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Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on Behavior of Monkeys in Peer Groups

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SCHANTZ, S. L., S. A. FERGUSON AND R. E. BOWMAN. *Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin on behavior of monkeys in peer groups.* NEUROTOXICOL TERATOL 14(6) 433–446, 1992.—Adult female rhesus monkeys were fed diets containing 0, 5, or 25 ppt 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) for approximately 4 years. They were bred to unexposed males during TCDD exposure (Experiment 1) and again after TCDD exposure ended (Experiment 2). Offspring from both experiments were weaned at 4 months and socialized for 1.5 h/day in groups of four monkeys each beginning at approximately 8 months of age. Each social group contained both control and TCDD-exposed monkeys. In Experiment 2, the offspring were later placed in new social groups containing only monkeys from the same TCDD exposure condition. The TCDD-exposed offspring born concurrent with maternal TCDD exposure (Experiment 1) initiated more rough-tumble play, retreated less during play bouts, and were less often displaced from preferred positions in the playroom. They also engaged in more self-directed behaviors. The behavior of offspring born after maternal TCDD exposure ended (Experiment 2) was not altered when they were socialized with control monkeys. However, some behavioral changes did emerge when they were placed in social groups containing only TCDD-exposed monkeys.

2,3,7,8-tetrachlorodibenzo-*p*-dioxin TCDD Monkey Perinatal exposure Peer group social behavior

2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN (TCDD) is a widespread environmental contaminant which is considered to be a prototype for a large class of chemical pollutants including the polychlorinated and polybrominated biphenyls (PCBs and PBBs), the dibenzofurans, and the dibenzodioxins. Chemicals of this class produce a characteristic pattern of effects and appear to share the same mechanism of action (27,28). The toxicity of TCDD and related chemicals in laboratory animals, together with their widespread presence in the environment, has created a great deal of scientific and public concern regarding potential human exposure. Exposure is believed to be of particular concern for females of childbearing age because these compounds accumulate in adipose tissue and are mobilized and transferred to the developing infant during gestation and lactation (3,16,26). However, the risks to human infants from perinatal TCDD exposure remain to be determined.

TCDD is fetotoxic and teratogenic in laboratory animals at doses well below those overtly toxic to the mother (6,23). The behavioral teratology of TCDD has not been widely investigated, but behavioral effects have been reported in rodents, monkeys, and humans following perinatal exposure to the structurally and toxicologically similar PCBs (39).

We conducted a series of studies on the long-term behavioral effects of perinatal TCDD exposure in rhesus monkeys. Because these were among the first studies to evaluate the behavioral teratology of TCDD, monkeys exposed during gestation and lactation were screened on a broad selection of behavioral tests (2). TCDD, at the doses studied (5 or 25 ppt in the maternal diet), did not affect reflex development, visual exploration, locomotor activity, or fine motor control in any consistent manner (2), but did produce a deficit in cognitive function (29). The TCDD-exposed offspring were impaired in their ability to learn an object discrimination-reversal series but were unimpaired in their ability to learn spatial discrimination-reversals or delayed spatial alternation. TCDD exposure also produced changes in the social interactions of mother-infant dyads (31). TCDD-exposed mother-infant dyads spent more time in close social contact, particularly ventral-ventral contact and infant-mother nipple contact. This suggested that mothers were providing increased maternal care to TCDD-exposed infants. A strikingly similar pattern of effects has been observed in rhesus monkeys rearing lead-exposed infants (31).

The effect of perinatal TCDD exposure on early peer interactions is the focus of this article. Adequate early peer group

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social experience is critical to a normal course of social development in primates (14,35). In nonhuman primates, social interaction with peers is essential for the acquisition of appropriate heterosexual behavior and reproductive success (11), as well as for appropriate aggressive behavior, predatory behavior, and tool use (34). In humans, early peer relations mediate and predict adult social adjustment across a wide range of measures including school performance, work history, criminal activity, and psychiatric hospitalization (7,24).

Clearly, toxicant exposure that interferes with the ability of the young organism to interact adequately with its peers in a social context may have important long-term consequences for the social and reproductive competence of that individual. Previous studies have shown that peer group social behavior of young monkeys is disrupted by perinatal exposure to lead or methylmercury (4,5,18). In those studies, interactive play behaviors were particularly sensitive to disruption and lead- or methylmercury-exposed monkeys exhibited suppression of social play. In the current study, interactive play and other behaviors of young rhesus monkeys born to TCDD-exposed mothers were assessed.

EXPERIMENT 1

METHOD

Subjects and Maternal TCDD Exposure

The monkeys tested in Experiment 1 were the offspring of female rhesus monkeys fed diets containing 0 or 5 ppt TCDD. Initially, 24 feral-born, individually housed adult female rhesus monkeys were assigned, 8 each, to one of three treatment groups. One group was fed a diet of Purina monkey chow (Ralston Purina Co., St. Louis, MO) prepared to contain 5 ppt TCDD. A second group was fed a diet prepared to contain 25 ppt TCDD. The third group was maintained on a control diet of Purina monkey chow which was devoid of any added TCDD. The procedures used to mix the TCDD-treated feed and to verify its TCDD content have been described in detail elsewhere (3) and are only summarized here. Initially, 58 μL of TCDD was pipetted from a stock solution in benzene (19.8 μg TCDD/mL), diluted to 200 mL in acetone and mixed into 8 kg of ground monkey chow. The 8 kg of ground chow was then mixed with additional ground chow to yield exactly 22.7 kg (50 lb) of a 50 ppt TCDD "premix." The 5 and 25 ppt diets were prepared by taking either 5 or 25 lb of the premix and bringing it up to 50 lb by mixing with additional ground monkey chow. The chow was then pelleted by adding 2 liters of water and 1 liter of glycerine per 50 lb of chow. The final TCDD content was calculated gravimetrically from the final weight of the feed.

Representative samples of the treated feed from throughout the study were analyzed for TCDD content by gas chromatography/high resolution mass spectrometry (13). The feed bags were numbered consecutively throughout the study and a total of 90 bags were fed over the four years. For the 5 ppt diet, four bags opened in 3/79, 9/79, 1/81, and 2/81 were assayed for TCDD content. For the 25 ppt diet, 11 bags opened in 11/78, 3/79, 9/79, 1/81, 4/81, 6/81, 8/81, 2/82, 8/82, and 8/82 were assayed. Five of the bags were also assayed for PCBs and dichlorodiphenyl dichloroethene (DDE). These were the 9/79 5 ppt bag and the 9/79, 1/81, 4/81, and 6/81 25 ppt bags. As detailed by Bowman et al. (3), the analytical results were in very close agreement with the gravimetrically calculated values for TCDD content. The samples averaged 5.9 ± 1.4 ppt TCDD (mean ± SE, n = 4) for the 5 ppt diet and 26 ± 3.0 ppt (n = 11) for the 25 ppt diet (3). The five samples analyzed for other contaminants averaged 7.8 ± 0.09 ppb PCBs and 0.95 ± 0.2 ppb DDE (John Van Miller, unpublished data).

The females were fed 200 gm of pelleted monkey chow each morning and food intake was monitored by counting the number of pellets remaining the following morning (each pellet weighed approximately 3.8 gm). The TCDD intake of each monkey was assessed daily by multiplying the estimated number of gm of food consumed by the gravimetrically calculated TCDD concentration in the food.

