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Sexually Dimorphic Behavioral Responses to Prenatal Dioxin Exposure

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**Pregnant Sprague-Dawley rats received a single oral dose of 0, 20, 60, or 180 ng/kg 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on day 8 of gestation. Each litter contributed a single male–female pair trained to press a lever to obtain food pellets under two operant behavior procedures. Initially, each lever press was reinforced. The fixed-ratio (FR) requirement was then increased every four sessions from the initial setting of 1 to values between 6 and 71. We then studied responses for 30 days under a multiple schedule combining FR 11 and another schedule requiring a pause of at least 10 sec between responses (DRL 10-sec). TCDD evoked a sexually dimorphic response pattern. Generally, TCDD-exposed males responded at lower rates than control males. In contrast, exposed females responded at higher rates than controls. Each response measure from the mult-FR DRL schedule yielded a male–female difference score. We used the differences in response rate to calculate benchmark doses based on the relative displacement from modeled zero-dose performance of the effective dose at 1% (ED_{01}) and 10% (ED_{10}), as determined by a second-order polynomial fit to the dose–effect function. For the male–female difference in FR rate of responding, the mean ED_{10} was 2.77 ng/kg with a 95% lower bound of 1.81 ng/kg. The corresponding ED_{01} was 0.27 ng/kg with a 95% lower bound of 0.18 ng/kg. For the male–female difference in DRL rate, the mean ED_{10} was 2.97 ng/kg with a 95% lower bound of 2.02 ng/kg. The corresponding ED_{01} was 0.30 ng/kg with a 95% lower bound of 0.20 ng/kg. These values fall close to, but below, current estimates of human body burdens of 13 ng/kg, based on TCDD toxic equivalents. *Key words:* behavioral toxicology, benchmark dose, neurobehavioral function, operant behavior, prenatal exposure, sexual dimorphism, TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Environ Health Perspect* 110:247–254 (2002). [Online 6 February 2002]
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2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is the prototype and most toxic member of a class of halogenated compounds, the polychlorinated dioxins (PCDDs), which are distributed widely in the environment. PCDDs are now recognized as potent developmental toxicants, provoking adverse effects in virtually every organ system studied. Public health concerns have been raised especially because of their ubiquitous presence in the environment and their retention in body tissues for extended periods. The half-life in humans of TCDD is in the range of 7–10 years. The developing organism may be particularly sensitive to TCDD exposure; some laboratory studies have reported that the fetus appeared to be 100-fold more sensitive than the adult (*1*). Because of its lipophilic properties, structural stability, and long half-life (*2*), TCDD stored in fat tissue is transferred via the placenta and maternal milk to developing offspring during gestation and lactation (*3,4*).

Disorders of sexual development are among the best-documented outcomes of prenatal exposure, and include genital abnormalities, impaired sexual performance, and reduced reproductive success. Some of these effects have occurred at levels in animals close to the human estimated background body burden of 13 ng/kg, as calculated from the sum of TCDD equivalents (TEQs) (*5*). PCDDs are believed to exert their effects through a ligand-activated transcription factor, the aryl hydrocarbon receptor (AhR). AhR is expressed in most organs and cells in the body. A crucial role for AhR in development is shown by the numerous defects observed in transgenic mice lacking it (*6,7*).

Because of TCDD's effects on gonadal and thyroid hormone function (*8,9*), which are essential elements in brain development, it is also reasonable to assume that its actions will be reflected in neurobehavioral indices. The enormous TCDD literature, however, contains surprisingly little information on this topic.

Regarding behavior, only a handful of studies are available. Schantz and Bowman (*10*) conducted a pioneering study in monkeys exposed to TCDD prenatally. Although the exposed offspring displayed retarded learning of shape reversals, they performed equivalently to controls on spatial and color reversals. The dose administered to the pregnant monkeys (0.126 ng/kg/day) would have produced a body burden of 19 ng/kg, equivalent to the lowest dose used in our own studies and close to human background levels. In a later rat study (*11*), offspring whose mothers were exposed to a total of 175 or 700 ng/kg TCDD during gestation days (GDs) 10–16 showed decreases in error scores on a radial maze, particularly in males, but the exposed animals did not differ from controls on a delayed spatial alternation task based on a T-maze. Subsequent studies from Schantz's laboratory (*12,13*) also showed a change in spatial learning and memory in exposed male offspring. Although the change facilitated responding so that exposed animals performed more efficiently, the apparent improvement appears to be an artifact of the experimental contingencies (i.e., a TCDD-induced behavioral stereotypy would account for the changes). Learning deficits were seen in both sexes on a discrimination reversal learning task.

We recently reported that female offspring of Holtzman rats that had been exposed to a single oral dose of 0, 20, 60, 180 ng/kg of TCDD on GD 18, showed dose-related changes in behavior (*14*). In that study, the rats pressed a lever under a fixed-ratio schedule of reinforcement to obtain a 30-sec opportunity to run in a running wheel. Benchmark dose analyses located the mean ED_{10} (effective dose at 10%) and BMD_{10} (benchmark dose at 10%) for two measures of performance in a range between 7 and 10 ng/kg.

Schedule-controlled operant behavior (SCOB) provides a powerful tool for examining neurobehavioral function. In this class of behavioral procedures, a relationship is defined between the behavior of a subject and its consequences in a defined environment. SCOB provides numerous procedures for the analysis of learning, performance, and memory (*15*), as well as providing the ability to tailor tasks to model complex cognitive activities in humans. Under both transitional and steady-state conditions, SCOB studies have been used extensively to detect

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and interpret outcomes of exposures to pharmacologic and toxicologic agents (*16*), including developmental exposures (*17,18*).

