Neurobehavioral Assessments of Rats Perinatally Exposed to a Commercial Mixture of Polychlorinated Biphenyls

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Received October 17, 2001; accepted February 11, 2002

Because of behavioral deficits associated with gestational exposure to PCBs in children, we sought to quantify neurobehavioral effects of perinatal exposure to Aroclor 1254® (A1254), a commercial mixture of PCBs, in rats. Pregnant Long-Evans rats were fed A1254 at doses of 0, 1.0, or 6.0 mg/kg/day throughout gestation and nursing. The growth and behavior of their male and female offspring were assessed both during development and as adults, using a variety of behavioral tests that included a neurobehavioral screening battery (functional observational battery [FOB] and automated tests of locomotor activity), habituation of motor activity, acquisition of a visual discrimination, and performance of a visual signal-detection task. During the suckling period, A1254 at 6 mg/kg reduced survival and body weight gain of offspring of both sexes; however, locomotor activity was unaffected, and only small and transient changes in other measures were evident. In adulthood, perinatal exposure to A1254 did not affect habituation of locomotor activity, acquisition of the visual discrimination, or sustained attention. Rats performing the signal-detection task were challenged with cocaine (0, 1.25, 2.5, 5.0 mg/kg) and haloperidol (0, 0.003, 0.010, 0.030 mg/kg) to probe the integrity of dopaminergic systems in the central nervous system (CNS). A1254 did not alter the impairment of attention caused by haloperidol. Cocaine reduced false alarms more in controls than in rats exposed to A1254, but the effect was not clearly related to the dose of A1254. Perinatal exposure to this commercial PCB mixture had very little effect on these tests of behavior during development and in adulthood.

Key Words: attention; cocaine; neurobehavioral screening; functional observational battery; habituation; haloperidol; learning; motor activity; PCBs; perinatal exposure; signal detection; Aroclor 1254.

Polychlorinated biphenyls (PCBs) were manufactured in the United States from 1929 to 1977, mainly as nonflammable alternatives to mineral oils for capacitors and transformers in the electrical industry. These stable compounds were produced as complex mixtures of congeners that differ in the number and pattern of chlorine substitutions on the biphenyl rings. Because of their chemical stability, their use was expanded to include immersion and cutting oils and dispersants for pesticides. However, this stability also inhibits their degradation, leading to their ubiquitous presence in the biosphere (Tanabe, 1988), and their lipophilicity has facilitated bioaccumulation of the compounds and their metabolites in the food chain.

Concern over the systemic toxicity of PCBs arose during two tragic episodes of poisoning from rice oil contaminated with PCBs and dibenzofurans, which resulted in the birth of children with a variety of physical, emotional, and intellectual problems (Rogan et al., 1988). PCBs have also been associated with neurotoxic effects in humans and animals (Seegal, 1996; Tilson and Kodavanti, 1998), particularly after exposure during development (Jacobson and Jacobson, 1997; Lonky et al., 1996; Palanza et al., 1999; Schantz et al., 1991; Stewart et al., 2000; Tilson et al., 1990). The present study was undertaken as part of an ongoing research program to explore the developmental neurotoxicity of PCBs, using the rat as a model species. This work focuses on a commercial PCB mixture, Aroclor 1254® (A1254), which we chose because it contains a high proportion of ortho-substituted, highly chlorinated biphenyls. Both of these characteristics have been associated with developmental neurotoxicity (Frame, 1999; Kodavanti et al., 2001; Stewart et al., 2000). In addition, assessment of risk to humans from environmental PCB toxicity has been based on the reference doses derived from animal studies with A1254 and other commercial mixtures (Coglano, 1998).

Recent studies have confirmed that perinatal administration of A1254 can be neurotoxic to rats. When tested as adults, these animals show elevated hearing thresholds and changes in behavior that suggest impaired cognitive function. Thus, male offspring of rats dosed with 6 mg/kg/day of A1254 from gestational day 6 through weaning made more reference mem-
ory errors in a 12-arm radial maze than did controls, although female offspring were unaffected (Roege et al., 2000), and similar effects on working memory errors did not reach statistical significance in an overall analysis. Siblings of these animals were unimpaired in acquisition and performance of a hippocampus-dependent water maze task, yet nevertheless they showed a decrement in the magnitude of hippocampal long-term potentiation (LTP) evoked by electrical stimulation in situ, as well as an increase in the train density required to induce the LTP (Gilbert et al., 2000). Other siblings showed sex-dependent differences in learning a series of spatial discrimination reversals: whereas males perseverated on the first reversal, females exhibited an associative deficit in reversals later in the series (Widholm et al., 2001). In still other siblings of both sexes, postnatal exposure to A1254 elevated auditory thresholds at 1 kHz (Crofton et al., 2000b), confirming previous evidence for low-frequency hearing loss associated with exposure to this PCB mixture (Crofton et al., 2000a; Goldey and Crofton, 1998; Goldey et al., 1995; Herr et al., 1996). This low-frequency hearing loss has again been replicated in a study that found no effects of perinatal A1254 on electrophysiological indexes of visual, somatosensory, or peripheral nerve function (Herr et al., 2001). Reduced concentrations of thyroid hormones, developmental delays in weight gain and motor activity, and acceleration of eye opening also followed perinatal exposure to A1254 (Goldey et al., 1995).

In contrast to the developmental effects of A1254, its toxicity in adult rats is much reduced. Motor activity was decreased and flavor aversion conditioning was induced by repeated doses greater than 10 mg/kg/day and 7.5 mg/kg/day, respectively, and higher doses were needed to cause these effects acutely (Nishida et al., 1997). Consistent with these observations, dietary exposure of adult rats to A1254 at doses up to 6.9 mg/kg/day was without significant neurobehavioral toxicity (Freeman et al., 2000). However, acute exposure of hippocampal slices to A1254 blocked long-term potentiation and synaptic transmission, indicating that high concentrations of the mixture can impair neural functioning in vitro (Niemi et al., 1998).

