TOXICOLOGICAL SCIENCES **77,** 315–324 (2004)
DOI: 10.1093/toxsci/kfg252
Advance Access publication November 4, 2003

Motor Impairment in Rats Exposed to PCBs and Methylmercury during Early Development

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Received on July 30, 2003; accepted on September 15, 2003

Epidemiological and laboratory studies indicate that polychlorinated biphenyls (PCBs) and methyl mercury (MeHg) may have additive or interactive adverse effects on nervous system function. Prior studies have shown that high doses of MeHg target the cerebellum and impair balance and coordination, but the effects of PCBs on cerebellar function were unknown. In addition, the combined effects of PCBs and MeHg on cerebellar function have not been studied previously. Therefore, we investigated the effects of developmental exposure to PCBs, MeHg, or PCBs + MeHg on three motor tasks that involve cerebellar functions. Female Long-Evans rats were exposed to MeHg (0.5 ppm in drinking water), PCBs (6-mg/kg/d Aroclor 1254), PCBs + MeHg, or vehicle only beginning 4 weeks prior to breeding, through pregnancy, and continuing through postnatal day (PND) 16. Starting at approximately PND 60, one male and one female from each litter were tested on three motor tasks that involve cerebellar function. PCB + MeHg–exposed rats were impaired relative to the controls on a task requiring them to traverse a rotating rod. Rats exposed to PCBs alone were also somewhat impaired relative to the controls, whereas MeHg-exposed rats were not significantly different from the controls. There were no statistically significant deficits related to PCB or MeHg exposure on a vertical rope-climbing test or a parallel bar test. Our results demonstrate that the possibility of additive neurotoxic effects of PCBs and MeHg needs to be seriously considered.

Key Words: **polychlorinated biphenyls (PCBs); methylmercury; cerebellum; motor learning; rotating rod; rope climb; parallel bars; rats**

Polychlorinated biphenyls (PCBs) and methyl mercury (MeHg) are widespread environmental contaminants that are known to be neurotoxic in laboratory animals and humans (Newland and Paletz, 2000). Both chemicals accumulate in aquatic ecosystems, and the primary source of human exposure is through consumption of contaminated fish, seafood, and marine mammals (Grandjean *et al*., 2001). Conflicting results from two epidemiological studies suggest that the two neurotoxicants may interact to cause neuropsychological deficits in children exposed during early development (Davidson *et al*., 1998; Grandjean *et al*., 2001).

Children in the Seychelles Islands have been examined for MeHg neurotoxicity due to the population's relatively high consumption of MeHg-contaminated ocean fish. These studies have failed to find any reliable neurological deficits associated with prenatal mercury exposure (Davidson *et al*., 1998). Another population was studied in the Faroe Islands originally for their exposure to MeHg. In contrast to the Seychelles Islands, the children in the Faroe Islands whose mothers consumed pilot whale meat and blubber were found to have language, attention, and memory deficits, along with a trend for visuospatial and motor dysfunctions (Grandjean *et al*., 1997). The two studies administered different tests at slightly different ages and used different measurements of mercury exposure (maternal hair vs. umbilical cord blood), but perhaps the most important difference between the two cohorts is that the children in the Faroe Islands were exposed to PCBs in addition to MeHg. Analyses of the children's blood in the Seychelles found no detectable exposure to PCBs (Davidson *et al*., 1998). In addition, average MeHg levels in maternal hair samples indicate that MeHg exposure was actually higher in the Seychelles (where no neurological deficits were observed) than in the Faroe Islands (6.8 vs. 4.27 ppm, respectively) (Davidson *et al*., 1998; Grandjean *et al*., 1997). Thus, it has been speculated that the effects in the Faroe Islands could be due to the additive or interactive effects of PCBs and MeHg. In fact, reanalysis of the Faroe Islands data found PCB-associated deficits within the highest tertile of MeHg exposure (Grandjean *et al*., 2001).

In vitro studies also suggest that the two chemicals may have additive or interactive effects on nervous system function.

