- ters recorded during the resident-intruder tests.

There was a significant effect of maternal treatment with DES, $F_{2, 394} = 0.05$, on latency to attack. Males exposed to the 0.018 ng/dose of DES ($p < 0.05$) and the 0.18 ng/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 in 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, in- terattack interval appeared to decrease as dose increased.

We conducted a separate analysis on data for aggression measures (bite frequency, total attack, and tail rat- tling) for only the males that attacked the intruder during the
\[10 \text{-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 rattrating \((F = 2.88, p < 0.06)\). Specifically, males exposed to 18 ng DDT showed lower bite frequency \((23.3 \pm 6.2; p < 0.05)\) and total attack time \((26.4 \pm 5.9; p < 0.05)\) than control males \((45.0 \pm 8.5; p < 0.11)\), respectively; males exposed to the 180 ng/dose of DDT also showed significant lower bite frequency \((53.0 \pm 6.1; p < 0.05)\), total attack time \((20.5 \pm 5.1; p < 0.05)\) relative to controls. In addition, 180 ng DDT males showed significantly less tail rattrating \((0.88 \pm 0.45; p < 0.05)\) relative to control males \((107.4 \pm 4.4)\). 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 (15). 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 glands in adulthood. These organs were examined because they both play a role in the regulation of sociosexual behavior in mice. Testosterone is secreted by the testes, and influences male-aggressive and sexual behaviors (16). 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: \(47.1 \pm 0.6\) g; DES 0.018 ng/g: \(47.8 \pm 1.4\) g; DES 0.18 ng/g: \(48.3 \pm 2.1\) g; DDT 18 ng/g: \(44.7 \pm 1.2\) g; DDT 180 ng/g: \(47.4 \pm 1.2\) 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 weights 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 \((p < 0.05)\).

**Female Intrasexual Aggression**

Based on studies of some domesticated stocks of mice, females that were not lactating had been considered to be non-aggressive 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 mouse social units (25,34,46). Female mice become ag-

**Table 1**

**Aggression by Individually Housed Males Prenatally Exposed to Different Doses of DES, DDT, or No Chemical (Combined Oil and Unhandled Controls) Against Male Intruders in a 4-Hour Test**

<table>
<thead>
<tr>
<th>Prenatal Treatment</th>
<th>Latency to Attack (s)</th>
<th>Number of Bites</th>
<th>Total Attack Time (s)</th>
<th>Tail Rattrating (%)</th>
<th>Social Behavior</th>
<th>Definitive Behavior</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>360 ± 48.4</td>
<td>24.2 ± 6.3</td>
<td>27.5 ± 7</td>
<td>5.8 ± 2</td>
<td>84.8 ± 12.2</td>
<td>1.7 ± 1</td>
</tr>
<tr>
<td>DES 0.018 ng/g</td>
<td>216.6 ± 40.8</td>
<td>28.3 ± 8.5</td>
<td>37.2 ± 8.8</td>
<td>8.5 ± 2.7</td>
<td>92.6 ± 14.6</td>
<td>2.0 ± 1.3</td>
</tr>
<tr>
<td>DES 0.18 ng/g</td>
<td>160 ± 34.81</td>
<td>39.3 ± 8.8</td>
<td>38.7 ± 7.4</td>
<td>9.3 ± 2.9</td>
<td>53.8 ± 7</td>
<td>62.3 ± 0.1</td>
</tr>
<tr>
<td>DDT 18 ng/g</td>
<td>268 ± 63.7</td>
<td>19.4 ± 5.7</td>
<td>22 ± 5.7</td>
<td>5 ± 2.2</td>
<td>77.5 ± 15.3</td>
<td>65 ± 0.3</td>
</tr>
<tr>
<td>DDT 180 ng/g</td>
<td>378.6 ± 56</td>
<td>12.8 ± 4.9</td>
<td>13.2 ± 4.2</td>
<td>0.5 ± 0.3</td>
<td>93.2 ± 10.8</td>
<td>5 ± 4.7</td>
</tr>
</tbody>
</table>

Mean and SEM are given. *p < 0.05; **p < 0.01 vs. control.
gressiveness toward 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 (24,36).