We report here the results of assessments of the physical and behavioral development of male and female offspring of rats dosed orally with A1254 during gestation and lactation. Growth and neurological integrity were evaluated using behavioral screening tests throughout development, and assessments of learning, attention, and vision were conducted in adulthood. Previous observations of sensory and motor changes during development after perinatal exposure to A1254 (Goldey et al., 1995) suggested that the observational and manipulative measures of a functional observational battery (FOB) and motor activity might detect similar effects in the present study. In addition, we undertook assessments of nonassociative learning (inter-session habituation of motor activity), associative learning (acquisition of a lever-press response via autoshaping and acquisition of a visuospatial discrimination), and sustained attention (visual-signal detection) to look for evidence of cognitive dysfunction, an important concern after developmental exposure to PCBs (Jacobson and Jacobson, 1997; Lonky et al., 1996; Palanza et al., 1999; Roege et al., 2000; Schantz et al., 1991; Tilson et al., 1990; Widholm et al., 2001). Habituation of motor activity was taken as an index of nonassociative learning (e.g., Kling, 1971; Mackintosh, 1974; Platel and PorsoI, 1982). Challenges with haloperidol and cocaine, dopaminergic drugs that affect a wide spectrum of dopamine receptors, were administered in light of evidence for persistent changes in dopamine concentrations in the brains of rats exposed to PCBs, either in adulthood (Seegal et al., 1991) or perinatally (Seegal et al., 1997). The results of autoshaping and visual threshold studies conducted with these animals have already been reported (Geller et al., 2001).

**MATERIALS AND METHODS**

**Subjects.** Adult female Long-Evans hooded rats were inseminated at Charles River Laboratory (Portage, MI) on gestational day 0 (GD 0), shipped on GD 3, and housed at the EPA laboratory in animal facilities approved by the Association for Assessment and Accreditation of Laboratory Animal Care International. The animals were housed in standard plastic hanging cages with sterilized pine shavings as bedding; they were housed in same-sex and same-litter pairs from weaning until postnatal day (PND) 84, and singly thereafter. Food (Purina lab chow) and water were provided ad libitum until PND 84, after which feeding was scheduled to maintain constant body weights in the rats in the operant experiments (see below). Temperature was maintained at 21 ± 2°C and relative humidity was maintained at 50 ± 10% with a 12-h light-dark cycle (0600–1800 h). All of the experiments were approved in advance by the institutional animal care and use committee of the National Health and Environmental Effects Research Laboratory, U.S. EPA.

The primary study was conducted in two replications (cohorts) of offspring of dosed rats. Each of six litters in Cohort 1 and six litters in Cohort 2 contributed one male and one female pup to each dose group in the study. Cohorts 1 and 2 were born 2 months apart. The schedule of treatment and assessments is shown in Figure 1. The FOB and motor activity tests were administered on PND 17, 28, 43, and 65; body weight data were also collected 2–3 times per week from PNDs 3–65. Habituation of motor activity was assessed from PND 73–77 for Cohort 1 and PND 81–85 for Cohort 2. Acquisition of lever pressing via autoshaping was assessed from PND 112 to 137 in both cohorts. Four of the six males and females from each litter in each cohort were then selected randomly for further assessments of the effects of treatment on visual discrimination learning, sustained attention, and visual thresholds (α = 8 per sex and A1254 treatment group). Visual signal detection training occurred from 4.5 to 8 months of age, and sustained attention was assessed between 9 and 12 months of age. Visual threshold testing, using a modification of this task, occurred between 17 and 19 months of age. Sustained attention was then challenged by haloperidol and cocaine between 21 and 24 months of age. The autoshaping and visual threshold experiments have been reported by Geller et al. (2001).

**Dosing with Aroclor 1254.** A commercial PCB mixture, A1254 (lot #124–191; purity >99%) was purchased from AccuStandard, Inc. (New Haven, CT) and dissolved in corn oil. The congener composition of this mixture has been published elsewhere (Kodavanti et al., 2001). The selected doses were 0, 1.0, and 6.0 mg/kg/day. Pregnant female rats were given either the corn oil vehicle or one of the concentrations of A1254 in corn oil (2 ml/kg) by oral gavage from GD 6 through PND 21, except on the day of parturition, when the dams were not disturbed. Dams were assigned randomly to treatment groups with the restriction that the groups were balanced for body weight gain from GD 2 to GD 6. In Cohort 1, nine dams were assigned to the control group,
FIG. 1. Time line of the assessments performed on the animals in this experiment. Shaded areas indicate the age range at which treatment with A1254 and each assessment occurred. Results of the Autoshaping and Visual Threshold studies are reported in Geller et al. (2001). FOBs: Horizontal lines indicate the 4 days on which the functional observational battery was performed. The vertical line to the left of Weight Maintenance indicates the period during which the animals’ body weights were maintained at 85% of their free-feeding weights for visual discrimination training and signal detection testing.

12 to the 1-mg/kg group, and 14 to the 6-mg/kg group. In Cohort 2, 15 dams were assigned to the control group, 17 to the 1-mg/kg group, and 12 to the 6-mg/kg group. Dosing occurred once per day between 0800 and 1000 h. Each dam was weighed daily before dosing. Six litters from each group in each cohort were randomly selected for the present study. Other litters were used either by Herr et al. (2001), or for neurochemical analysis or measurement of PCB levels in brain (Kodavanti et al., 2000).