In the present study, we examined schedule-controlled operant performances of male and female littermates whose dams had been administered TCDD on GD 8. Although GDs 9–10 in the rat represent the onset of organogenesis and brain development unfolds later (*19*), GD 8 was chosen for a number of reasons specific to TCDD. Abbott et al. (*20*) found in mouse embryos that AhR mRNA and protein were expressed in GD 10–13 neuroepithelium, and that, as development progressed, levels in brain decreased. Also, Abbott and Probst (*21*) found that GD 10–11 mouse embryos showed the highest levels of aryl hydrocarbon nuclear translocator (ARNT) in neuroepithelial cells of the neural tube. They did not evaluate ARNT at earlier times. If the toxic effects of TCDD are closely linked to AhR binding, it is critical to determine the consequences of exposure during the period of elevated AhR expression in accord with the concept that tissue concentration at the critical window of sensitivity is a key dose metric. Hurst et al. (*22*) administered a dose of 200 ng/kg to pregnant Long Evans rats on GD 8 and found that fetal TCDD levels were maximum (39.6 pg/g) on GD 9 and then fell slowly.

In the present study, we administered a single oral dose of 0, 20, 60, or 180 ng/kg TCDD to pregnant female rats on GD 8. We studied the behaviors of both male and female offspring under two different schedule-controlled, food-reinforced operant procedures: fixed ratio (FR) and multiple FR 11, differential reinforcement of low rate (DRL) 10 sec (mult-FR 11, DRL 10 sec). Two general hypotheses were examined: TCDD would alter the operant behavior; TCDD effects would depend partially on sex.

We studied the FR initially under an incremental FR condition. The FR schedule specifies that every *n*th response is reinforced. In the incremental FR condition, the FR value was increased every 4 days in an ascending series of values ranging between 1 and 71. Such a progression allowed us to study the transition-state performances (*23,24*) that occur in response to changes in experimental conditions. Transition-state performances are of particular interest because they reflect the ability of the subject to learn, adapt, or adjust to changing environmental circumstances. The rate and form of such behavioral adjustments may indicate an adverse effect not seen under final steady-state conditions during which compensatory factors have had an opportunity to emerge. Other investigators have also demonstrated the sensitivity of transition states, particularly under FR schedules, in studies of prenatal or early developmental effects of ethanol (*25,26*), methyl and elemental mercury (*27,28*), cadmium (*29*), and lead (*27,30,31*). Our recent TCDD experiment (*14*), showed that running-wheel FR transition-state performances of rats were especially sensitive to gestational exposure to TCDD.

A transition-state procedure can also be viewed as a dynamic challenge that requires the subject to adjust to a new set of circumstances and may thereby reveal deficits or vulnerabilities not seen under steady-state conditions. Unmasking silent toxicity can be achieved using behavioral or other forms of challenges, such as pharmacologic agents (*32*) or conditions that impose stress on the subject. Such challenges have been used to reveal delayed neurotoxicity (*33*) after developmental exposures to neurotoxic agents, as well as to evaluate its mechanisms (*34*).

In a multiple schedule, two or more simple schedules of reinforcement are presented in successively alternating components, with unique stimulus conditions such as visual or auditory stimuli signaling which component is in effect. Typically, the performance of a well-trained rat in which good discriminative control has been established switches between the components so that responding in each component resembles that seen in a rat trained only under that specific schedule. A DRL component was combined with an FR component in the present study. Under a DRL schedule, a clock begins at the onset of the component and after each lever press. Only a press emitted after the specified interval (10 sec in this experiment) has elapsed is reinforced with a food pellet. If the rat responds too early, the clock is reset, and the 10-sec waiting period begins again. Under this contingency, then, lower rates of responding yield higher rates of pellet delivery. In contrast, high FR rates yield high rates of food delivery. In this experiment, we expected to see high rates of responding in the FR component and low rates in the DRL component. Performances under the DRL schedule, like those under the FR schedule, have proven sensitive to development neurotoxicants (*35*).

A multiple schedule offers several advantages. First, by combining schedules of potentially different sensitivities to the exposure agent, we increase the likelihood of measuring exposure effects. Second, interpreting the nature of the toxicity may be facilitated by comparing the results across the component schedules (*16*). The results may assist in identifying nonspecific influences because, in a sense, one component schedule acts as a baseline control for the other; performance on the two components may suggest sensory deficits (*36*); they may implicate cognitive processes involved in complex learning and memory; or they may suggest a role for a specific neurochemical involvement or other mechanisms of action (*37*).

Sex differences often emerge under SCOB contingencies. Because gonadal hormones can influence differences in responding between males and females in operant behaviors such as lever-pressing, neurotoxicants that disturb the organizational effects of these hormones on brain development could potentially produce enduring performance changes (*38*). Should developmental TCDD exposure interfere with sexual differentiation of the brain, we would expect to observe an altered pattern of sex differences in behavior.

Normal male rats, for example, tend to emit higher overall response rates than females under ratio schedules (*39*) or under schedules that differentially reinforce high rates of responding (*40*), both of which appear to elicit a food-motivated function called behavioral perseverance. Male rats, in fact, display food-motivated perseverance across several behavioral manipulations. Male rats spend more time than females holding down a lever if holding is food reinforced (*41*). Male rats are more likely than females to continue to respond using a lever that no longer produces reinforcement (*42*). Also, under ratio schedules, the performance of castrated males resembles the lower response rates more typical of control females, suggesting the influence of testosterone (*43*). Females, on the other hand, tend to respond more efficiently than males under a DRL reinforcement schedule (*41*).