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Bemis and Seegal (1999) exposed tissue punches taken from the striatum of the rat brain to PCBs, MeHg, or both PCBs and MeHg. They found that PCBs significantly reduced tissue dopamine (DA) and increased media DA in a dose-dependent fashion. MeHg alone at low concentrations did not change DA levels. However, when tissue was exposed to both PCBs and low concentrations of MeHg, they observed a greater effect on DA than when PCBs were given alone (Bemis and Seegal, 1999). Bemis and Seegal (2000) also found that low concentrations of PCBs and MeHg synergistically increased intracellular calcium concentrations, but at higher concentrations or longer exposure times intracellular calcium concentration was reduced. Both the synergistic and antagonistic effects on calcium release were hypothesized to be due to the interactions of PCBs and MeHg at a common site, the ryanodine receptor (Bemis and Seegal, 2000).

Motor disturbances have been reported following developmental exposure to high levels of either PCBs (Harada, 1976; Rogan *et al*., 1988) or MeHg (Amin-Zaki *et al*., 1981). In the Faroe Islands study, MeHg exposure was associated with poorer performance on the finger-tapping task (Grandjean *et al*., 1997), which can be indicative of cerebellar damage (Ivry and Keele, 1989). However, the Seychelles Islands study did not find an association between MeHg exposure and finger tapping in either the pilot (Davidson *et al*., 2000) or the main study (Myers *et al*., 2003). Epidemiological studies of exposure to lower levels of either chemical have not focused on assessing the full range of motor functions, including balance and coordination tasks that are heavily dependent on the cerebellum. Relatively few studies of developmental exposure to PCBs in animals have tested motor function. Tilson *et al*. (1979) found reduced forelimb grip strength and impairments traversing a wire rod in mice given high doses of a single PCB congener. Developmental motor reflexes (Overmann *et al*., 1987; Pantaleoni *et al*., 1988; Rice, 1999) and general locomotor activity (reviewed in Schantz, 1999) have also been examined. For MeHg, it is clearer that high doses (2–18 mg total dose) cause motor deficits, such as impaired rotarod performance (Sakamoto *et al*., 1993, 2002), retarded or abnormal walking ability (Inouye *et al*., 1985; Watanabe *et al*., 1999), retarded development of swimming ability (Elsner *et al*., 1988; Geyer *et al*., 1985; Olson and Boush, 1975; Vorhees, 1985), hind-limb dysfunction (Inouye *et al*., 1985; Kobayshi *et al*., 1981; Magos *et al*., 1985; Sakamoto *et al*., 1993), and even severe movement and postural disorders (O'Kusky *et al*., 1988) in laboratory animals. MeHg is known to target the cerebellum in humans (Eto, 1997) and in rats (Leyshon and Morgan, 1991). In laboratory studies, prenatal exposure to high doses of MeHg (total dose 2–50 mg in rats and 0.01–0.4 mg in mice) can cause pathological changes in the cerebellum, such as degeneration of Purkinje and granule cells (Chang *et al*., 1977), decreased dendritic arborization of Purkinje cells (Choi *et al*., 1981), and reduced thickness of the internal granular and molecular layers (Sager *et al*., 1984). The effects of PCBs specifically on the cerebellum have only recently been investigated. Morse *et al*. (1996) found that the concentration of glial fibrillary acidic protein (GFAP), a glial cell marker, was increased in the cerebellum of the prenatally PCB-exposed rats on PNDs 21 and 90, indicating reactive gliosis following direct damage of the neurons or glia (Morse *et al*., 1996). More recently, Mervis *et al*. (2002) reported that rats perinatally exposed to PCBs had smaller Purkinje cell branching areas on PND 22 that recovered by PND 60.