Beginning on GD 22, rats were checked twice daily (A.M. and P.M.) for births, and the date that birth was first discovered was assigned PND 0. All dams (>90% successful pregnancies) gave birth within a few h and the litter size varied between 4 to 17 pups. On PND 4, litters were culled to eight pups per litter (4 males and 4 females); any litter with fewer than eight pups was terminated immediately. Three male and three female pups from each litter were randomly selected for neurochemical analyses (one each on PND 7, 14, and 21; to be reported elsewhere). Neurobehavorial assessments were conducted on the fourth male and female pup from each litter. Body weights of the offspring were recorded twice per week during lactation, prior to dosing the dam, and once per week from weaning at PND 21 to PND 65. These pups were housed in pairs matched for sex and treatment from PND 21 to the beginning of operant testing at PND 84–88, after which time they were housed singly (see below).

Assessments of reproductive outcome and development. Litter size was determined on PND 1. Postpartum mortality was assessed by noting the number of deaths in each litter during PND 0–4. Mortality during lactation was noted between PND 5–22. Body weights of the dams were collected from GD 5 to PND 21, and weights of the pups that were selected for behavioral assessments were taken from PND 3 to PND 65. Maternal weights before parturition were analyzed separately from weights after parturition.

Mortality data were converted to a percentage (100 × number of deaths/number of pups in each litter) and analyzed by two-way analyses of variance (ANOVAs) with A1254 dose and cohort as factors. Maternal body weight data were analyzed by a mixed-model ANOVA, with A1254 dose and cohort as between-group factors and day as a repeated measure. The pup weights were analyzed similarly but included sex, nested as a within-litter factor. Significant interactions were followed by simple-effects ANOVAs for the interacting variables (SAS, 1990), and Huynh-Feldt degree-of-freedom (df) corrections were used to reduce the effects of asymmetrical variance-covariance matrices for repeated measures. The Type I error rate was 0.05 for each comparison.

Behavioral Procedures

Functional observational battery and assessment of motor activity. Neurobehavioral evaluations began in both cohorts on PND 17, prior to weaning. Pups were also tested on PND 28, 43, and 65. Procedural details and scoring criteria for the FOB endpoints and automated assessment of motor activity for postweanling rats are provided in McDaniel and Moser (1993). Previous studies with preweaning rats indicated the need for modifications in some FOB test measures (e.g., changes in scoring criteria); these changes are described in Moser (2000). Behavioral testing required approximately five min per rat, and was followed by a 30-min (for PND 17 rats) or a 60-min (older rats) session in the motor activity chambers.

On test days, rats were transported to an isolated laboratory and allowed at least one h to acclimate before testing began. On PND 17, rat pups were kept in pairs similar to the acclimation period. The observer removed the rat, held it, and scored lacrimation, salivation, and handling reactivity, according to defined criteria. The rat was then placed on a laboratory cart covered with a clean absorbent pad (60 × 90 cm) and surrounded with a perimeter barrier (6.5 cm high). The rat moved about on the cart undisturbed for 3 (PND 17 rats) or 3 (older rats) min, during which time the observer evaluated and scored its level of activity, arousal, ataxia, and any gait abnormalities. Any tremor or other involuntary motor movements were recorded. Next, the rat’s responses to sensory stimuli were scored: these included auditory (metal clicker), somatosensory (touch to the rump), and nociception (tail pinch using forceps) stimuli. In postweanling rats, the ability of the pupil to constrict in response to light was also assessed. Landing foot splay and grip strength (using digital strain gauges) were measured in the older rats. Aerial righting ability was evaluated in postweanling rats; the PND 17 pups were placed on their backs and the ability to attain normal posture was ranked. Rectal temperature data were collected in postweanling rats, and body weight was measured in all rats. The same observer conducted all tests and was unaware of the rat’s treatment level. All rats were tested in the same day, with sex and dose counterbalanced across the time of day.

Immediately after FOB testing, motor activity data were collected in automated devices. These chambers are composed of a series of interconnected alley's in the shape of a figure 8, with two blind alley's projecting from the central arena (Ruppert et al., 1984). Six infrared phototransmitter-dieode pairs were equally spaced around the figure-8 portion of the maze, and one pair was located in each of the blind alley's (total of eight detectors). Activity, defined as photocell interruptions, was recorded in 5-min blocks throughout the session.
Habituation of Motor Activity (Nonassociative Learning)

Apparatus. Six photocell devices (Motron Electronic Motility Meter, Motron Produkter, Stockholm, Sweden) were used to measure motor activity. Each device had a 5 × 8 matrix of photodetectors in the platform that were illuminated by a single overhead lamp; movements that occluded these detectors were recorded as horizontal activity. Each device also had an array of six photomultipliers and detectors that were placed 16.5 cm above the platform to record vertical activity (rearing). Each device was housed in a sound- and light-attenuating ventilated cubicle in a dedicated test room. A clear plastic chamber (33 × 21 × 26 cm) with removable lid was placed over each platform to contain the rat during testing.

Procedure. Motor activity was recorded during five successive 6-min blocks in each of five daily 30-min sessions (M-F) to establish patterns of change in activity over time for each rat, both within each test session and across test sessions. Horizontal and vertical counts were subjected to separate mixed-model ANOVAs with A1254 dose, sex, and cohort as between-group variables and sessions and 6-min blocks as repeated measures (Proc GLM: SAS, 1990). Habituation was quantified as a decrease in activity counts across time, and could occur both across blocks within each session and across sessions.