Beginning after seven months of TCDD exposure, the females were bred repeatedly with unexposed males until conception occurred or to a maximum of 10 matings. For each breeding, the female was placed with one of nine male breeders for four days which bracketed the expected day of ovulation as estimated from the length of the last menstrual cycle. Pregnancies were detected via assay of serum chorionic gonadotropin on about day 15–16 after the estimated day of ovulation and later confirmed via palpation of the uterus on about day 30–35 after the estimated day of ovulation. Eight of the eight control females delivered viable offspring and six of the eight 5 ppt TCDD-exposed females delivered viable offspring, whereas only one of the eight 25 ppt TCDD-exposed females delivered a viable infant (3). The one 25 ppt offspring was not studied behaviorally. The six offspring of 5 ppt TCDD-exposed females were born after 16.2 ± 0.4 months of maternal TCDD exposure. Estimated total maternal TCDD intake at that time averaged 59.6 ± 5.0 ng/kg. All TCDD-exposed females were maintained on the TCDD diet throughout gestation and lactation. Thus, exposure of the offspring to TCDD was transplacental, transmammary and potentially through access to the mother's food. However, the infants were only rarely observed eating monkey chow and usually only in the month just prior to weaning, so it is unlikely that significant TCDD exposure occurred via that route. Exposure to TCDD terminated when the offspring were weaned at 4 months of age. At that time they were placed on a diet of regular Purina monkey chow and housed individually in wire mesh cages that allowed auditory, visual, and olfactory contact with other monkeys. After weaning, they had no physical contact with other monkeys until peer-group testing began.

The monkeys tested in the current experiment consisted of the six 5 ppt TCDD-exposed offspring (3 females and 3 males) and six of the eight control offspring (3 females and 3 males) whose ages most closely matched those of the TCDD offspring. Data from one of the six control monkeys were not included in the statistical analyses because it was learned that its father had been exposed to PCBs in an earlier study. There was no evidence that the behavior of the other three monkeys in that animal's social group was influenced in any way by the presence of the animal, so their data were retained in the analysis. With the exception of TCDD exposure, all control offspring were subjected to the same experimental procedures as were TCDD-exposed offspring.

Mesenteric fat samples were collected via laparotomy from each of the offspring at 5 months of age and analyzed for TCDD content via gas chromatography/high resolution mass spectrometry (13). TCDD concentrations in the mesenteric fat of offspring born to and nursed by the 5 ppt TCDD-exposed females averaged 377 ± 141 ppt (range: 290–950). TCDD was not detected in any of the control offspring at detection limits ranging from 2–200 ppt.

None of the 5 ppt TCDD-exposed or control mothers and

offspring exhibited any unusual or serious health problems during the course of Experiment 1. Furthermore, none of the TCDD-exposed offspring exhibited any signs of developmental toxicity at any point during the study. Birth weights and growth rates of the TCDD-exposed offspring did not differ significantly from those of the controls.

Apparatus

Peer groups were tested in a large playroom which measured 2.0 m long × 2.4 m wide × 2.2 m high (Fig. 1). The room was equipped with wire mesh ledges and ramps and a horizontal metal bar that transversed the entire room half-way between the ceiling and floor. Testers observed the monkeys through a glass observation window measuring 2.4 × 1.4 m which was mounted in the front wall of the room. Differential lighting (darkened outer room; lighted interior) facilitated the observer's view of the monkeys and partially obscured the monkeys' view of the observer. Behavior was recorded on an Apple IIe microcomputer. The software (Kraemer Behavioral Scoring System, Copyright Situation Software Co., 1984) used a syntactic encoding process which preserved the frequency, duration, coincidence, and sequence of behaviors. Features of the encoding process, randomization of scoring order, recording of date and time of testing, data evaluation, and data storage were software controlled.

Procedure

Beginning when they were 8.6 ± 0.3 months of age, the monkeys were placed in peer groups of four monkeys each, for 1.5 h per day, Monday through Friday, and allowed to interact without interference. Each group consisted of two control and two 5 ppt TCDD-exposed monkeys (1 male and 1 female from each condition), matched as nearly as possible for age (Table 1). The maximum age differences within each of the three social groups were 2, 2.5, and 2.8 months. Each monkey was socialized only with the three other animals in its group. Data collection began on day two of socialization and was conducted 4 days per week (Tuesday through Friday) for a total of 36 sessions in 9 consecutive weeks. Each test session began 30 min after the monkeys were placed in the playroom and 5 min after the tester entered the test area. The times of testing for the three groups were counterbalanced across days such that each group was tested an equal number of times at 10:45 a.m. and 12:30 p.m.

TABLE 1

COMPOSITION OF SOCIAL GROUPS IN EXPERIMENT 1

Group	Monkey #	Gender	TCDD (ppt)	Mother/Father		D.O.B.
1	AI79	F	5	113	5	5/22/80
	AI81	M	5	101	4	6/07/80
	AI82	F	0	93	1	6/10/80
	AI89	M	0	92	1	7/22/80
2	AI71	F	0	P47	824	8/06/80
	AI77	M	0	Z12	CL1	8/29/80
	AI84	F	5	103	5	6/14/80
	AI86	M	5	97	6	7/04/80
3	AI88	M	5	111	2	7/16/80
	AI91	F	5	102	1	8/05/80
	AI92	F	0	96	3	8/10/80
	AI93*	M	0	108	1632	10/10/80

*Excluded from statistical analyses because father had prior PCB exposure.

Behavior was scored by either of two experienced testers who were blind to the treatment conditions of the monkeys. Prior to the start of testing, testers were trained to intertester reliability coefficients of at least 0.90 on the frequency and duration of each of the 36 possible behaviors. Periodic reliability checks assured that interobserver reliability was maintained at 0.90 or greater during the 9 weeks of testing.

A focal animal scoring technique was used for all observations (1). Animals were scored in a computer-generated random test order, and each monkey's behavior was observed and recorded for a period of 5 min during each test session. The scoring system used operationally defined behavioral categories, each of which was assigned a two-letter mnemonic alphanumeric code (Table 2). With the exception of noncontact social play, all behaviors which involved social interaction were scored as initiate, if the focal animal was the initiator of the behavior, receive if the focal animal was the recipient of

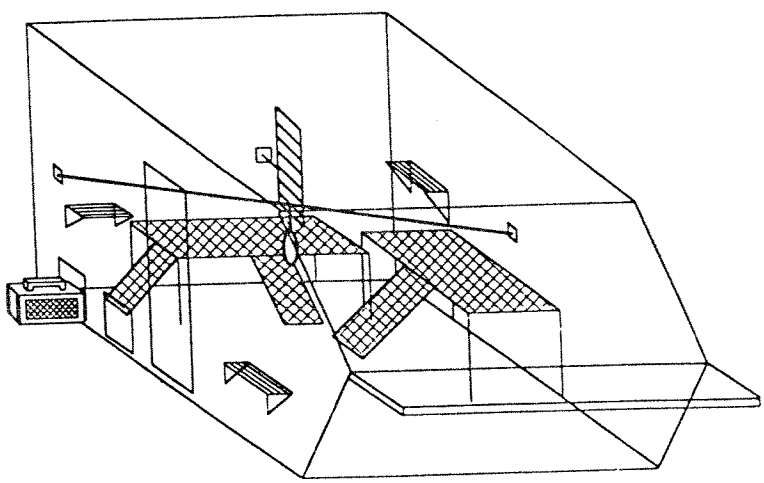

FIG. 1. The playroom. Reprinted with permission from Ferguson and Bowman (9).