Only one *in vivo* laboratory study to date has examined the effects of combined exposure to PCBs and MeHg. Tanimura *et al*. (1980) found that mice exposed to both PCBs and MeHg during development showed impairments on cliff avoidance, visual placement, and hind-limb support tasks. Swimming ability was retarded in all exposed mice, especially in the PCB-exposed groups. Locomotor activity was increased in the high-dose MeHg group on PND 21 and at 10 weeks, while the groups receiving either PCBs alone or the high dose of MeHg plus PCBs showed decreased locomotor activity at 10 weeks postnatal. Learning impairments were also observed in the PCB-exposed groups and PCBs plus the low dose of MeHg group on the water-filled T-maze, while the MeHg or PCBs alone caused impairments on an active avoidance task. The present study is the first to examine the effects of developmental exposure to PCBs alone, MeHg alone, or PCBs and MeHg in combination on motor function in rats. Motor function was examined using three tasks upon which performance is impaired in animals with cerebellar damage (Klintsova *et al*., 1998)—climbing a vertical rope, locomotion on parallel bars, and locomotor performance on a rotating rod.

MATERIALS AND METHODS

Animals and Exposure. Female Long–Evans rats were purchased from Harlan Sprague Dawley (Madison, WI) in three cohorts spaced 6 months apart. Dams within each cohort were evenly distributed across dosing groups. Cohorts 1, 2, and 3 resulted in 16, 12, and 16 litters, respectively, for a total of 44 litters used in the study and $n = 10–13$ litters/group with one male and one female tested per litter. The females were housed individually in standard plastic cages with corn-cob bedding in a temperature- and humidity-controlled room (22°C, 40–55% humidity). The animal facility was AAALAC-approved, and all procedures were performed in accordance with protocols approved by the Institutional Animal Care and Use Committee at the University of Illinois at Urbana-Champaign. All rats were maintained on a 12-h reverse light–dark cycle (lights off at 0900) with food and tap water available *ad libitum*. After a 2-week adaptation period, the females were assigned to one of four exposure groups: (1) vehicle control; (2) PCBs only; (3) MeHg only; or (4) PCBs + MeHg. Exposure began 4 weeks prior to breeding and continued through postnatal day (PND) 16 for all groups.

The PCB-exposed groups received a commercial mixture of PCBs known as Aroclor 1254 (A1254; Accustandard, Lot #124–191) at a dose of 6 mg/kg/day. Dose selection was based on previous studies (Crofton *et al*., 2000b; Gilbert *et al*., 2000; Roegge *et al*., 2000). The 6-mg/kg/d dose of A1254 eliminated the postnatal mortality associated with higher doses, but produced a transient hypothyroxinemia and a transient (10–15%) decrease in postnatal body weight gain that recovered by weaning (Crofton *et al*., 2000b). This dose also caused impairments in spatial learning (Roegge *et al*., 2000; Widholm *et al*., 2001)

and hippocampal long-term potentiation (LTP) (Gilbert *et al*., 2000) along with low-frequency hearing loss (Crofton *et al*., 2000b). A1254 was diluted in corn oil, pipetted onto one-half of a vanilla wafer cookie (Keebler Golden Vanilla Wafers®), and these treated cookies were fed to the female rats daily throughout the exposure period. The dose was adjusted daily to account for weight gain in the dams.

MeHg was mixed with the drinking water at a concentration of 0.5 ppm. This dose was selected based on previous studies of chronic gestational and lactational exposure to MeHg in rats (Newland and Reile, 1999; Rasmussen and Newland, 2001). Using the same exposure protocol, Rasmussen and Newland (2001) found that rats exposed to MeHg during development had altered sensitivity to drug challenges as adults on an operant schedule known as differential reinforcement of high rates (DRH). The female rats had constant access to the water bottles except during the daily weighing of the bottles for determination of water consumption. During breeding, the water bottles were also unavailable to the females for the 9 h of pairing to prevent MeHg exposure to the breeding males. The MeHg females did not have access to unadulterated tap water during the exposure period. There was no difference between groups in the amount of water consumed prior to mating and through gestation. The average MeHg exposure was 41.90 μg/kg/day prior to mating and 58.90 μg/kg/day during gestation. Newland and Reile (1999) found that a similar MeHg exposure resulted in 0.49 ppm Hg in the brain at birth, dropping to 0.045 ppm Hg in the brain at weaning.