Materials and Methods

Subjects: breeding and exposure. We used Sprague-Dawley rats (Harlan Sprague-Dawley, Inc., Madison, WI) as subjects. On arrival at the University of Rochester Medical Vivarium, 40 females 6 weeks of age and 20 males 12 weeks of age were housed singly in polycarbonate cages in a temperature-controlled (± 22°C) barrier facility provided with independent, filtered air and were maintained on a 12-hr light/12-hr dark cycle. Food and tap water were supplied *ad libitum*. Breeding began after 2 weeks of acclimation to the vivarium quarters. For breeding, two females were placed with one male overnight (approximately from 1600 to 0830 hr) in hanging wire cages. GD 0 was designated as the day on which sperm were detected in the vaginal smear obtained from each female at approximately 0830 hr; at that time, each dam was placed in a separate polycarbonate cage.

On the morning of GD 8, we assigned 36 pregnant dams to each of 3 treatment groups: 20, 60, 180 ng/kg TCDD, or a control group, according to a randomized block design. TCDD, 98% purity (Cambridge Isotope Laboratories, Inc, Andover, MA), suspended in corn oil, was administered by gavage in the Supertox facility in the University of Rochester Environmental Health Sciences Center. For control animals, an equivalent volume of corn oil was administered.

Animal care and welfare procedures complied with National Institutes of Health guidelines. The vivarium is certified by the Association for Assessment and Accreditation of Laboratory Animal Care. Health surveillance of all animals was conducted under the direction of the Laboratory Animal Services Shared Facility of the Environmental Health Sciences Center.

Litters. Postnatal day (PND) 0 was designated as the first day on which a new litter was discovered by 0830 hr. Gestational length, number of live offspring, and sex distribution and appearance of the offspring were assessed. We recorded pup weights on PNDs 1, 4, 8, 12, 16, and 20. On PND 4, litters were culled to 5 females and 5 males, when possible. After weaning on PND 21, offspring were housed in pairs with same-sex littermates until PND 60. After PND 60, all offspring were housed individually in standard polycarbonate cages. A total of 22 healthy, appropriately distributed litters were generated from the breeding. The number of litters in dose groups assigned to control, 20, 60, and 180 ng/kg TCDD were 5, 6, 6, and 5, respectively, except for the multiple-schedule measures, where there were 5, 5, 6, and 5, respectively. Offspring were fed *ad libitum* until PND 80, at which time a fixed amount of food was supplied daily to maintain constant body weights (males, 290–330 g; females, 235–255 g) throughout the experiment. On PND 80, we randomly selected one male and one female from each litter for the current experiment.

Apparatus. Behavioral testing was conducted in 12 matched operant chambers (Model E 10-10RF; Coulbourn Instruments, LLC, Allentown, PA) containing two levers along one wall, with one active and the other not active, which will not be considered further. The levers were centered 4 cm above the floor and 12 cm apart from one another. Reinforcers, 45-mg standard lab animal diet pellets (Noyes Precision Food Pellets; Rodent Diet, P.J. Noyes Co., Inc., Lancaster, NH), were delivered to a recessed feeder receptacle mounted between the levers 8 cm above the floor. When a pellet was delivered, both the feeder light and an audible clicker were turned on for 0.5 sec. Pressing the lever with a force of 25 N or greater closed a microswitch sensed by the computer controlling the experimental events. A house light was mounted in the center of the ceiling. The operant chambers were housed inside sound-attenuating chambers, and a fan provided ventilated air. Schedule control and data acquisition were accomplished by means of the SKED software system (State Systems, Kalamazoo, MI) run on a PDP 11/93 computer (Digital Equipment Corporation, Maynard, MA). Data were collected as intervent times with a 10-msec resolution for all responses and schedule events.

Behavioral methods. We initiated the behavioral procedures (Table 1) when rats were 90 days old. Sessions were conducted once per day, 5 days per week (Monday–Friday). For both condition 1 and condition 2, each session remained in effect for 45 min or for 50 reinforcements, whichever occurred first. A 5-sec timeout (TO) started with each pellet delivery. During TO, responses to the lever were ineffective. The TO ensured that brief overruns in responding, which can occur at the time of pellet delivery, affected neither the FR nor DRL schedule consequences, and they were excluded from analyses.

Preliminary training. During preliminary training, the rats were first exposed to a concurrent variable-time 30.5-sec schedule (VT) FR 1 schedule. Under a VT schedule, a series of intervals of different durations ends with delivery of a pellet, independent of the rat's behavior. Under this concurrent schedule, a pellet was delivered whenever the rat pressed the lever once (FR 1) or the variable interval had elapsed. A session terminated after 100 pellets were delivered. This training step was completed either after two sessions in which at least 25 reinforcers had been obtained by lever pressing or after six sessions. Training then started under a FR 1 reinforcement schedule. Each rat was trained to a criterion of two successive sessions, in each of which 50 pellets were obtained. The incremental FR procedure began after all animals met this criterion.

Condition 1: incremental fixed-ratio. Responses were reinforced according to successively larger FR values in the following sequence: 1, 6, 11, 21, 31, 41, 51, 61, and 71. A new criterion was established at the beginning of every four sessions and remained constant within the sessions.