Signal Detection Behavior (Associative Learning)

Beginning on PND 84 (Cohort 1) or PND 88 (Cohort 2), 35 male (18 from Cohort 1 and 17 from Cohort 2) and 34 female (18 from Cohort 1 and 16 from Cohort 2) rats were housed individually and placed on a restricted feeding schedule. After autoshaping (Geller et al., 2001), the number of rats was reduced by random selection of litters to 48 (n = 8 per dose-group per sex). A target body weight was calculated for each rat at 85% of its free-feeding weight, with an upper limit of 420 g for males and a lower limit of 210 g for females. Target weights for three females in each treatment group were set to 210 g; target weights for two control males, two males in the 1 mg/kg group, and one male in the 6 mg/kg group were set to 420 g. Target weights were achieved over the course of a 2-week period of restricted feeding, with a minimum allotment of 5 g/day. Daily food rations were calculated according to an algorithm developed to maintain constant body weights (Ali et al., 1992). These weights (+10 g) were maintained thereafter by scheduled feeding of rodent chow (Ralston Purina, St. Louis, MO) in the home cage after daily behavioral testing and on weekends. Water was supplied ad libitum in the home cage. All training and testing occurred during the light phase of the diurnal cycle.

After autoshaping, the rats were trained to perform the signal detection task, which involved learning a visual discrimination under the response rule: press one lever in the presence of a visual signal, and the other lever in its absence. Rats from the two cohorts were trained and tested in the same apparatus during alternating periods of 1 to 3 months, with Cohort 1 preceding Cohort 2 at each stage. While rats of one cohort were being tested, the other rats remained in their home cages under weight maintenance conditions. One male rat in the 1-mg/kg group died during training of causes unrelated to treatment, leaving seven rats in that group.

Apparatus. Four standard operant conditioning chambers were used as previously described (Bushnell, 1999, 2001; Bushnell et al., 1997). Each chamber was equipped with a food cup, two retractable levers (a signal lever and a blank lever), a signal light, a loudspeaker, and a 4-kHz tone generator. The food cup was located in the center of the panel, with the two retractable levers on either side. The left lever was designated as the “signal” lever in half the boxes and as the “blank” lever in the other half; the opposite designations were made in the other boxes. The loudspeaker delivered continuous, masking white noise at 60 dB(A). For training, the signal light was initially located directly above the signal lever; it was moved to the top of the box above the food cup after all rats achieved criterion accuracy (≥80% correct).

Training for signal detection. Training followed previously described steps (Bushnell, 1999, 2001) for 12 sessions in Cohort 1. Then, in preparation for visual threshold assessments (Geller et al., 2001), the opaque aluminum top and back of each test chamber was replaced with clear plastic; a new house light was installed on the ceiling of the containment shell surrounding the test chamber; the interior of the shell was painted white; and the original house light was replaced by a tone generator. All trial-dependent changes in illumination were thereafter restricted to the signal light: the food cup light was not used to signal delivery of a food pellet, and the house light was not turned off after each error. These conditions applied for the remainder of training of Cohort 1 and for all training of Cohort 2.

Visual discrimination training began when the animals were 4.5 months old, and it was divided for analysis into two phases: Phase 1 consisted of 12 sessions with the signal light located immediately above the signal lever, and Phase 2 (10 sessions) began after the signal light was moved to the top of the test chamber. Training after each change in conditions continued until each animal achieved criterion accuracy (≥80% correct). Testing conditions for sustained attention were achieved during 34 (Cohort 1) or 39 (Cohort 2) daily 1-h sessions when the rats were 10 to 12 months of age (Fig. 1). For the first 30 (Cohort 1) or 35 (Cohort 2) sessions, test parameters were gradually adjusted to their final values (see Bushnell, 1999, 2001).

Asymptotic performance was quantified in the last four 300-trial sessions, with parameters chosen to challenge sustained attention. Thus, signals were brief (300 ms) and were presented in a highly variable schedule: the pre-signal interval ranged from 0.3 s to 24.4 s, with a mean of 7 s (Fleschler and Hoffman, 1962), and the post-signal interval was 2.3, 4 s (selected randomly) prior to the insertion of the levers. These temporal parameters yielded a rate of about five trials per min. In addition, the signal intensity was varied among seven values (0.003, 0.010, 0.025, 0.066, 0.183, 0.494, or 1.190 lux).

A signal consisted of a 300-ms increase in the brightness of the signal light. “Signal” and “blank” trials were presented in equal number in each session in a pseudorandom sequence. Signal and blank trials differed only in that no signal was presented during a blank trial. Both levers were inserted simultaneously after the post-signal interval. Both levers were retracted when one was pressed or if 5 s passed without a press. If no press occurred, a response failure was recorded and the trial was not repeated. Each correct response (a press on the signal lever on a signal trial, “hit” or a press on the blank lever on a blank...
trial, “correct rejection”) was followed by 4-kHz, 90-dB, 200-ms tone on every trial and delivery of a food pellet into the food cup on 80% of trials. Each incorrect response (a press on the signal lever on a blank trial, “false alarm,” or a press on the blank lever on a signal trial, “miss”), or response failure, was followed by a 0.5-s increase in the intensity of the white noise (from 65 to 72 dB) during a 2-s period of timeout.

Drug challenges. Drug challenges were administered between 22 and 24 months of age (Fig. 1), after completion of visual threshold tests (Geller et al., 2001). Challenge drugs were cocaine hydrochloride (Sigma, St. Louis, MO) and haloperidol (Sigma). Haloperidol was administered sc in saline 15 min before testing in a volume of 1 ml/kg. Cocaine was administered sc in saline 30 min before testing in a volume of 2 ml/kg. Doses of haloperidol were 0, 0.003, 0.010, and 0.030 mg/kg; doses of cocaine were 0, 1.25, 2.50, and 5.00 mg/kg. Cohort 1 received haloperidol before cocaine; Cohort 2 received cocaine before haloperidol. Within each drug series, the doses were administered in an order counterbalanced for A1254 treatment, sex, test time, and test box. Injections were given twice per week with at least 48 h between successive doses.