For breeding, each female was paired daily with an unexposed male for a maximum of 1 week. Breeding sessions were from 1100 hours to 2000 hours, which was during the dark period of their diurnal cycle. Pregnancy was determined by the appearance of a sperm plug, and the pregnant females were then removed and housed individually. On PND 2, the litters were reduced to 10 pups (five males and five females where possible), cross-fostering with extra pups from the same treatment group as needed. The pups were weaned on PND 21. One male and one female from each litter were randomly selected on PND 28 for motor testing. Other littermates were selected for cognitive testing and hearing assessments (Schantz *et al*., 2003) and for organ weight assessment at weaning (see below). The pups were housed in same-exposure, single-sex pairs or triplets after PND 28.

Motor Testing. The rats began the motor testing after PND 60 (62–67 days of age for Cohort 1, 75–81 days for Cohort 2, and 60–68 days for Cohort 3). Motor testing lasted for 4 weeks. During the first week, the rats were tested on the rope-climb task. The rats were tested on the parallel-bars task during the second week. Half of the rats were tested on the rotating rod during the third week, while the other half were tested during the fourth week. The motor tests were selected based on the involvement of the cerebellum in mediating performance (Klintsova *et al*., 1998; Pellegrino and Altman, 1979). All testers were trained by the same individual and were blind to the treatment conditions of the rats throughout testing. A second person, also blind to treatment, assisted each tester by recording the data and worked with all testers within a cohort to ensure consistency across testing procedures.

Rope Climb. The rats were trained on four consecutive days to climb 2-ft-long vertical ropes leading to a square horizontal platform (15 cm^2) mounted 1 m above the floor. On the first day, the rats were pretrained to climb the thickest of four ropes (3 cm in diameter). For the first trial of pretraining, the rat was placed near the top of the rope close to the platform and guided to climb onto the platform. For the second trial of pretraining, the rat was started midway up the rope, and on the last trial the rat started at the bottom of the rope. On the day after pretraining, the rats were tested using a 2.5-cm rope followed by decreasing rope diameters of 1.9 and 1.25 cm on days 2 and 3, respectively. The rats were given three consecutive trials on each day of testing. For each trial, the rat was placed at the bottom of the rope. If the rat did not start climbing or stopped climbing, then the testers would prod the rat from below or tap the rope above them to lead them up. The time to climb to the platform was recorded on every trial. In addition, every trial was given a subjective rating between 1 and 5 according to the following scale: 1 = excellent performance, no prods or help needed; 2 = small amount of prodding (usually to begin) required; 3 = rat would climb with extensive prodding but would try to go down when prodding was interrupted; 4 = heavy prodding whole length of rope; 5 = could not climb or even hold rope, slid down.

Parallel bars. The parallel-bar apparatus consisted of two parallel wooden rods (0.48 cm in diameter, 91 cm long) that were connected to two wooden platforms (15 cm^2) mounted 1 m off the floor. Four sets of parallel rods were used with varying inter-rod distances, and the distance between the rods was increased on each day of testing. On day 1 of testing the inter-rod distance was 2.5 cm, followed by inter-rod distances of 3.75, 5, and 6.25 cm on testing days 2–4, respectively. The rats were given three consecutive trials on each day of testing. A trial started by placing the rat on the starting platform. The rats were given up to 30 s to explore the platform and to begin crossing the rods voluntarily. If a rat did not start to traverse the bars on its own after 30 s, the tester placed the rat on the bars. Once on the bars, if the rat stopped moving forward, the testers would gently prod it from behind and cue the rat to move forward by lightly tapping the rods in front of the rat. The testers recorded the time to traverse the bars to the end platform along with the number of hind-limb slips made while crossing.