For this procedure, the dependent variables consisted of *a*) rate of FR responding: responses per session minutes; and *b*) local response rate: responses per session minutes excluding the time to the first response in a FR run of responses.

At the end of the sequence, four FR-71 extinction sessions were conducted. During those sessions, all conditions were the same as FR-71 except that the pellets were delivered to a location behind the foodcup where the rat could not obtain the pellet. The houselight remained on throughout the session under condition 1.

Condition 2: multiple-fixed ratio 11, DRL 10 sec. The multiple schedule was introduced after the FR acquisition sequence had been completed. In this multiple schedule, FR 11 comprised one component schedule that replicated the previously studied FR 11. During the FR component, the chamber houselight remained on. A DRL 10-sec schedule comprised the second component. Under the DRL schedule, a clock began at the onset of the component and after each lever press. Only a press that occurred after the criterion interval 10 sec had elapsed was reinforced with a food pellet. During the DRL component, the chamber houselight flickered at a rate of 200 msec on and 200 msec off. Component changes occurred in strict alternation independent of responding. The FR component duration was 1 min; the DRL component duration was 5 min.

For this procedure, the dependent variables were designated as follows: *a*) rate of FR responding: FR responses per FR component minutes; *b*) local response rate: responses per session minutes excluding the time to the first response in a fixed-ratio run of responses; *c*) rate of DRL of responding: DRL responses per DRL component minutes; *d*) DRL rate of reinforcement: DRL pellets per DRL component minutes; *e*) proportion of DRL responses reinforced; and *f*) FR relative rate of responding: the ratio of FR responses per FR component minutes to total responses

Table 1. Number of sessions conducted for the incremental fixed ratio and mult-FR 11, DRL 10-sec schedules.

Value	Number of sessions
Preliminary training	
VT + FR 1	2
FR 1	3–4
Incremental FR	
1	4
6	4
11	4
21	4
31	4
41	4
51	4
61	4
71	4
Retrain	
VT + FR 1	1
FR 11	2
Extinction	
FR 11	4
Retrain	
VT + FR 1	1
FR 11	2
Multiple	
FR 11 DRL 10 sec	30
Extinction	
FR 11 DRL 10 sec	2

per session minutes, which served as an index of schedule discrimination

Statistical methods. The General Linear Model procedure (*44*) was used to examine the behavioral data, primarily by repeated-measures analysis of variance (ANOVA; using SAS version 8, SAS Institute, Cary, NC). Prenatal treatment was the between-subject factor. Because one male and one female littermate were drawn from each litter, the statistical unit of analysis was litter, with sex included as a within-subject factor. For the incremental FR reinforcement schedule, four sessions at each of the nine FR values were treated as within-subject factors for repeated measurements. For the multiple FR 11 DRL 10 sec reinforcement schedule, the six dependent variables were analyzed separately. For each variable, the data were averaged over five consecutive sessions (six blocks) preceding the ANOVA, which included the factors sex, treatments, and blocks (the last being repeated measurements). To evaluate the dose by sex interaction, the data of the male and female offspring were collapsed across the six blocks. We then analyzed these data for linear and quadratic contrasts between sexes. For both incremental FR and mult-FR DRL reinforcement schedules, we used the Huynh-Feldt (*45*) adjustment to the degrees of freedom when appropriate. For the multiple schedule, we used a mixed procedure to evaluate local FR responses/minute because not all animals responded under the FR schedule at sufficient levels to evaluate complete sets of male–female littermate pairs.

Benchmark dose analysis. Dose–response relationships were described by benchmark dose modeling software, version 1.3, provided by the U.S. Environmental Protection Agency (BMDS, U.S. EPA, Research Triangle Park, NC). The benchmark approach (*46*) is a useful alternative to the more traditional no-observed-adverse-effect level calculations used to derive exposure standards. Benchmark calculations consider the entire dose–response relationship and do not involve extrapolations far below experimental observations. The benchmarks we calculated represent doses that are associated with specific operant behavior performance. With the continuous model, we calculated benchmark doses representing the model-estimated control mean minus proportional deviations equivalent to a 10% (ED_{10}) or 1% (ED_{01}) change. The BMDS software also provides a 95% lower bound that can be divided by a standard uncertainty factor, such as 100 to calculate a reference dose or provide a margin of exposure.

Table 2. Mean ± SD dam body weights across the gestational period.

Dose (ng/kg)	Body weight (g) on gestation day 0	4	8	12	16
Control	270.83 ± 14.11	306.00 ± 5.66	298.33 ± 18.99	311.67 ± 22.11	346.83 ± 18.05
20	275.00 ± 19.62	299.33 ± 13.95	302.00 ± 18.67	321.50 ± 19.68	353.75 ± 27.01
60	262.00 ± 22.29	275.50 ± 24.08	288.29 ± 22.19	306.00 ± 18.58	337.57 ± 21.60
180	278.00 ± 16.40	292.86 ± 17.54	304.29 ± 20.99	320.57 ± 17.65	346.00 ± 21.29

Results

Maternal and postpartum data. All dams delivered within 3 weeks after determination of pregnancy. The group mean weight gain across the gestational period, shown in Table 2, ranged from 68 to 79 g. The number of

Table 3. Mean ± SD pup sex distribution and weight gain across the lactational period.