TABLE 2
BEHAVIOR CATEGORIES

A. Social Interaction
Rough tumble play: The animal initiates (*Initiate Rough Tumble Play*), is the recipient of (*Receive Rough Tumble Play*), or engages in mutual (*Mutual Rough Tumble Play*) oriented wrestling activity.
Nonresponse: The animal receives rough tumble play, but does not reciprocate (scored in Experiment 2 only).
Noncontact social play: Mutual nonagonistic orientation of two or more monkeys which involves little or no physical contact (e.g., chase play).
Playface: A relaxed, open mouth expression, usually occurring at the beginning of a play interaction.
Social explore: The animal initiates (*Initiate Social Explore*), is the recipient of (*Receive Social Explore*), or engages in mutual (*Mutual Social Explore*) nonagonistic manual, oral or perinasal contact with another animal (excluding grooming, play or sexual behavior).
Groom: The animal initiates (*Initiate Groom*), is the recipient of (*Receive Groom*), or engages in mutual (*Mutual Groom*) discrete picking and/or spreading of an animal's fur.
Aggression: The animal initiates (*Initiate Aggression*), is the recipient of (*Receive Aggression*), or engages in mutual (*Mutual Aggression*) vigorous and/or prolonged biting, hair pulling, or fighting.
Threat: An aggressive facial expression characterized by a stare, with ears retracted, lower jaw pulled down and out and lips pursed into a tight "O".
Fear grimace: A submissive expression characterized by avoidance of eye contact, with lips retracted, exposing teeth.
Approach: Any oriented reduction in physical distance.
Retreat: Any oriented increase in physical distance.
Play retreat: An oriented increase in physical distance that occurs during a chase play sequence (scored only in Experiment 1).
Displacement: The animal approaches another animal and takes the position that animal is occupying.
Yield to displacement: The animal yields to another animal by moving away so that the other animal can assume its position.
Proximity: The animal assumes a static posture and is within one arm's length of another animal.
Contact cling: The animal initiates (*Initiate Contact Cling*), is the recipient of (*Receive Contact Cling*), or engages in mutual (*Mutual Contact Cling*) sustained ventral-ventral or ventral-dorsal contact.
Clump: Contact cling which includes at least three animals.

B. Other Behaviors
Vocalization: Coo, bark, screech, or other vocalization.
Locomotion: Ambulation or brachiation in which the animal moves at least one body length through space.
Environmental exploration: Manual, oral, or pedal manipulation of the physical environment.
Self-motion play: Play activity not directed toward another animal.
Inactive: The animal assumes a static posture and shows an absence of all social and exploratory activity.
Stereotypy: Patterned movement maintained in a rhythmic and repetitive fashion.
Self-directed behaviors: Oral, manual or pedal self manipulation.
Huddle: A fetal-like posture in which the animal's back is hunched and its head is at or below its shoulders.
Nonspecific contact: Any physical contact between two animals not covered by any of the above categories (scored in Experiment 1 only).
Misjudgment: Any loss of balance (scored in Experiment 1 only).

the behavior, or mutual if the behavior was mutually initiated by the focal animal and one or more other animals. The scoring system was not mutually exclusive. Rather, it allowed for more than one behavior to be scored simultaneously. For example, if the focal animal was engaged in a chase play sequence with another animal, both noncontact social play and locomotion would be scored.

Data Analysis

A total of 21 behaviors occurred with sufficient frequency to warrant statistical analysis in Experiment 1. Four behaviors (aggression, threat, fear grimace, and contact cling) occurred very infrequently and were not analyzed. As suggested by Altmann (1), durations were analyzed for behaviors generally regarded as behavioral states. These included all play, social explore, groom, clump, environmental exploration, self-directed behaviors, locomotion, and inactivity. Frequencies were analyzed for behaviors regarded as events. These included facial expressions, vocalizations, approaches, retreats, and displacements. Weekly means were calculated for each behavior category and these were analyzed via a series of separate, three-way repeated measures analyses of variance (ANOVA) in which treatment and gender were between groups factors and weeks was the repeated measure. Significant interactions were further analyzed via post hoc tests for simple main effects (17). The relationship between TCDD concentrations in body fat and the overall mean for each behavioral measure was analyzed via linear regression.

RESULTS

Summary Data

Table 3 summarizes the social and nonsocial behaviors that occurred with the longest durations or greatest frequencies. Nonsocial behaviors that occurred with greatest duration in all social groups were *locomotion, inactive, environmental exploration*, and *self-directed behavior*. Social behaviors with the longest durations included *proximity, nonspecific contact, rough-tumble play* and *social explore*. For behaviors regarded as events rather than behavioral states, *approach* and *retreat* occurred most frequently.

TABLE 3

OVERALL BEHAVIOR PATTERNS OF THE MONKEYS IN EXPERIMENT 1

Behavioral States	Seconds/5 Min (Mean ± SE)
Nonsocial	
Locomotion	88.16 ± 6.00
Inactive	206.51 ± 4.80
Environmental exploration	48.60 ± 2.88
Self-directed behavior	14.42 ± 2.05
Social	
Proximity	23.22 ± 2.50
Nonspecific contact	25.34 ± 3.60
Social explore	17.01 ± 3.00
Rough-tumble play	12.05 ± 2.50
Behavioral Events	**Frequency/5 min (Mean ± SE)**
Approach	3.70 ± 0.59
Retreat	1.59 ± 0.30

Treatment Effects

Play behaviors. The treatment × weeks interaction was significant for the initiate rough-tumble play category, $F(8, 56) = 2.68$, $p < 0.025$; Fig. 2. This analysis compared the duration of play initiated by TCDD animals to the duration of play initiated by controls. Post hoc tests of simple main effects for each week indicated significant group mean differences ($p < 0.05$) for weeks 5, 8, and 9 of testing, and a marginally significant difference ($p < 0.10$) for week 6 of testing. During these weeks, TCDD-exposed animals spent more time in self-initiated rough-tumble play than did controls. Such an effect could be due to an increase in the number of play bouts initiated by TCDD-exposed monkeys or to an increase in the length of the play bouts they initiated or both. Further analysis of the data suggested the effect was primarily due to the former. That is, more play bouts were initiated by the TCDD-exposed animals, $F(8, 56) = 2.35$, $p < 0.05$), but the average duration of the bouts was not different than the average length of play bouts initiated by control animals. The main effect of gender and the gender × treatment interaction for initiate rough-tumble play were not significant. There were no significant group differences for the receive or mutual rough-tumble play categories, and when initiate, receive, and mutual rough-tumble play were combined to form a single category which included duration of all rough-tumble play behavior, there were no TCDD-related differences in the overall amount of rough-tumble play.

A significant main effect of treatment was also observed for frequency of play retreats, $F(1, 7) = 6.13$, $p < 0.05$; Fig. 3. TCDD-exposed monkeys retreated less often during play than did control monkeys.

Displacement. The main effect of treatment was significant for the yield to displacement category, $F(1, 7) = 6.42$, $p < 0.05$; Fig. 4. TCDD-exposed monkeys were less often displaced from positions than were control monkeys. There was also a significant main effect of gender for this behavior, $F(1, 7) = 10.51$, $p < 0.025$. Males were displaced less often than females.

Self-directed behavior. There was a significant main effect of TCDD for self-directed behavior, $F(1, 7) = 9.28$, $p < 0.025$; Fig. 5. The TCDD-exposed monkeys engaged in more self-directed behavior. The main effect of gender was also significant for this behavior, $F(1, 7) = 7.04$, $p < 0.05$. Females engaged in more self-directed behavior than did males.

Environmental exploration. There was a significant treatment × gender interaction for the category of *environmental exploration*, $F(1, 7) = 7.97$, $p < 0.05$; Fig. 6. However, post hoc tests for simple main effects of treatment (control males vs. TCDD males; control females vs. TCDD females), and gender (TCDD males vs. TCDD females; control males vs. control females) were not significant.

Other behaviors. None of the other behavioral categories were affected by TCDD exposure.

Linear regressions. There were no significant relationships

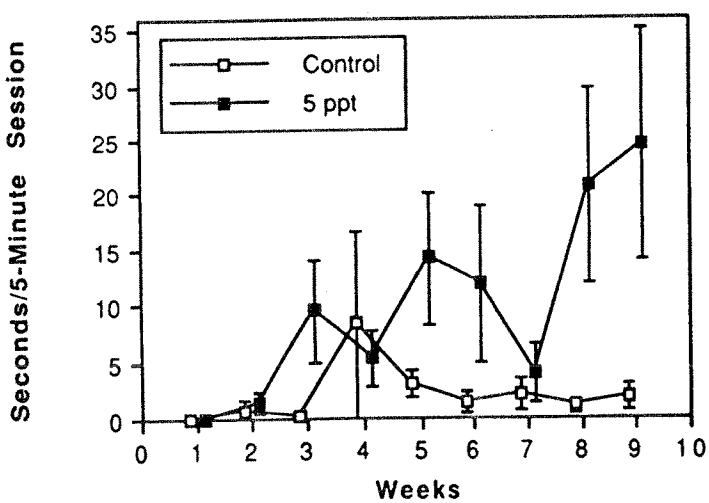

FIG. 2. Mean ± SE for duration of initiate rough tumble play in control and 5 ppt TCDD-exposed monkeys during 9 weeks of testing.