Rotating rod. The rotating rod was a PVC pipe approximately 2 m long and 10 cm in diameter that was suspended 1 m above a foam cushion on the floor by a plywood structure (see Altman and Bayer (1997) for a schematic diagram). This three-sided plywood structure supported the rod and had four plywood flaps on hinges that rested on top of the rod, allowing it to be completely covered. Unlike the commonly used rotarod task, where rats are trained to run in place on a rather narrow rod, for the rotating-rod task used here the rats were trained to cross the 2-m-long rod lengthwise. During pretraining, the rat was guided to cross the apparatus three times. On the first crossing, all plywood flaps were down and the rod underneath the flaps was not visible. On the second crossing, two alternating plywood flaps were lifted so the rat walked on alternating plywood and exposed-rod sections. Lastly, all four plywood flaps were lifted, exposing the entire rod, and the rat walked all the way across on the immobile rod. During pretraining and day 1 of testing, a rod with a rough sandpaper-like surface was used. Day 2 of testing used a smooth PVC rod with two foam sections (each 7.5 cm in length and 1 cm in height). The first foam section was approximately one-third of the way across the rod, while the second foam section was two-thirds of the way across. Day 3 of testing used a smooth PVC rod without the foam sections. On days 1–3 of testing, each rat received three consecutive trials at each of the following rotation speeds: 0, 10, 15, 25, and 30 rpm. For each trial, the rat was placed on the start platform and given 30 s to step onto the rod voluntarily. After 30 s, the tester placed the rat onto the rod. Once on the rotating rod, the rat was guided by the tester's hands to move forward. If necessary, the testers would make a barrier with one of their hands to prevent the rat from retreating back to the start platform. On each trial the time to traverse the rod and the number of slips or falls were recorded. Testing was terminated for the day if a rat made more than five slips or falls in one trial. For analysis, unfinished trials were assigned the maximum score of five slips.

Data Analysis. Data were analyzed via repeated measures analysis of variance (ANOVA) using SPSS 7.5 for MS Windows. The litter was used as the unit of analysis nested within exposure group, and sex was treated as a nested within litter variable. For all motor tasks, the rats were given three consecutive trials, and the mean of those three trials was used for each of the following variables analyzed. For the rope-climb task, the climbing time and the subjective rating were analyzed via separate four-way ANOVAs (EXPOSURE(4) $\times$ COHORT(3) $\times$ SEX(2) $\times$ DAY(3)). For the parallel bars, the traverse time and number of hind-limb slips were also analyzed via separate four-way ANOVAs (EXPOSURE(4) $\times$ COHORT(3) $\times$ SEX(2) $\times$ DAY(4)). For the rotating rod, the number of slips was analyzed via a five-way ANOVA (EXPOSURE(4) $\times$ COHORT(3) $\times$ SEX(2) $\times$ DAY(3) $\times$ RPM(5)). Significant effects were further analyzed via tests for simple main effects and/or planned comparisons of the exposed groups to the control group, as appropriate (Keppel, 1982). Statistical significance was ascribed as $p < 0.05$.

RESULTS

Reproductive and Developmental Outcomes

There were no overt signs of clinical toxicity in the dams from any of the treatment groups. The dams in all exposure groups had gestational lengths, gestational weight gains, liver weights, litter sizes, percent male pups, and percent live births similar to the controls (Table 1). The birth weight of the pups exposed to PCB + MeHg was on average slightly decreased relative to the controls ($p = 0.062$), while no other groups differed from the controls (Table 2). On PND 2, the average weights of the PCB + MeHg pups were significantly decreased ($p < 0.01$) compared to the controls, while the PCB pups were also slightly smaller on average than the control pups ($p = 0.088$) (Table 2). The average pup weights for the PCB and PCB + MeHg groups were consistently significantly smaller than the controls on PNDs 7, 14, and 21 (Table 2). The body weights for the MeHg-exposed pups did not differ from the controls at any preweaning time point (Table 2). In addition, the body weights did not differ between the PCB alone and PCB + MeHg groups, indicating that the effect on growth was PCB-related.