Dose group (ng/kg)	Pups/litter	Weight gain (g) on postnatal day 4	8	12	16	20
Male						
Control	6.43 ± 2.07	11.26 ± 2.39	20.01 ± 3.02	30.07 ± 3.63	38.68 ± 4.67	53.81 ± 6.93
20	6.88 ± 2.10	11.22 ± 1.22	20.26 ± 2.81	33.28 ± 4.73	42.22 ± 3.47	56.73 ± 4.86
60	7.29 ± 2.75	11.02 ± 1.11	20.23 ± 1.96	30.01 ± 2.15	40.65 ± 2.66	54.77 ± 4.58
180	5.71 ± 3.55	11.49 ± 1.70	21.84 ± 2.72	31.39 ± 3.07	41.75 ± 3.44	55.31 ± 5.10
Female						
Control	5.71 ± 2.29	11.04 ± 2.58	19.67 ± 3.28	28.44 ± 5.08	38.23 ± 4.96	53.14 ± 7.14
20	6.88 ± 2.80	10.57 ± 1.07	19.34 ± 1.93	30.11 ± 2.87	39.59 ± 2.84	54.19 ± 4.63
60	5.14 ± 1.57	10.49 ± 1.26	19.62 ± 2.07	29.43 ± 1.82	39.53 ± 2.47	53.77 ± 4.17
180	5.29 ± 1.60	11.42 ± 1.50	21.63 ± 2.43	31.80 ± 3.96	40.98 ± 6.46	55.65 ± 5.60

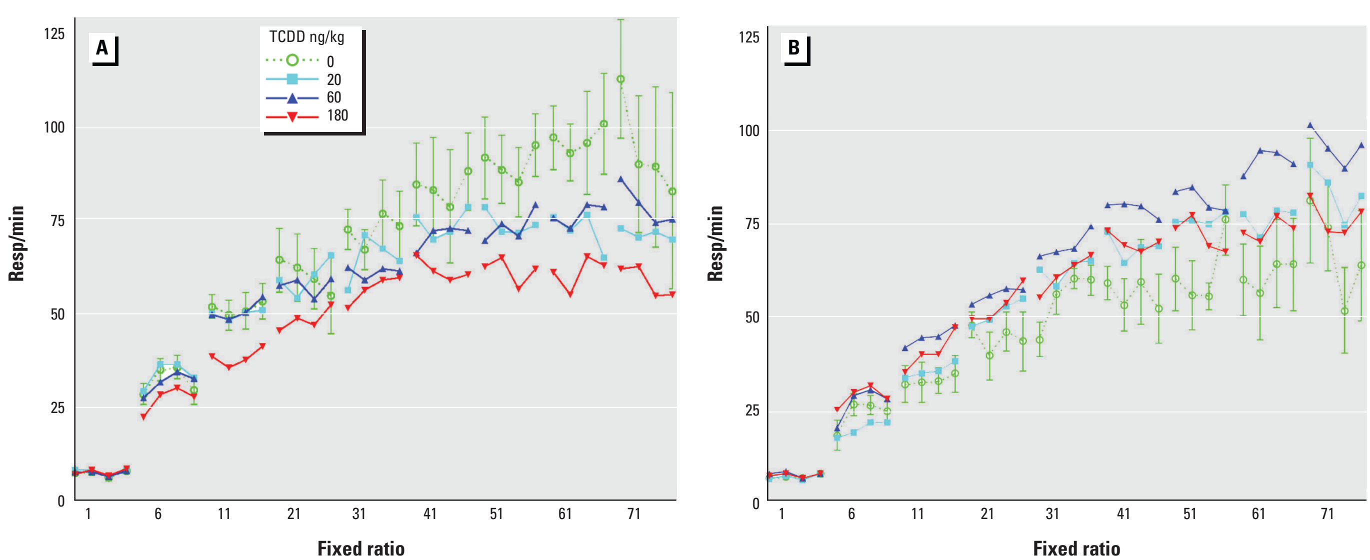

Figure 1. Mean (± SEM for controls) rate of responding per session for the four TCDD exposure groups during the incremental fixed-ratio condition: (*A*) males; (*B*) females. Resp, responding.

male and female pups per litter and their body weights are summarized in Table 3. None of those observations indicated an effect of exposure.

Behavioral data. All animals acquired the lever-press response within 3 or 4 days of preliminary training.

Incremental fixed-ratio condition. Mean response rates (responses/min) for each FR value are shown in Figure 1. The ANOVA evaluated the contribution of TCDD treatment, and also several interactions including treatment by sex and treatment by sex by FR value. None of those results were statistically significant. We also examined the local rate of responding (i.e., the rate of responding corrected for the postreinforcement pause; data not shown), and similarly observed no significant effects.

Multiple FR 11, DRL 10-sec reinforcement schedule. TCDD treatment affected almost all of the variables studied. The ANOVA results showed that although neither main exposure nor sex effects per se were seen, interactions were observed for every response measure except FR relative rate (Table 4). These significant results are examined below.

FR component. Mean response rates of the males and females across blocks of five sessions during the FR component are shown in Figure 2. For the males, all three groups exposed to TCDD responded at lower rates than the controls. For the females, all three TCDD-treated groups responded at higher rates than controls. The significant treatment-by-sex interaction (p = 0.036) for FR response rate is depicted in Figure 3, which plots the mean response rates of males and females collapsed across session blocks. Although the mean rate for control males exceeded that for control females, this relationship changed across doses. For example, the 60 ng/kg females responded at higher rates than did the 60 ng/kg males. The ANOVA of sex differences in FR response rate revealed a significant quadratic trend (p = 0.01).