Data analysis. Separate ANOVAs (general linear model) were conducted for each endpoint (Proc GLM; SAS, 1990). Each analysis was dictated by the experimental design: A1254 dose, cohort, and sex were treated as between-subject variables in all analyses, and day of testing, drug dose, and/or signal intensity were treated as repeated measures, as appropriate. Sex was not analyzed as nested within litter, because not all litters were represented in the experiment by one male and one female. No litter was represented by more than one animal of each sex, however. In all analyses, Huynh-Feldt degree-of-freedom (df) corrections were used to reduce the effects of asymmetrical variance-covariance matrices for repeated measures. Statistical significance was tested with a Type I error rate of 0.05 for each ANOVA.

During acquisition of the signal detection rule, performance was quantified by accuracy, as percent correct for each 100-trial session. Percent correct during the first 12 days of acquisition was subject to a four-way ANOVA with day, cohort, sex, and A1254 dose as independent variables. To quantify sustained attention in the signal detection task, the number of hits, misses, correct rejections, and false alarms were recorded for each signal intensity during each of the last four test sessions. The proportion of hits (P(hit) = [number of hits]/[number of hits + number of misses]) and the proportion of false alarms (P(fa) = [number of false alarms]/[number of false alarms + number of correct rejections]) were calculated for each signal intensity. P(hit) was then adjusted for guessing (Green and Swets, 1974) by the following formula: P*(hit) = (P(hit) – P(fa))/[1 – P(fa)]. P*(hit) values were subjected to a four-way ANOVA with A1254 dose, sex, and cohort signal intensity as independent variables. Response failures were counted as the number of times in each session that a rat did not press either lever during the 5-s limited hold period, and were analyzed by Friedman’s nonparametric ANOVA due to non-normal distributions of scores (Conover, 1971). Latency was recorded for each response and was defined as the time between insertion of the levers into the chamber and the subject’s lever press. P(fa), latencies for hits, and response failures were analyzed with separate three-way ANOVAs with A1254 dose, cohort, and sex as independent variables. Intensity was excluded as a factor, because these endpoints did not change reliably across intensity (Bushnell et al., 1994). Drug challenge data were analyzed analogously, adding drug dose as a repeated measure in the analyses of P*(hit), P(fa), latency, and response failure.

RESULTS

Maternal weight and pregnancy outcome. Treatment with A1254 did not alter the maternal body weights during gestation or lactation (Fig. 2); neither the main effect of A1254 dose nor the dose × day interaction was significant (Fs < 1 for both effects during both time periods). Body weights of dams in Cohort 1 were significantly higher than those in Cohort 2, both during gestation and lactation (cohort main effects during gestation: F(1,72) = 44.29, p < 0.0001; during lactation: F(1,68) = 47.72, p < 0.0001). Moreover, these differences did not interact with A1254 dose (cohort × dose interaction Fs < 1 during both gestation and lactation). The pregnancy rate for both cohorts was 88% for dams in the control group, 97% in the 1-mg/kg group, and 94% in the 6-mg/kg group. One dam appeared to be pregnant in the 6-mg/kg group but failed to deliver pups. One dam in the control group showed signs of stress, including shivering and chromodacryorrhea during the first days of dosing, and subsequently died; no necropsy was performed. Three dams, one in each dose group, died during dosing; necropsy verification was not performed.

Litter size ranged from 4 to 17 pups with an overall mean of 11.6 pups per litter, and did not differ significantly across cohorts or treatment groups. Litters with fewer than 8 pups on PND 4 were discarded (litters discarded: Cohort 1: control; 1; 1 mg/kg; 2; 6 mg/kg, 1; Cohort 2: control, 1; 1 mg/kg, 1; 6 mg/kg, 0). Pup mortality between the date of birth until culling on PND 4 was not affected by A1254 (Dose main effect F[2,78] = 1.37, p = 0.26) in either of the cohorts (cohort main effect and cohort by dose interaction Fs < 1). However, more pups from dams given 6 mg/kg A1254 died between PND 5 and PND 22, compared to pups from control dams (dose main effect F[2,78] = 6.55, p = 0.0024) (Table 1). Pup mortality did not differ between the cohorts at any time (cohort main effect F[1,78] = 2.04, p = 0.16; cohort by dose interaction F[2,78] < 1).

Body weight of offspring. Body weight during development was lowered by A1254, but this effect differed across age (dose by day interaction F[34,476], p = 0.0070); there were no significant interactions of dose with cohort or sex. Analyses of each day showed lowered body weight in the 6-mg/kg group, from PND 10 to 38 (Fig. 3).

In adulthood, A1254 reduced the body weights of high-dose male and female offspring in Cohort 1 but not in Cohort 2 (dose by cohort interaction F[2,68] = 4.11, p = 0.022) (Table 2). When weighed from PND 80 to 84 under ad libitum feeding conditions, the 6-mg/kg females in Cohort 1 were 8% lighter than their controls and the 6-mg/kg males were 4% lighter than their controls. This difference was not apparent in Cohort 2. Limiting the upper and lower bounds of target weights (see Materials and Methods) reduced, but did not eliminate, these effects. Averaged across cohorts, there was no significant effect of A1254 on body weight of either sex.