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 toward 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 female 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 30 min. An attack was considered to have occurred if the resident experimental female attacked and attempted to bite the intruder, which often occurred when the resident was trying to groom the intruder. Rough grooming (which often preceded 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 a significantly higher proportion of females exposed in utero to the 0.18 ng/g dose of DES exhibited being attacked toward same-sex intruders relative to control females (p < 0.05). Because a few control females (2 of 10) exhibited attacks, all of the measures of intensity of aggression differed significantly (p < 0.05) when the data for all females tested were analyzed (including zero 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 sociotaxal behavior in male and female mice. Specifically, prenatal exposure to low, 18, or 180 part per billion (ppb) 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. 2.** Weight of paired prolactin glands (in mg) in adult male mice produced by females fed DES, o,p'-DDT, or no chemical (combined oil exposed and unhandled controls) prior to mating days 11-17. *p < 0.06 vs. control.

**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) prior to mating day 11. *p < 0.01 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) prior to mating with male-mated rats; *p < 0.05, Fisher exact probability test.
Males exposed to the 0.018 mg/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 addition, preputial gland phenotypes are involved in social communication between males and females (8), and preputial gland secretions influence aggressiveness between males (15,22). Preputial gland secretions pass through ducts that empty into the prepuce, which is especially adapted in mice for depositing urination marks (21). The placing of these pheromones into a male mouse's environment is thus a 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 urination marks in a novel environment is increased by prenatal exposure to the same doses of DES and oP-DTD used in the present study (37). Based on our current findings regarding the decrease in latency to attack an intruder in prenatal treated males, it is also possible that this previously observed increase in urination 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 dose 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 urination marking.

A slightly different set of effects on behavioral and organ development resulted from exposure to oP-DTD than occurred with DES. Although the lowest DDT dose examined (18.8 ng/g) tended to increase the proportion of males that attacked a male intruder, analysis of the behavior displayed by aggressive males suggested that males from these 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 oP-DTD 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 the intensity of territorial and social status (52), we hypothesize that these animals may be less effective at achieving and/or maintaining dominance. However, whatever the difference between control and DDT-exposed individuals is only quantitative will require further investigation.

The finding that males exposed to the lower oP-DTD dose had smaller testes suggests the possibility that exposure to oP-DTD 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 finding is consistent with the possibility of effects on testicular sperm production. Fetal exposure to a low (30 ng/g body weight) 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 our present findings, it is clear that not all effects of prenatal exposure to DES or oP-DTD (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 to give rise to different outcomes 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., (23,37,39)]. 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. Here and in our previous study (37) showed that testis weight can increase while sperm count decreases due to interference with epithelial, reabsorption 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 0.018 mg/dose of DES and to the 180 ng/g dose of oP-DTD tended to exhibit lower rates of intrasexual than control males. It is known that perinatal testosterone-one has opposite effects on adult intermale aggression and intrafetale in males of CF-1 mice (32). Our finding here suggests that during fetal life, estrogenic chemicals may also have opposite effects on intrasexual aggression and intrasexual. However, it appears that the effects of estrogenic chemicals on intrasexual aggression may occur at lower doses than do effects on behavior toward pups.

In the house mouse competition for resources and matings, 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, intra-terrestrial aggression is a primary determinant of reproductive success in this species (37). Reproductive competition in female mice has been observed in several studies (12,20,35,31). Females can be exclusively territorial (aggressively excluding other females) or form a dominance hierarchy that determines reproductive success (12,51). In social species such as the house mouse, intersexual aggression regulates the density of animals, leading to an appropriate spacing in relation to resources and territories. Thus, our finding that the low (0.018) mg/dose of DES significantly increased the proportion of females 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 social/sexual behaviors of females as well as males.

It is generally accepted that natural selection operates on developmental and behavioral traits that fitness is maximized; that is, animals have evolved an optimum phenotype for the environment that they inhabit. Perurbation of systems that differentiate 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, hormones, levels of exposure to environmental factors, etc. In 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 social interactions, but may also differ in the degree of 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 den), whereas nonaggressive males cope better with changing conditions (e.g., migratory circumstances (29,31)). This range of phenotypic variation of a particular species is adapted to specific environmental (ecological) conditions
REFERENCES