This TCDD prenatal treatment-by-sex interaction was examined further with benchmark dose analyses. The fitted polynomial (Figure 4) was based on the following data: For each of the six blocks of five sessions each, the mean response rate of a female was subtracted from the mean response rate of its male littermate. Those littermate, male–female differences were then averaged across the six blocks to yield a mean difference for each litter within each dose group. The means and standard deviations of those data (Table 5) complied with a second-order polynomial function (p = 0.01), as seen in Figure 4. With the BMDS continuous model, we calculated the ED_{10} or ED_{01} as well as a 95% lower bound (Table 6). The ED_{10} for FR response rate was 2.77 ng/kg, with a 95% lower bound of 1.81 ng/kg.

DRL component. Mean response rates of male and female offspring across blocks of five sessions during the DRL 10-sec component are shown in Figure 5. As with the FR component, the ANOVA indicated a significant sex-by-treatment interaction (p = 0.01). Duplicating the FR analysis, all three male dose groups responded at lower rates than controls. For the females, all three TCDD-treated groups responded at higher rates than controls. The interaction is seen in Figure 6, which shows the mean response rates of both males and the females collapsed across session blocks. The mean rate for control males exceeded that for control females, but this relation changed across doses (e.g., the 60 ng/kg females responded at higher rates than did the 60 ng/kg males). The ANOVA analysis of the sex difference in DRL response rate again revealed a significant quadratic trend (p = 0.01).

A benchmark dose analysis of the treatment-by-sex interaction in DRL response rate, based on the data in Table 5 as for FR response rate, showed it to be accurately modeled by a second-order polynomial (p = 0.01), as seen in Figure 7. As shown in Table 6, the ED_{10} was 2.97 ng/kg, with a 95% lower bound of 2.02 ng/kg. The ED_{01} was 0.30 ng/kg, with a 95% lower bound of 0.20 ng/kg.

Response rate under an FR schedule directly controls the rate of reinforcement. Under the DRL schedule, however, identical rates of responding need not produce identical rates of reinforcement because reinforcement depends on the distribution of responses across time. Efficiency of responding, (i.e., the ratio of reinforced responses to total responses), shown in Figure 8, measures how precisely responding meets the DRL criterion. It indicates that control females responded more efficiently than control males . The plot also depicts the nature of the sex-by-treatment interaction shown in

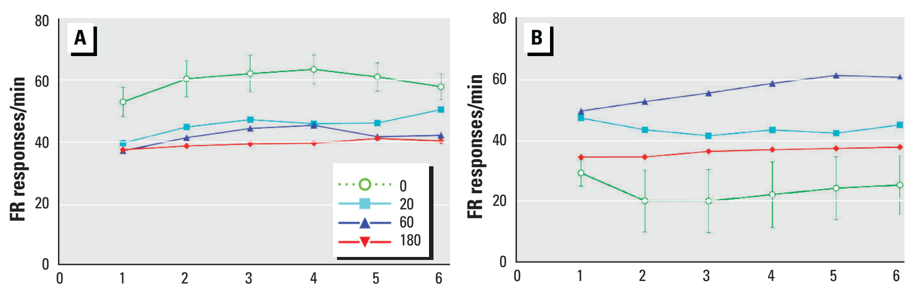

Figure 2. Mean (± SEM for controls) rate of responding across the six 5-session blocks for the four TCDD exposure groups during the fixed-ratio component of the mult-FR 11, DRL 10-sec condition: (*A*) males; (*B*) females.

Table 4. Results from the general linear models procedure, repeated-measures ANOVA: factor, degrees of freedom, and p-values for the mult-FR 11, DRL 10-sec response measures.

Factor	df	FR responses/min	DRL responses/min	DRL reinforcements/ DRL responses	FR relative rate	DRL reinforcements/min	Local FR responses/min[a]
TCDD Dose (treatment)	3, 17	0.67	0.43	0.37	0.63	0.59	0.69
Sex	1, 17	0.25	0.20	0.03	0.21	0.07	0.52
Sex × treatment	3, 17	0.04	0.01	0.09	0.40	0.03	0.04
Block	5, 85	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Block × treatment	15, 85	0.49	0.58	0.02	0.33	0.18	0.86
Sex × block	5, 85	0.07	0.16	< 0.01	0.46	0.27	0.21
Sex × block × treatment	15, 85	0.28	0.27	0.14	0.36	0.30	0.91
Linear trend: sex difference (M – F)	1	0.23	0.09	0.20	0.73	0.20	No analysis
Quadratic trend: sex difference (M – F)	1	0.01	0.01	0.03	0.10	0.01	No analysis

Abbreviations: df, degrees of freedom; F, female; M, male. The Huynh-Feldt correction is indicated where block is a factor, except for the local FR responses/min.
[a]No analysis if < 5 ratios were completed.

Table 4. The ANOVA analysis of the sex difference in DRL efficiency showed a significant quadratic trend ($p = 0.03$).

The ANOVA contrast of the sex difference across treatments was also conducted for two other measures. DRL reinforcements per minute showed a significant quadratic trend ($p = 0.01$); the mean of the sex difference across doses in Table 5 shows the nature of the trend for this measure. The contrast of sex difference was not significant for FR relative rate ($p = 0.10$).

Extinction. Examination of the graphical data did not suggest any effects of treatment on performances during the four extinction sessions after the incremental FR procedure or the two sessions after the mult-FR 11 DRL 10-sec reinforcement schedule (data not shown). No further analyses of those data were conducted.