Functional observational battery and motor activity. Few functional domains were altered overall. Excitability was significantly affected by A1254 (F[2,30] = 4.39, p = 0.0027) on PND 17. Analyses of individual endpoints revealed that handling reactivity scores were significantly lower in the 6-mg/kg-dose group (sexes combined; mean scores: control = 2.5; 6 mg/kg = 2.0). No other domains were altered.
Postweanling rats showed significant cohort interactions in the sensorimotor (dose main effect $F[2,30] = 16.88, p = 0.0001$; dose by cohort interaction $F[2,30] = 6.76, p = 0.0038$) and activity (dose main effect $F[2,30] = 7.60, p = 0.0021$; dose by cohort $F[2,30] = 4.19, p = 0.0248$) domains. Step-down analyses (sexes combined) of the sensorimotor measures showed general increased responses in Cohort 1 only: increased click response across time (mean score: control = 2.8, 6 mg/kg = 3.1; dose $\chi^2[2] = 9.26, p = 0.0097$) and increased tail-pinch response on PND 28 only (mean score: control = 4.1, 6 mg/kg = 4.5; dose by day interaction $\chi^2[4] = 9.73, p = 0.0452$). Transient, inconsistent, and non-dose-related changes in a few activity measures were also observed: decreased open-field activity in 1-mg/kg females only on PND 28 in Cohort 1, and increased home-cage activity in 1 mg/kg males only on PND 28 in Cohort 2.

**Habituation of motor activity (assessment of nonassociative learning).** Habituation of horizontal motor activity, defined as a decrease in activity counts across blocks, occurred within each test session (main effect of block $F[4,268] = 607.54, p < 0.0001$). Habituation was not affected differently in rats exposed perinatally to A1254 (insignificant dose by block interaction). Exposure to A1254 did not affect overall horizontal activity level, nor did A1254 dose interact significantly with any other independent variable.

Habituation of vertical motor activity also occurred within the test sessions (block effect $F[4,268] = 469.79, p < 0.0001$). However, exposure to A1254 did not affect total vertical activity counts or habituation of vertical activity. In addition, there were no significant interactions among A1254-dose animals and the other independent variables.

Significant three-way interactions among cohort, session, and block for both horizontal ($F[16,52] = 2.29, p < 0.0129$) and vertical activity ($F[16,52] = 3.03, p < 0.0013$) indicated that the two cohorts displayed slightly different patterns of habituation. Graphic display of within-session habituation data, collapsed across sex and dose, revealed that the within-session decrease in activity was more acute across sessions in Cohort 1 than in Cohort 2.

**TABLE 1**

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<th>Control</th>
<th>1 mg/kg/day</th>
<th>6 mg/kg/day</th>
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<td>Postpartum (PND 0–4)</td>
<td>10.2 ± 5.5</td>
<td>1.1 ± 0.5</td>
<td>9.7 ± 4.9</td>
</tr>
<tr>
<td>Lactational (PND 5–22)</td>
<td>2.1 ± 1.2</td>
<td>6.5 ± 2.1</td>
<td>12.0 ± 2.7*</td>
</tr>
</tbody>
</table>

*Note. Values are mean (±SEM) percent mortality for litters of both cohorts. *Significantly different from control.

**FIG. 2.** Maternal body weights during perinatal exposure to A1254. Values shown are mean (± SE) body weights of pregnant female rats from Cohorts 1 and 2 (combined) dosed from GD 6 through PND 21 with 0 ($n = 24$), 1 ($n = 29$), or 6 ($n = 26$) mg/kg/day A1254. PND 0 was defined as the day of birth. A1254 did not significantly affect maternal body weight.
Signal detection behavior (assessment of associative learning). Acquisition of the signal detection response rule was assessed during 12 sessions across 3 weeks of training (Fig. 4): the day of training had a large effect on accuracy (day main effect $F[11,396] = 90.68$, $p < 0.0001$). Cohort 1 learned the discrimination more quickly than did Cohort 2 (cohort main effect $F[1,36] = 13.85$, $p = 0.0007$; cohort-by-day interaction $F[11,396] = 4.79$, $p < 0.0001$). However, neither A1254 dose nor sex significantly affected accuracy nor interacted significantly with day or cohort (all $p$’s $> 0.07$).

Perinatal exposure to A1254 did not affect sustained attention in adulthood: no significant differences were observed in the accuracy of signal detection as a function of A1254 dose in either males or females (Fig. 5). Whereas $P^*(\text{hit})$ increased monotonically with signal intensity in all groups (intensity main effect $F[6,210] = 856.6$, $p < 0.0001$), neither the main effect of A1254 dose ($F < 1$) nor the A1254 dose-by-intensity interaction ($p > 0.33$) was significant. The only significant effect on $P^*(\text{hit})$ involved the sex-by-intensity interaction ($F[6,210] = 2.82$, $p < 0.03$), which resulted from lower $P^*(\text{hit})$ values for females at signal intensities of 0.025 and 1.19 lux and values not different from those of the males at the other signal intensities (Fig. 5). Neither $P(\text{fa})$ nor response latency was affected significantly by any factor in the analyses (all $p$’s $> 0.12$). No rat failed to respond more than 10 times per 300-trial session; the response failure rate did not differ significantly across the treatment groups (Friedman’s $\chi^2(5) = 3.0$, $p > 0.25$).