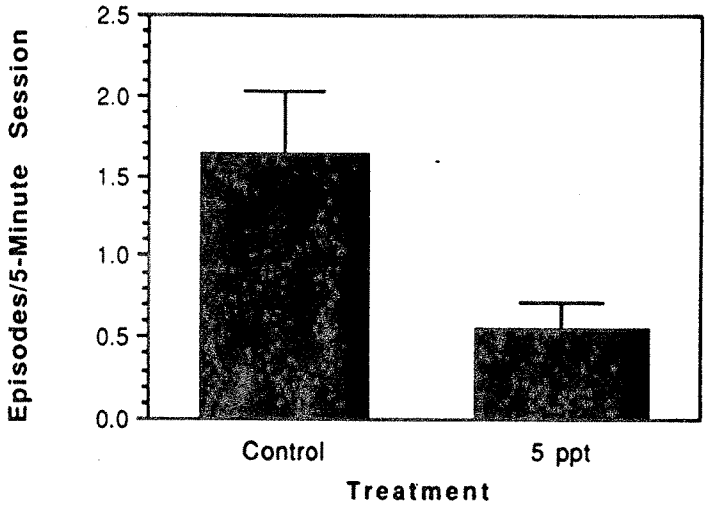

FIG. 3. Mean ± SE for episodes of play retreats for control and 5 ppt TCDD-exposed monkeys during 9 weeks of testing.

between TCDD concentrations in body fat and the above behavioral effects.

DISCUSSION

Several aspects of peer group interactive behavior were altered in perinatally TCDD-exposed rhesus monkey. TCDD-exposed monkeys initiated more rough-tumble play, retreated less during play and were less often displaced from preferred positions in the playroom. TCDD exposure also induced increased levels of self-directed behavior. These behavioral effects were exhibited in the absence of any significant health effects due to TCDD exposure. Although the treatment × gender interaction was significant for environmental exploration, there was not a clear effect of TCDD on this behavior. Post hoc comparisons for simple main effects of treatment and gender were not significant.

Decreased social play has been reported in monkeys following perinatal exposure to either lead or methylmercury (4,5,18). Unlike those other toxicants, TCDD did not alter the overall amount of social play. However, it is difficult to draw comparisons with those studies because infants in those studies were nursery reared, whereas the TCDD-exposed monkeys in the current study were mother-reared. Also, the age at which the animals in the current experiment were tested and their prior test experience differed from that of monkeys in the other studies.

The finding that TCDD-exposed animals initiated more play bouts is interesting, but is difficult to put into perspective because few other studies have recorded the initiator and recipient in play bouts. Interestingly, gonadectomized rhesus monkeys of both sexes exhibited more episodes of play than unoperated controls (21). This finding may be relevant to the current study given that TCDD is a potent anti-estrogen and

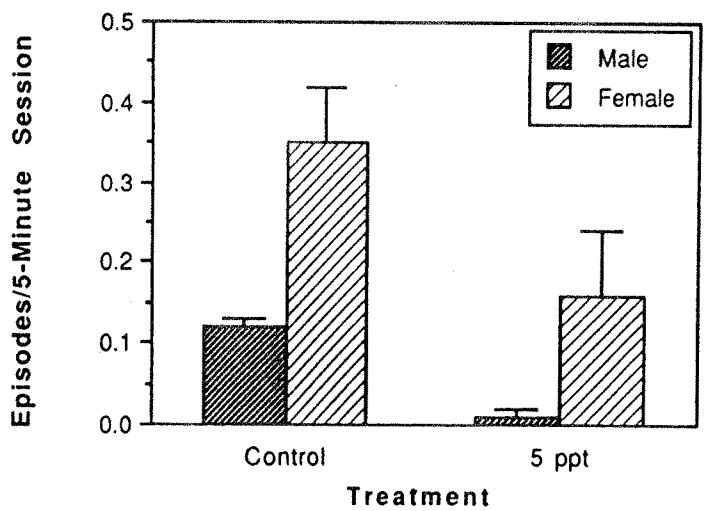

FIG. 4. Mean ± SE for episodes of yield to displacement for male and female control and 5 ppt TCDD-exposed monkeys during 9 weeks of testing.

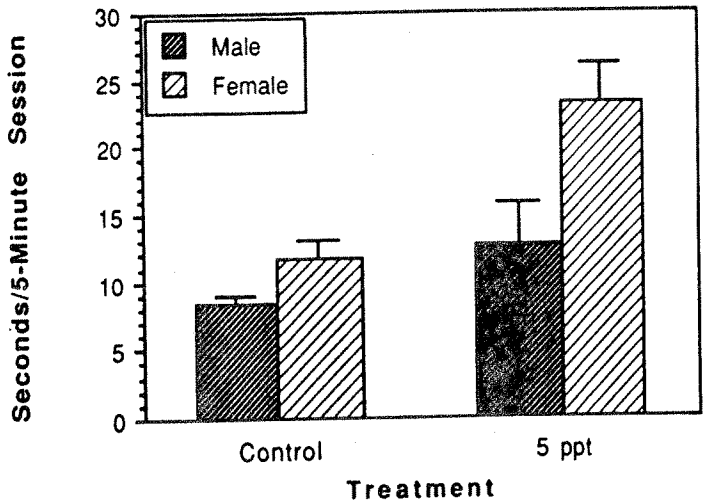

FIG. 5. Mean ± SE for duration of self-directed behaviors for male and female control and 5 ppt TCDD-exposed monkeys during 9 weeks of testing.

that perinatal TCDD exposure has been shown to demasculinize and feminize male rats (25). The mechanism through which TCDD might masculinize female behavior is not as clear. However, a recent study does suggest that perinatal TCDD-exposure masculinizes the behavior of female rats (32).

The biological significance of increased initiation of social play in terms of the overall social adjustment of the animal is difficult to assess. Most studies that have addressed the functional importance of play have used play deprivation procedures and have not separated play into initiate and receive categories. Another finding was that TCDD-exposed monkeys were less often displaced from preferred positions in the playroom. Together with their increased initiation of social play, this might suggest that the TCDD-exposed monkeys were more dominant than controls.

TCDD-exposed offspring also engaged in significantly more self-directed behavior than did control offspring. Significant increases in self-directed behavior have also been seen in lead-exposed rhesus monkeys (9,18). Increased self-directed behavior has generally been considered to be a maladaptive behavior pattern. Increased self-directed behavior is a well known hallmark of the abnormal behavioral syndrome seen in monkeys reared in various conditions of social deprivation and has also been shown to have its counterparts in emotionally disturbed children (12,36).