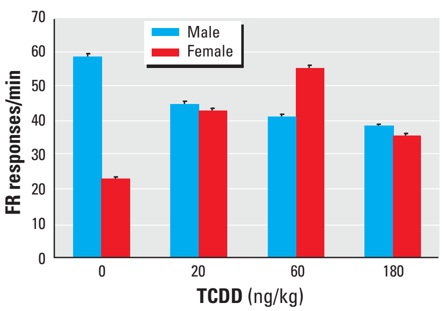

Figure 3. Mean (± SEM) response rate for male and female littermates during the fixed-ratio component for the 30 sessions of the mult-FR 11, DRL 10-sec condition for each TCDD exposure group.

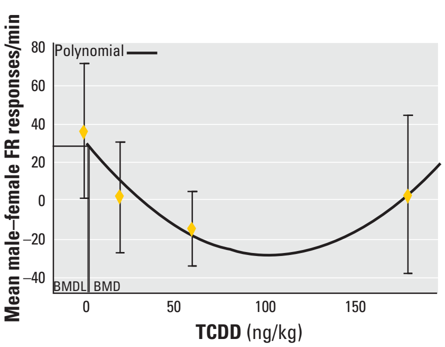

Figure 4. Polynomial model for benchmark dose ED_{10} value and 95% lower confidence level for the male–female littermate differences in FR response rate during the fixed-ratio component of the mult-FR 11, DRL 10-sec schedule. The polynomial was calculated from a quadratic fit to the dose–effect data shown for FR responses/min in Table 5.

Discussion

Administration of TCDD on GD 8 to pregnant rats altered the schedule-controlled performance of their offspring. The most striking result is the sexually dimorphic pattern of responses. This pattern was seen most clearly under the mult-FR DRL schedule. Figure 1 indicates a similar pattern of sex differences under the incremental FR schedule. Under both conditions, TCDD-exposed males responded at lower rates than control males. Females displayed an opposite pattern, with TCDD exposure associated with higher rates.

When the multiple schedule was introduced, conditions during the FR component replicated those of the incremental FR 11 condition. The DRL component, however, offered a marked contrast in response requirements and stimulus conditions. In particular, while the FR contingency selectively reinforced short inter-response times (IRTs) and high rates of lever pressing, the DRL contingency selectively reinforced long IRTs and low rates of lever pressing. The FR relative rate measure (see Table 5) describes how well the subjects discriminated between the response requirements of the two component schedules. This index did not differ among groups, indicating that under those specific condtions TCDD did not affect acquisition of the discrimination.

Sexually dimorphic patterns of responding have been observed in many schedule-controlled operant behaviors. For example, under a random ratio schedule, which generally maintains high rates of responding, males respond at higher rates than females. Under DRL schedules, females generally perform more efficiently than males (*38,47*). Similar response patterns were also observed in control offspring in the present experiment. Such behavioral differences between the sexes appear not to be a function of sex differences in food motivation. Instead, they are influenced at least partly by the presence or absence of gonadal hormones, specifically the male gonadal hormone testosterone (*48*). These response patterns can be altered by external hormonal exposure (*38*).

Although we did not directly measure gonadal function in the offspring, our data support a role for TCDD-induced alterations in neuroendocrine function. Previous studies have repeatedly reported that TCDD, even at relatively low doses, interferes with normal development of reproductive function, including sex-specific patterns of reproductive

Table 6. BMDs and 95% lower bound (95% LB) calculations based on a 1% or a 10% shift from the control group mean (ED_{01} or ED_{10}) for mult-FR DRL response measures.

	FR responses/min		DRL responses/min	
	ED_{01}	ED_{10}	ED_{01}	ED_{10}
BMD (ng/kg)	0.27	2.77	0.30	2.97
95% LB	0.18	1.81	0.20	2.02

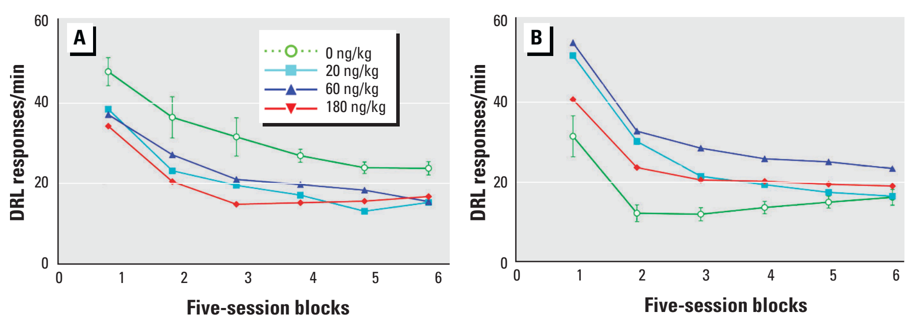

Figure 5. Mean (± SEM for controls) rate of responding across the six 5-session blocks for the four TCDD exposure groups during the DRL component of the mult-FR 11, DRL 10-sec condition: (*A*) males; (*B*) females.

Table 5. Male–female littermate differences for each response measure for mult-FR 11, DRL 10-sec.