Drug Challenges

Haloperidol. The high dose of haloperidol (0.030 mg/kg) significantly reduced $P^*(\text{hit})$ and increased $P(\text{fa})$ in both males and females (Fig. 6) in all A1254 treatment groups, equivalently. For $P^*(\text{hit})$, the significant main effect of haloperidol dose ($F[3,102] = 25.59$, $p < 0.0001$) and the haloperidol dose-by-sex interaction ($F[3,102] = 3.00$, $p < 0.04$) resulted from a greater drug-induced reduction in $P^*(\text{hit})$ in the males than in the females. The main effect of A1254 dose was not significant; the only significant interaction involving this factor

### Table 2

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Females</th>
<th>Males</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>1 mg/kg/day</td>
</tr>
<tr>
<td>1</td>
<td>263.4 ± 7.8</td>
<td>275.9 ± 5.4</td>
</tr>
<tr>
<td>2</td>
<td>263.0 ± 5.9</td>
<td>250.3 ± 6.7</td>
</tr>
<tr>
<td>Mean</td>
<td>263.2 ± 5.6</td>
<td>263.1 ± 5.6</td>
</tr>
</tbody>
</table>

Note. Body weights are measure as mean ± SEM, g. Ad libitum values reflect weights from free-feeding conditions just prior to establishing weight maintenance by scheduled feeding on PND 84 to 88.
was a 4-way interaction among haloperidol dose, cohort, sex and A1254 dose ($F(6,102) = 2.55, p < 0.03$). Step-down analyses of this interaction revealed an anomalous increase in $P^*(\text{hit})$ with increasing haloperidol dose only in the group of females given 1 mg/kg of A1254 in Cohort 1 (not shown); this change attenuated the overall effect of haloperidol in the females.

$P^*(\text{hit})$ increased with signal intensity (intensity main effect $F(6,204) = 457.44, p < 0.0001$) and haloperidol also affected the shape of this function (dose-by-intensity interaction $F(18,612) = 2.89, p < 0.0001$). This interaction resulted from small, intensity-dependent reductions in $P^*(\text{hit})$ by the two lower doses of haloperidol ($p = 0.05$) and a clear, intensity-independent effect of the high dose ($p < 0.0001$). Contrasts across doses at each intensity showed that the high dose reduced $P^*(\text{hit})$ significantly at all intensities; 0.01 mg/kg reduced it at 0.49 lux, and 0.003 mg/kg reduced it at 0.003 lux. Step-down analyses of a significant four-way interaction among haloperidol dose, intensity, cohort, and sex ($F(18,612) = 2.28, p < 0.002$) indicated that the effect of haloperidol was more strongly dependent upon the signal intensity in the males of Cohort 1 than in the males of Cohort 2 or in any of the groups of females.

Haloperidol also increased $P(\text{fa})$ (main effect of dose $F(3,102) = 3.05, p < 0.035$), this effect being significant only at the highest dose (Fig. 6). No other factor in the analysis significantly affected $P(\text{fa})$. Hit latency was not affected by any factor in the analysis. Females failed to respond more frequently than did males (main effect of sex $F(1,34) = 7.12, p < 0.012$), but this difference was not affected by haloperidol or A1254.

To summarize, haloperidol decreased $P^*(\text{hit})$ at all signal intensities in all PCB groups, with a greater effect in males than in females, and increased $P(\text{fa})$ in all groups at the highest (0.03 mg/kg) dose. Haloperidol neither slowed responding nor engendered response failure at these doses.

Cocaine. Cocaine exerted no detectable effect on $P^*(\text{hit})$ in rats of either sex (not shown). The main effect of intensity was highly significant $F(6,204) = 536.65, p < 0.0001$; in addition, significant intensity by sex $F(6,204) = 2.89, p < 0.025$ and intensity by sex by A1254 dose $F(12,204) = 2.41, p < 0.02$ interactions involved sex-dependent differences in visual threshold similar to those reported previously (Geller et al., 2001). In addition, the maximum $P^*(\text{hit})$ was lower in females than in males, partly because of a higher false alarm rate in the females, suggesting a sex-dependent difference in attentiveness. Finally, a significant five-way interaction ($F(36,612) = 1.66, p < 0.015$) was traced by step-down analyses to a greater flattening of the $P^*(\text{hit})$ by intensity gradient by A1254 in the males in Cohort 1 compared with Cohort 2 and a cocaine dose by A1254 dose-by-intensity interaction in the females of Cohort 2 only. Because these effects were not consistent across cohorts, they were not considered further.

Cocaine did reduce $P(\text{fa})$ slightly (Fig. 7). In addition, treatment with A1254 altered the effect of cocaine on $P(\text{fa})$ (A1254 dose by cocaine-dose interaction $F(6,102) = 2.25, p < 0.05$). Step-down analyses showed that the middle dose of cocaine (2.50 mg/kg) reduced $P(\text{fa})$ in the control group, 1.25 mg/kg cocaine reduced $P(\text{fa})$ in the 1-mg/kg-A1254 group, and none of the doses of cocaine significantly affected $P(\text{fa})$ in the 6-mg/kg-A1254 group. Examination of Figure 7 suggests that the high dose of A1254 may have caused a dextral shift of the cocaine dose-response function, although this effect was small and not clearly related to the dose of A1254.

No independent variable significantly affected response latency (all $p$’s > 0.06). As in the haloperidol study, female rats failed to respond more often than did males (main effect of sex $F(1,34) = 5.81, p < 0.025$), but neither cocaine nor A1254 altered this effect.

**DISCUSSION**

The behavioral consequences of exposure to A1254 during gestation and nursing were subtle at best, given that this dosing...
regimen increased pup mortality and decreased body weight gain during development. The lack of effect of A1254 on maternal weight argues against maternal toxicity affecting the pups. However, measures of maternal care were not obtained; thus, possible influences of maternal behavior on the development of the pups cannot be ruled out. Regardless of its source, and even considering the positive findings from other assessments of these animals (Geller et al., 2001), the effects of perinatal A1254 on detailed assessments of complex behavior in adulthood were no more dramatic than was its effect on the physical growth of the pups.