It is unclear why TCDD-exposed offspring exhibited this putatively maladaptive behavior pattern and at the same time showed an increased tendency to initiate social play and a decreased tendency to be displaced, behaviors that are usually considered to be adaptive. One possible explanation is that the overall level of behavioral arousal was increased in the TCDD-exposed offspring. Increased behavioral arousal has

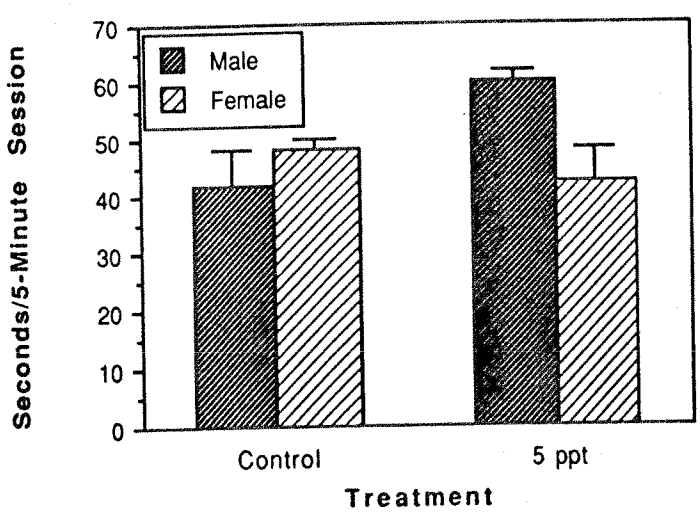

FIG. 6. Mean ± SE for duration of environmental exploration for male and female control and 5 ppt TCDD-exposed monkeys.

been proposed by Siviy et al. (33) to explain the increased play, activity, and exploration seen in juvenile rats of both genders treated with idazoxan, an alpha-2 noradrenergic antagonist. Consistent with the theory of behavioral arousal is the finding that these TCDD-exposed offspring were more active than control offspring when they were tested in a crossed photobeam activity cage from 5.5 to 6.5 months of age (30).

There were no clear relationships between TCDD concentrations in body fat and any of the behavioral effects. Given the variability of social behavioral data (both between and within subjects), the gender differences in some of the affected behaviors, and the small *n* in this study, the failure to detect significant relationships between TCDD concentrations in body fat and behavioral effects is not surprising. As Tachibana (38) has illustrated, an *n* much larger than that in the present study would be needed to reliably detect a dose–effect relationship.

EXPERIMENT 2

METHOD

Subjects and Maternal TCDD Exposure

Following weaning of the offspring used as subjects in Experiment 1, the two TCDD-exposed groups were maintained on the TCDD diets for a total of 3.5 (5 ppt group) or 4.0 (25 ppt group) years. During this time, the control and TCDD-exposed adult females were bred a second time with unexposed males. One 25 ppt female died as a result of injuries received from a male during that breeding. Social behavioral data were not collected from offspring of the second breeding round and they are not discussed further here. At the end of TCDD exposure, the adult female monkeys were removed from the experimental diets and placed on a control diet of Purina monkey chow. Estimated total TCDD intake over the course of the experiment was 163 ± 8 ng/kg for the 5 ppt group and 938 ± 36 ng/kg for the 25 ppt group.

Beginning 10 months after exposure to the TCDD diets ended, 20 of the 23 remaining females were bred with unexposed males. The procedures used for mating and pregnancy detection were the same as those described for Experiment 1, except that 10 unexposed males were used as breeders. The three 25 ppt females with the least reproductive success were not bred and were used instead for a kinetics study of TCDD clearance (3). The remaining four 25 ppt females conceived. One gave birth to a stillborn infant at term. The other three all gave birth to viable offspring. Seven of the eight 5 ppt females conceived and gave birth to viable offspring. All eight control females conceived. One gave birth to a stillborn infant at term. The other seven delivered viable infants. The subjects tested in Experiment 2 consisted of three 25 ppt TCDD-exposed offspring (2 males and 1 female), seven 5 ppt TCDD-exposed offspring (5 males and 2 females), and six control offspring (2 males and 4 females).

Except for TCDD exposure, all control offspring were subjected to the same experimental procedures as were TCDD-exposed offspring. As in Experiment 1, both control and TCDD-exposed offspring were weaned at 4 months of age, placed on a solid diet of Purina monkey chow, and housed individually in wire mesh cages that allowed auditory, visual, and olfactory contact with other monkeys. After weaning, they had no physical contact with other monkeys until peer-group testing began. Exposure of the offspring to TCDD was transplacental and transmammary and ended when they were weaned at 4 months. There was no TCDD exposure through access to the mother's food because all mothers were on a control diet at this time.

Mesenteric fat samples were collected via laparotomy from each of the offspring at weaning (4 months of age) and analyzed for TCDD content via the same method described in Experiment 1 (13). TCDD concentrations in the mesenteric fat of offspring born to and nursed by the previously exposed 5 ppt TCDD females were 188 ± 58 ppt (range: 99–523), while concentrations in the mesenteric fat of offspring born to and nursed by the previously exposed 25 ppt TCDD females were 827 ± 284 ppt (range: 380–1400).

None of the surviving TCDD-exposed or control mothers exhibited any significant health problems during the course of Experiment 2. However, one female control infant died during the preweaning period, apparently as the result of a shigella infection. None of the other TCDD-exposed or control offspring suffered from any unusual or serious health problems, or altered incidence of health problems, and none of the TCDD-exposed offspring exhibited any signs of developmental toxicity during the course of the study.

The birth weights of the 5 and 25 ppt TCDD-exposed offspring were not significantly different from those of controls. Both groups of TCDD-exposed infants gained about 10% less weight than controls prior to weaning. The weights of animals in all three groups were very similar until day 40. At that time they began to diverge, with the controls gaining weight more rapidly than the exposed animals during the last two-thirds of the nursing period. The 10% weight deficit at weaning was not significant, and after weaning the exposed animals' weights caught up to those of the controls within a few weeks. There were no significant differences in body weight at the time of behavioral testing.

Procedure

Phase 1. The test apparatus and methods of data collection were the same as described in Experiment 1 except that three behaviors *nonspecific contact, misjudgments*, and *play retreats* were dropped in an effort to simplify the behavioral scoring system. Testers required extensive training to score these behaviors reliably. Experiment 2 was initiated before the results from Experiment 1 were completely analyzed and data on a potentially interesting behavior, *play retreats*, were lost. Beginning when they were 8.7 ± 0.2 months of age, the monkeys were placed in peer groups of four monkeys each, for 1.5 h per day, Monday through Friday, and allowed to interact without interference. Groups were matched as nearly as possible for age, gender, and maternal TCDD exposure (Table 4). The maximum age differences within the four social groups were 2.5 weeks to 2.2 months. As in Experiment 1, each social group contained both control and TCDD-exposed offspring and each monkey was socialized only with the three other monkeys in its group. Behavioral testing began on day two of socialization and was conducted 4 days per week (Tuesday through Friday) for a total of 48 sessions in 12 consecutive weeks. Test time was counterbalanced such that each social group was tested an equal number of times at each of four test times (11:30 a.m., 12:40 p.m., 1:50 p.m., and 3:00 p.m.).

Phase 2. After data collection for Phase 1 was completed, the monkeys were socialized daily in the same peer groups until they were 18.0 ± 0.5 months of age. At that time, the monkeys were assigned to new social groups of three or four animals each. The new groups were matched as nearly as pos-

TABLE 4

COMPOSITION OF SOCIAL GROUPS IN EXPERIMENT 2

Group	Monkey #	Gender	TCDD (ppt)	Mother/Father		D.O.B.
			Phase 1: Mixed Groups			
1	AL20	F	0	108	T78	3/19/84
	AL21	F	5	102	S30	3/20/84
	AL25	M	5	106	T14	4/03/84
	AL27	M	5	101	T78	4/15/84
2	AL26	M	5	97	CL1	4/15/84
	AL28	F	25	94	X50	4/16/84
	AL31	M	0	109	R11	4/26/84
	AL34	F	0	96	CL1	5/02/84
3	AL32	M	25	95	S74	4/30/84
	AL36	M	5	111	Z52	5/10/84
	AL38	F	0	112	X93	5/15/84
	AL53	F	5	113	X93	6/02/84
4	AL77	M	25	98	S74	7/17/84
	AL80	F	0	93	R11	8/03/84
	AL81	M	5	103	Q96	8/08/84
	AL88	M	0	90	Z52	9/23/84
			Phase 2: Treatment Groups			
1	AL20	F	0	108	T78	3/19/84
	AL31	M	0	109	R11	4/26/84
	AL38	F	0	112	X93	5/15/84
2	AL21	F	5	102	S30	3/20/84
	AL26	M	5	97	CL1	4/15/84
	AL36	M	5	111	Z52	5/10/84
3	AL25	M	5	106	T14	4/03/84
	AL27	M	5	101	T78	4/15/84
	AL53	F	5	113	X93	6/02/84
	AL81	M	5	103	Q96	8/08/84
4	AL28	F	25	94	X50	4/16/84
	AL32	M	25	95	S74	4/30/84
	AL77	M	25	98	S74	7/17/84
5	AL34	F	0	96	CL1	5/02/84
	AL80	F	0	93	R11	8/03/84
	AL88	M	0	90	Z52	9/23/84

sible for age and gender, but each group contained only monkeys from the same treatment condition (Table 4). Age differences within the new groups were from 1.75 to 4.5 months. The new groups were socialized for 1 h per day, Monday through Friday. They were tested 4 days per week (Tuesday through Friday) for a total of 72 consecutive sessions. Test times (11:00 a.m., 12:45 p.m., 2:30 p.m. and 4:15 p.m.) were counterbalanced across groups.