		FR responses/min		DRL responses/min		DRL reinforcements/ DRL responses		FR relative rate		DRL reinforcements/min	
Dose (ng/kg)	No.	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control	5	36.113	28.051	18.436	7.986	–0.182	0.093	0.079	0.145	–0.814	0.448
20	5	1.887	23.014	–0.987	10.963	–0.115	0.209	0.044	0.111	–0.364	0.821
60	6	–14.301	18.143	–4.522	7.194	0.048	0.081	–0.035	0.046	0.374	0.540
180	5	3.113	32.917	–0.406	15.231	–0.042	0.166	0.039	0.127	–0.163	0.443

For each of the six blocks of five sessions each, the mean response rate of a female was subtracted from the mean response rate of its male littermate. Those littermate, male-female differences were then averaged across the six blocks to yield a mean difference for each litter within each dose group. Littermate differences were then averaged across the number of litters for each dose of TCDD.

behavior (*49–52*). The remarkable differences between male and female offspring in how TCDD affected operant behavior strongly suggest that its influence is exerted through the effect of gonadal hormones on brain development. In addition, the current results provide an intriguing counterpoint to those of our recent study (*14*), in which TCDD was administered at doses of 0, 20, 60, and 180 ng/kg to pregnant dams on GD 18. The earlier experiment tested the performance of only the female offspring in a situation in which the subjects responded on FR schedules for the opportunity to exercise in a running wheel. In that experiment, TCDD produced significant dose-related reductions in performance. The results of the current study, coupled with the findings of the previous study, emphasize the need for further investigation of how TCDD modifies the course of brain development, especially in relation to the markers of sexual differentiation. In rats, markers of sexual differentation appear late in gestation (*53*).

The male–female differences in response to prenatal TCDD exposure followed a U-shaped function across the doses studied. This outcome is not unique. It is becoming increasingly recognized that, especially for endocrine-disrupting agents, monotonic dose–response functions may not be the prevalent pattern (*54,55*). Similar results were reported in our previous study in which rats were exposed to TCDD doses of 0, 60, 180 and 540 ng/kg on GD 15 (*56*). On a delayed visual discrimination task, the performance of both male and female offspring exposed to 180 ng/kg TCDD was significantly less accurate than the lowest and the highest exposure dose groups. Seo et al. (*13*) also observed U-shaped dose–effect functions. Male offspring exposed to a total dose of 700 ng/kg made significantly fewer errors in a radial arm maze, but males exposed to 1,400 ng/kg resembled controls. Moreover, vom Saal et al. (*55,57*) reported that perinatal exposure to estradiol and diethylstilbesterol (DES) increased prostate weight in rats described by an inverted-U relationship between dose and response. Prostate weight changed in response to the medium dose of estradiol or DES but did not react to the highest dose of estradiol or DES.

Generally, testing methods for systemic toxicants, which include endocrine disruptors, are based on the assumption of a monotonic dose relationship, where the response to an environmental chemical is assumed to increase as dose increases. Results from our experiments and others just noted reliably demonstrate a curvilinear response to dose. Curvilinear dose–response functions such as those seen in the hormesis literature (*58–60*) are difficult to explain in our case because of our limited understanding of the toxic mechanisms underlying perinatal TCDD exposure.

Whatever the mechanisms, the current findings, especially the benchmark dose analyses, indicate that current human body burdens based on TEQs, even though they may have fallen since 1995 (*5*), may represent a health hazard. Human data on the developmental neurotoxicity of this class of compounds are almost totally absent except for studies linking PCBs and impaired child development (*61,62*). Reductions of exposure, coupled with further research on the behavioral mechanisms and consequences of exposure to this class of chemicals, including studies of brain structure (*63*), are clearly warranted.

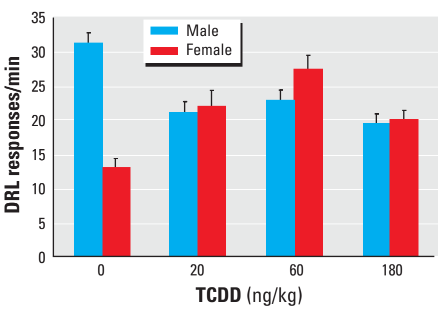

Figure 6. Mean (± SEM) response rate for male and female littermates during the DRL component for the 30 sessions of the mult-FR 11, DRL 10-sec condition for each TCDD exposure group.

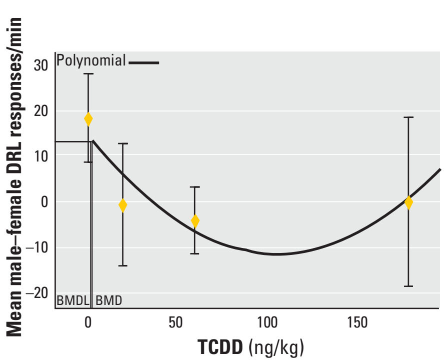

Figure 7. Polynomial model for benchmark dose ED_{10} value and 95% lower confidence level for the male–female littermate differences in DRL response rate during the DRL component of the mult-FR 11, DRL 10-sec schedule. The polynomial was calculated from a quadratic fit to the dose–effect data shown for DRL responses/min in Table 5.

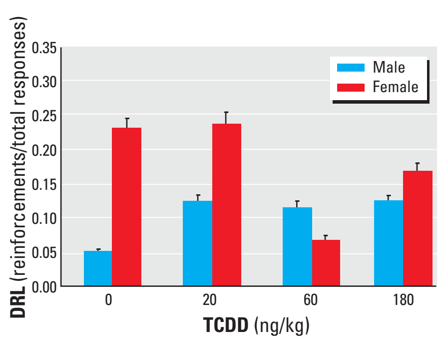

Figure 8. Mean (± SEM) DRL efficiency (DRL reinforcements/DRL responses) for male and female littermates during the DRL component for the 30 sessions of the mult-FR 11, DRL 10-sec condition for each TCDD exposure group. A value of 1 indicates that each DRL response was reinforced with a pellet delivery, and a value of 0 indicates that no DRL response was reinforced.

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