Repeated assessments of neurobehavioral function revealed very few effects of perinatal exposure to A1254. The data showed only a transient decrease in reactivity in the high-dose pups on PND 17, increased responses to some stimuli, and inconsistent changes in activity. Motor activity was not affected, as has been reported in previous rat studies (see Goldey et al., 1995; Tilson et al., 1990). In addition, other measures of motor function (e.g., gait, coordination, righting, and grip strength) were not altered in this study. These findings are not consistent with the gross motor alterations reported in children exposed to environmental levels of PCBs (reviewed in Brouwer et al., 1995; Tilson et al., 1990), and explanations for these discrepancies are unknown.

Learning, as assessed by habituation of unconditioned motor activity and by acquisition of the visual discrimination required
for the signal detection task, remained intact after perinatal exposure to A1254. The visual discrimination was slower in Cohort 2 than in Cohort 1 (probably because auditory cues replaced visual cues as feedback for correct and incorrect responses in Cohort 2), but A1254 had no significant effect on acquisition in either condition. These results are consistent with assessments of other rats exposed similarly to A1254; for example, Gilbert et al. (2000) observed no effects of A1254 in two tests of learning using the Morris water maze, in either sex of rats, and Zahalka et al. (2001) reported no effects from perinatal exposure with A1254 (8 mg/kg/day, GD 6–PND 21) on either spatial-delayed alternation in the T-maze or on spatial navigation in the water maze. Roegge et al. (2000) reported an increase in reference memory errors in the 12-arm radial maze in male rats, which was evident only when females were excluded from the analysis and did not extend to working memory errors in either sex. In addition, the parallel decline in errors across sessions in the two groups suggested that the treatment might have altered performance strategies in the maze in these animals, rather than the learning per se.

These results contrast with sex-dependent changes in autoshaping in these rats (Geller et al., 2001) and sex-dependent deficits in spatial reversal learning (Widholm et al., 2001) in other rats exposed perinatally to A1254 by the same scenario. Geller et al. suggested that behavior might be altered in a sex-dependent manner by perinatal exposure to A1254, given that the behavior patterns in treated animals could be interpreted as feminization of behavior in the males and masculinization of behavior in the females. Specifically, sex-dependent differences in control animals were reversed by A1254 in tests of autoshaping and visual increment thresholds. In contrast, whereas Widholm et al. observed a clear sex-dependent difference in the effect of A1254 on errors to criterion in reversal learning, these effects occurred in the absence of a sex difference in the behavior of control rats. Taken together, these observations suggest that the conditions under which learning is impaired by this PCB mixture do not generalize broadly, and that sexual dimorphism in controls is not sufficient to cause sex dependence of effects of A1254. This conclusion can be further generalized to include non-behavioral measures, as Herr et al. (2001) reported that A1254 did not affect electrophysiological measures of sensory function, despite observations of sex differences between male and female control animals.

Performance of the signal detection task under conditions challenging to sustained attention (short signal and variable trial timing) revealed no effects of perinatal exposure to A1254. The equivalent behavior of all groups under these conditions substantiates the lack of effect in this task of perinatal exposure to PCB126, a non-ortho-substituted PCB congenor (Bushnell and Rice, 1999). Conversely, test conditions favoring psychophysical measurements (long signal and constant trial timing) revealed consistent sex-dependent differences in visual threshold in these A1254 rats (Geller et al., 2001), whereas no such pattern was observed after exposure to PCB126 (Geller et al., 2000). These results support conclusions that (1) perinatal exposure to PCBs, regardless of substitution in the ortho position, do not affect sustained attention in rats, but that (2) perinatal exposure to the primarily ortho-substituted PCBs in A1254 may subtly alter visual perception.

Two drug challenges were performed to assess the integrity of dopaminergic pathways in these rats, given evidence that exposure of rats to PCBs, either in adulthood (Seegal et al., 1991) or perinatally (Seegal et al., 1997), has been associated with persistent changes in dopamine concentrations in the central nervous system (CNS). Haloperidol, a dopaminergic antagonist acting primarily at D2 receptors (Civelli et al., 1993), caused a dose-related decrease in signal detection consistent with attentional impairment: P*(hit) was reduced at all intensities and P/fa was increased. In contrast, cocaine, a dopamine reuptake inhibitor (Ritz et al., 1987), slightly decreased P/fa without affecting P*(hit), perhaps reflecting a subtle improvement in attending. Perinatal exposure to A1254 may have shifted the cocaine dose-effect function for P/fa, reducing the sensitivity of the 6-mg/kg group to cocaine, but this small effect was observed against an equally tenuous effect of cocaine in controls, casting doubt on the overall efficacy of cocaine in this task. Together, these results provide little support for potential mediation of PCB-induced functional differences by dopaminergic pathways.

We reiterate that robust, sex-dependent effects of this exposure to A1254 on autoshaping and visual function were observed in these same animals (Geller et al., 2001) on endpoints in which sex differences were observed in controls. However, sex-dependent changes in spatial reversal learning were observed in rats similarly treated with A1254 (Widholm et al., 2001) in the absence of sex differences in the behavior of control animals. Furthermore, clear sex differences were ob-
served on several behavioral measures in this study (motor activity, grip strength, some aspects of visual signal detection, and the response to haloperidol), but none were altered by A1254 treatment; Herr et al. (2001) reported a similar pattern. Thus sex-dependent differences in behavior are neither necessary nor sufficient for expressing effects of perinatal exposure to A1254, and these results do not clarify the role of sex in determining the neurobehavioral effects of perinatal exposure to PCBs.

ACKNOWLEDGMENTS

We thank Drs. Kevin M. Crofton and Deborah C. Rice for helpful comments on an early draft of this manuscript; and E. Baker Bailey, Jackie Farmer, Charles Hamm, Kimberley Jarema, Kay Riggsbee, and Tracey Samsam for cheerful, exemplary technical assistance with this project.

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