Data Analysis

All 24 behaviors scored in Experiment 2 occurred with sufficient frequency to warrant statistical analysis. As in Experiment 1, durations were analyzed for behaviors generally regarded as behavioral states, and frequencies were analyzed for behaviors regarded as events. For Phase 1, weekly means were calculated for each behavior and analyzed via a series of two-way repeated measures ANOVAs in which treatment was a between groups factor and weeks was the repeated measure. Significant interactions were further analyzed via post hoc tests for simple main effects (17) and Dunnett's test as appropriate (8). As in Experiment 1, overall means for each behavioral category were also calculated for each animal and the relationship to TCDD in body fat was analyzed via linear regression. For Phase 2 analysis, means were calculated for 2-week periods and analyzed similarly to Phase 1. Because of gender distribution, it was not feasible to analyze for gender-related effects in Experiment 2.

As discussed in a recent review by Hertzog and Rovine (15), the mixed-model ANOVA is not robust to violations of the assumption of homogeneity of variance across the repeated measure (the assumption of circularity). Violations of circularity can lead to an increase in Type 1 errors. In Experiment 2, all significant effects were treatment × repeated measures interactions, and thus, violation of the circularity assumption was a concern. Therefore, Huynh-Feldt df adjusted F tests were used to correct for heterogeneity of variance in the repeated measure (22). In the Huynh-Feldt procedure, a correction factor (Epsilon) which ranges from 0 to 1.0 is calculated. Degrees of freedom for both the numerator and denominator are then multiplied by the correction factor to yield a new, more conservative critical F value. Perfect homogeneity produces a correction factor of 1.0 and the critical F does not change. The Huynh-Feldt Epsilon is derived from the Greenhouse–Geisser correction and compensates for the fact that Greenhouse–Geisser is overly conservative when the sample size is small. All reported df's are the Huynh–Feldt corrected values.

RESULTS

Summary Data

Table 5 summarizes the social and nonsocial behaviors that occurred with the longest durations or greatest frequencies in Phases 1 and 2 of Experiment 2. As in Experiment 1, nonsocial behaviors that occurred with greatest duration in all social groups were *locomotion, inactive, environmental exploration,* and *self-directed behavior*. Social behaviors with the longest durations were *proximity, rough-tumble play,* and *social explore*. For behaviors regarded as events, *approach* and *retreat*

TABLE 5

OVERALL BEHAVIOR PATTERNS OF THE MONKEYS IN EXPERIMENT 2

Behavioral States	Phase 1 Seconds/5 Min (mean ± SE)	Phase 2 Seconds/5 Min (mean ± SE)
Nonsocial		
Locomotion	96.79 ± 7.65	111.21 ± 10.91
Inactive	75.41 ± 7.54	105.13 ± 8.68*
Environmental exploration	27.04 ± 2.94	38.87 ± 4.50*
Self-directed behavior	12.16 ± 1.83	12.13 ± 1.90
Social		
Proximity	27.04 ± 2.94	15.69 ± 3.07*
Social explore	23.66 ± 2.59	12.59 ± 2.05*
Rough-tumble play	19.01 ± 2.92	10.11 ± 2.13*
Behavioral Events	Frequency/5 min	Frequency/5 min
Approach	5.87 ± 0.97	6.06 ± 1.35
Retreat	2.41 ± 0.46	5.29 ± 0.96*

*$p < 0.05$.

occurred most frequently. The average durations and frequencies for most behaviors in Phase 1 were very similar to those observed in Experiment 1 (Table 3). The only significant differences were for inactive and environmental exploration. Animals in Experiment 1 spent more time exploring the environment (48.6 vs. 27.1 s). The large difference for inactive appears to be related to a difference in the way the behavior was scored in the two experiments. In Experiment 1 any pause in locomotion was scored as inactive, whereas in Experiment 2 inactive was scored only if the animal was not engaged in any social or exploratory activity or any self-directed behaviors.

In Experiment 2, the behavior of the animals in Phase 2 differed from their behavior in Phase 1 in several interesting ways. In Phase 2, they were more inactive, spent less time in proximity to other animals, and retreated from other animals more frequently. They also engaged in less social exploration and rough-tumble play and explored the environment more. In other words, there was less social interaction between the animals during Phase 2 testing.

Treatment Effects

Phase 1. For Phase 1, in which the monkeys were socialized in groups which contained both control and TCDD-exposed monkeys, there was one significant TCDD-related effect. There was a significant TCDD × weeks effect for the behavioral category, receive social explore, $F(21, 134) = 1.73$, $p < 0.05$. A visual examination of the data revealed that this was very likely a spurious effect related to a large peak in receive social explore which occurred during week 3 for the 25 ppt TCDD-exposed group.

Phase 2. Play behaviors. For Phase 2, in which the monkeys were socialized in groups containing only monkeys from the same TCDD exposure condition, the treatment × 2 week blocks interactions were significant for both the initiate and the receive rough-tumble play categories. The pattern of the effect was very similar for the two categories. Therefore, these categories were combined to form a single category which also included mutual rough-tumble play. The treatment × 2-week blocks interaction for the combined category, total rough-tumble play, was also significant, $F(10, 65) = 2.09$, $p < 0.05$; Fig. 7. The control monkeys showed very little variation in the amount of time they spent in rough-tumble play over the 8 two-week blocks of testing. The 5 ppt groups also showed very little variation in rough-tumble play. Although their means were above those of the control group for each 2-week block, these differences were not significant. On the other hand, the 25 ppt group engaged in virtually no rough-tumble play during the first two, 2-week blocks of testing (16 test sessions). By the third 2-week block, this group began to engage in more rough-tumble play and by the fourth 2-week block was similar to the 5 ppt group. Post hoc tests for simple main effects between groups at each block and within groups over blocks were not significant.

The treatment × 2-week blocks interaction was also significant for the category playface, $F(6, 38) = 4.90$, $p < 0.05$; Fig. 8. Again, post hoc tests indicated no significant differences between groups for any of the eight individual 2-week blocks. However, tests within groups across blocks were significant for the control group, $F(2, 11) = 10.73$, $p < 0.01$ and for the 25 ppt group, $F(7, 14) = 2.96$, $p < 0.05$. Monkeys in the control group showed a sharp increase in the number of playfaces starting after the sixth 2-week block and maintained this level until the end of testing. The 25 ppt group showed virtually no playfaces initially and then the frequency of playfaces gradually increased throughout the remaining seven 2-week blocks of sessions. In contrast, the 5 ppt groups showed very little variation over weeks. There were no significant group differences for the noncontact social play or self-motion play categories.

Displacement. There was a significant treatment × 2-week blocks interaction for the category yield to displacement, $F(8, 52) = 2.27$, $p < 0.05$; Fig. 9. Post hoc tests indicated no significant differences between groups at any given 2-week block. However, tests within groups over blocks were significant for the control group, $F(4, 20) = 4.31$, $p < 0.05$, and the 25 ppt group, $F(7, 14) = 9.23$, $p < 0.01$. The control group showed

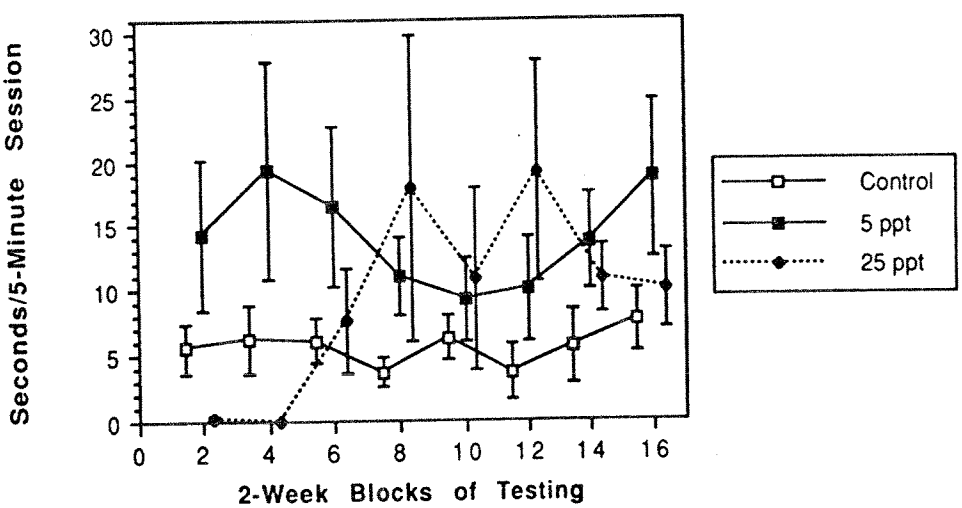

FIG. 7. Mean ± SE for duration of rough tumble play in control, 5 ppt TCDD-exposed, and 25 ppt TCDD-exposed monkeys across 2-week blocks of test sessions.

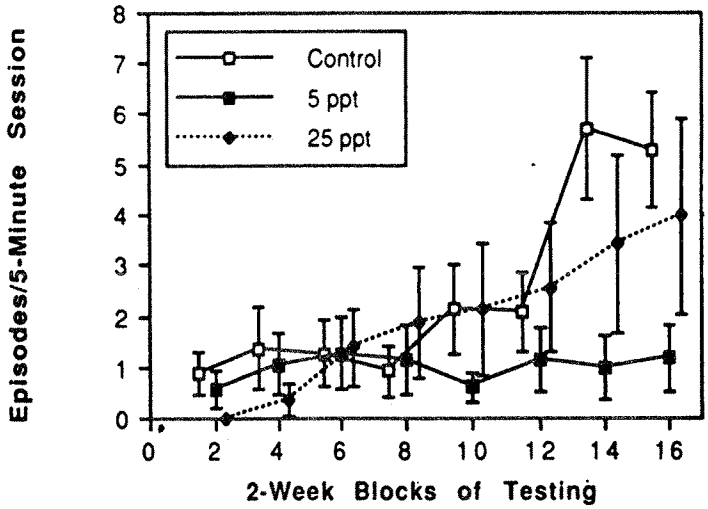

FIG. 8. Mean ± SE for episodes of play face in control, 5 ppt TCDD-exposed, and 25 ppt TCDD-exposed monkeys across 2-week blocks of test sessions.

a gradual increase over weeks and the 25 ppt group increased abruptly after the 6th block. In contrast, the 5 ppt group was fairly stable across blocks.

Self-directed behavior. The main effect of treatment, $F(2, 13) = 3.86, p < 0.05$, and the treatment × 2-week block interaction, $F(12, 78) = 2.44, p < 0.05$, were significant for the category of self-directed behavior (Fig. 10). *Post hoc* tests indicated no significant differences between groups at any given two-week block and no significant differences within groups across blocks.

Other behaviors. None of the other behavioral categories were affected by TCDD exposure.

Linear regressions. As in Experiment 1, there was no relationship between TCDD concentration in body fat and any of the behavioral effects.

DISCUSSION

Phase 1

The only significant TCDD-related effect observed in Experiment 2, Phase 1 was a treatment × weeks interaction for the behavioral category, receive social explore. Visual examination of the data indicated that the effect was related to a

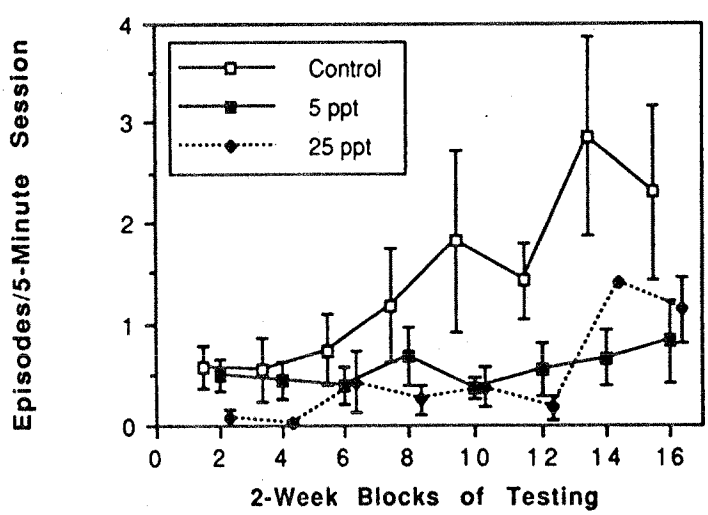

FIG. 9. Mean ± SE for episodes of yield to displacement for control, 5 ppt TCDD-exposed, and 25 ppt TCDD-exposed monkeys across 2-week blocks of sessions.

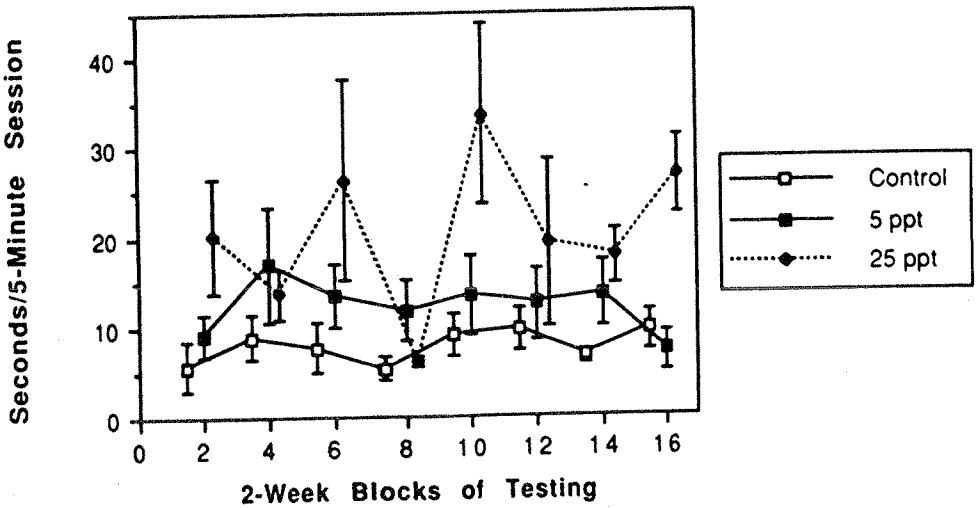

FIG. 10. Mean ± SE for duration of self-directed behaviors for control, 5 ppt TCDD-exposed, and 25 ppt TCDD-exposed monkeys across 2-week blocks of sessions.

large but transient increase in the incidence of this behavior in the 25 ppt TCDD-exposed group during week 3 of the 12-week test period. Given that 24 separate analyses were run, only one significant effect was seen, and the pattern of the effect was very unusual, it is quite likely that this was a spurious effect unrelated to the TCDD exposure of the animals.

Phase 2

The overall behavior of the animals in Phase 2 differed from that in Phase 1. During Phase 2, the animals were more inactive, spent less time in proximity to other animals, and retreated more often from other animals. They also engaged in less social exploration and less rough-tumble play and exhibited higher levels of environmental exploration. Together these findings suggest the animals were engaging in less social interaction during Phase 2. This was most likely an effect of age. Young animals of many species are more active and play more than older animals. However, the possibility that the change from four to three animals/group accounted for some of these differences cannot be excluded.

Significant TCDD-related differences in social play emerged when the monkeys of Experiment 2 were shifted to the Phase 2 social groups which contained only monkeys from the same TCDD exposure condition. Rough tumble play and the play related behavior, playface, were almost completely suppressed in the 25 ppt TCDD-exposed offspring for the first two, 2-week blocks (16 sessions) of testing. This effect was most pronounced for the category rough tumble play. Virtually no rough tumble play was observed in the 25 ppt offspring during the initial two blocks of testing. Rough-tumble play of the 5 ppt group was fairly stable across Phase 1 and Phase 2 and was somewhat elevated with respect to controls during both phases. This difference was probably related to the fact that the 5 ppt group was predominantly male, whereas the control group was predominantly female. It is well-known that male monkeys engage in more rough-tumble play than do females (37). Given that the 25 ppt group also contained more males than females, it seems unlikely that the gender distribution was responsible for the suppression of play seen in that group. However, the results should still be considered preliminary and interpreted with caution because of the small *n* in the 25 ppt group.

During later blocks of testing, both TCDD-exposed groups failed to show the increases in playface and yield to displacement that were seen in control offspring. The influence of gender imbalance on these behaviors is harder to assess because the differences between males and females have not been studied extensively. However, in our studies, females always yield to displacement more often than males. Therefore, the observed differences for this behavior could be related to the gender distribution of the groups.

In addition to altered patterns of social play, there was a dose dependent increase in self-directed behaviors in the TCDD-exposed groups during Phase 2 testing. Again, the effect was most pronounced in the 25 ppt group. Overall, the 25 ppt monkeys engaged in 2.5 times more self-directed behavior than the controls. In Experiment 1, females exhibited more self-directed behavior than males. It was difficult to assess whether there was a gender effect for self-directed behavior in Experiment 2 because of the uneven gender distribution in the groups. However, given the results of Experiment 1, it is possible that the increase in self-directed behavior may have been even more dramatic if the control group had not been mostly female and exposed groups had not been mostly male. As discussed earlier, self-directed behavior is generally considered to be a maladaptive behavior pattern and increased incidence of self-directed behavior has been associated with abnormal behavioral syndromes in both monkeys and humans (12,36).

The fact that suppression of social play and increased self-directed behavior in the 25 ppt monkeys did not emerge until Phase 2 of testing might have resulted from several factors. It is possible that the effects may have been maturational and did not emerge until after Phase 1 testing was completed. Alternatively, the behavioral differences may have been induced by the stress of adapting to new peers. It is also possible that the presence of unexposed and less highly exposed mon-

keys in the social groups during Phase 1 may have helped to facilitate normal social behavior in the 25 ppt TCDD-exposed monkeys.

A similar suppression of social play has been observed in lead-exposed rhesus monkeys when they were socialized in groups containing only lead-exposed monkeys (5). In that experiment, the monkeys were socialized on alternate days in one of two different play groups: one that contained only lead-exposed monkeys and one that contained both lead-exposed and control monkeys. Durations of play behavior were significantly reduced when lead-treated monkeys were socialized in the groups which contained only other lead-treated monkeys but not when they were socialized in the groups containing control monkeys. This finding suggests that the presence of unexposed animals in the play group may indeed help to "normalize" the behavior of toxicant-exposed animals. Although the findings for the present study are consistent with this interpretation, no firm conclusions can be reached because the study was not specifically designed to test this hypothesis and because the small *n* in the 25 ppt group and uneven gender distribution across groups may have influenced the results.

GENERAL DISCUSSION

Phase 1 of Experiment 2 was similar to Experiment 1 in that the monkeys were the same age at the time of testing and were socialized in groups which contained both TCDD-exposed and control monkeys. The behavioral patterns of the animals in the two experiments were similar in many respects. However, a similar pattern of treatment effects was not seen. The TCDD-exposed monkeys in Experiment 1 initiated more rough tumble play, retreated less during play, were displaced less often, and exhibited more self-directed behavior than control monkeys. The TCDD-exposed monkeys of Experiment 2 did not exhibit similar effects.

While the offspring tested in Experiment 1 were born concurrent with maternal TCDD exposure, those tested in Experiment 2 were born approximately 16 months after maternal TCDD exposure had ended. As a result, the mean tissue concentration of TCDD was substantially lower for the 5 ppt offspring in Experiment 2 (188 ppt vs. 377 ppt). This may account for the lack of effects in the 5 ppt offspring of Experiment 2.

The lack of behavioral effects is more difficult to explain for the 25 ppt offspring of Experiment 2. The mean tissue concentration of TCDD was substantially higher for that group (827 ppt) than for the Experiment 1 five ppt group, yet no significant effects were seen in Phase 1. One important difference between the two experiments is that the gender make-up of the experimental groups differed. In Experiment 1, both the TCDD-exposed and control groups were gender balanced (3 males and 3 females in each group); whereas in Experiment 2, both TCDD-exposed groups were predominantly male and the control group was predominantly female. Because self-directed behavior was higher in females in Experiment 1, the gender ratio of the groups may have served to mask any effects on self-directed behavior in Experiment 2.

Gender imbalance cannot easily explain the lack of effects in the 25 ppt monkeys on the initiate rough-tumble play or yield to displacement categories. However, the alterations in these behaviors that were observed in the 5 ppt monkeys of Experiment 1 could be viewed as facilitations of behavior. The animals initiated more play and were less often displaced from preferred positions by other monkeys. It is not uncommon to observe a facilitation of behavior with low levels of exposure to a drug or chemical and no effect or a deficit at higher levels of exposure. For example, monkeys exposed to low levels of TCDD, PCBs, or lead all show facilitated behavior on a spatial learning task (19,20,29). Monkeys exposed to higher doses of the same toxicants do not show a similar facilitation of behavior, and in fact, those exposed to the highest doses show profound deficits on the same behavioral task (19,20).

In conclusion, the results of the current study indicate that low level perinatal exposure to TCDD may alter the later peer group social behavior of offspring. Altered patterns of social play and an increased incidence of self-directed behavior were observed at TCDD exposure levels which did not produce clinical symptoms of toxicity or significantly depress the birth weights or weaning weights of offspring. In Experiment 1, offspring were born concurrently with maternal TCDD exposure and altered behavior patterns were observed when TCDD-exposed monkeys were socialized in groups which contained both control and TCDD-exposed peers. In Experiment 2, offspring were born after maternal TCDD exposure had ended and effects were not observed when the animals were socialized in mixed groups. **It was only when TCDD-exposed offspring were socialized with TCDD-exposed peers from the same exposure condition that significant effects emerged.**

It is important to point out that the TCDD-exposed infants in these studies were reared by TCDD-exposed mothers. As a result, we cannot rule out the possibility that the peer group behavioral changes reported here were indirect effects related to differences in the early socialization of the infants rather than direct effects of TCDD exposure. We have reported that TCDD-exposed mother-infant dyads spend more time than control dyads in close social contact, particularly in ventral-ventral contact and nipple contact (31). There are no studies that we are aware of to indicate whether or not these early behavioral changes might account for some or all of the peer group behavioral changes observed later.

The results of this study and others (4,5,18) suggest that social interactions with peers are very sensitive to disruption by perinatal toxicant exposure and point to the need to include assessments of social behavior when toxic agents are evaluated for neurobehavioral effects. Although adequate early social experience with peers has been shown to be critical to a normal course of social development (14,35), the effects observed in these TCDD studies were quite subtle and long-term follow-up studies would be needed in order to determine whether the early TCDD exposure of these monkeys had any permanent effects on their social adjustment.

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