Outcomes at Weaning

Pup brains were collected and weighed at weaning. Since the body weights of the PCB- and PCB + MeHg-exposed rats were decreased on PND 21 (see Table 2), the brain:body weight ratios were examined. The EXPOSURE main effect was significant for the brain:body weight ratio ($F_{3,19} = 6.254$, $p = 0.004$). The brain:body weight ratios were significantly elevated in the PCB- and PCB + MeHg–exposed rats (Table 3), indicating that the brain was at least partially spared from the toxic effects of PCBs on growth. Pup liver:body weight ratios were significantly increased on PND 21 in the PCB-alone and PCB + MeHg–exposure groups (Table 3), which is an effect that was reported previously with PCB exposure (Morse and Brouwer, 1995; Ness *et al*., 1993; Overmann *et al*., 1987; Seo *et al*., 1995) and is likely reflecting liver enzyme induction. Thymus:body weight ratios were significantly decreased on PND 21 in the PCB-alone and PCB + MeHg–exposure groups (Table 3), which is also a toxic effect of PCBs that has been reported previously (Overmann *et al*., 1987; Seo *et al*., 1995). MeHg-exposed pups did not differ from the controls on brain, liver, or thymus to body weight ratios on PND 21 (Table 3).

Body Weights Postweaning

The body weights of rats exposed to PCBs or PCB + MeHg were also significantly reduced postweaning ($F_{3,12} = 6.901$, $p = 0.006$). The body weights were significantly reduced by 5–11% for the PCB-exposed rats and by 11–15% for the PCB + MeHg–exposed rats up to PND 63. In contrast, the body weights of the MeHg-exposed rats were not significantly different from the controls up to PND 63, but the MeHg-exposed rats weighed on average 7% less than the controls during testing, which occurred between PNDs 62 and 102. During testing, the PCB rats weighed on average 8% less and the PCB + MeHg rats on average 11% less than the controls.

Motor Tests

Rope Climb. For the subjective rating score on the rope-climb test, there was a marginally significant EXPOSURE main effect ($F_{3,31} = 2.781$, $p = 0.057$). *Post hoc* comparisons revealed that the MeHg-exposed females performed significantly worse than the control females on day 3 (Fig. 1B). A similar pattern of effects was seen for climbing time (Fig. 2), although the EXPOSURE main effect was not significant ($F_{3,31} = 1.983$, $p = 0.137$). There was also a significant SEX effect. Males received worse rating scores ($F_{1,31} = 49.749$, $p < 0.001$) and took longer to climb ($F_{1,31} = 55.274$, $p < 0.001$) than females (Figs 1 and 2).

Parallel Bars. For the number of hind-limb slips on the parallel bars, there was a significant three-way (EXPOSURE × SEX × DAY) interaction ($F_{9,96} = 2.476$, $p = 0.014$). *Post hoc* analyses of the data revealed that the MeHg males made fewer hind-limb slips than the control males on testing days 3 and 4 (Fig. 3A). There were no significant differences in the number of hind-limb slips among the females (Fig. 3B). The two-way EXPOSURE × SEX interaction was also significant ($F_{3,32} = 3.710$, $p = 0.021$), while neither the EXPOSURE × DAY interaction ($F_{9,96} < 1$, $p = 0.937$) nor the EXPOSURE main effect ($F_{3,32} < 1$, $p = 0.448$) was significant. In addition, there was a significant effect of DAY ($F_{3,96} = 6.843$, $p < 0.001$).

TABLE 1
Reproductive Outcomes of Dams Exposed to MeHg, PCB, or PCB + MeHg during Gestation and Lactation

Exposure	Gestational length (days)	Dam gestational weight gain (g)	Dam liver weight (g)	Litter size	% Male	% Live births
Control	23.0 ± 0.0	120.2 ± 8.7	14.5 ± 0.4	10.5 ± 1.2	54.5 ± 5.7	98.5 ± 0.8
MeHg	23.1 ± 0.1	134.5 ± 4.9	15.0 ± 0.5	11.4 ± 0.9	54.1 ± 7.5	96.3 ± 2.2
PCB	22.8 ± 0.1	129.6 ± 2.8	14.2 ± 0.3	11.9 ± 0.4	51.4 ± 3.0	100.0 ± 0.0
PCB + MeHg	22.9 ± 0.1	128.7 ± 5.7	14.6 ± 0.4	11.6 ± 0.8	42.2 ± 5.7	98.7 ± 1.3

Note. Means (± SE).

TABLE 2
Average Body Weights (± SE) for Pups Born to Dams Exposed to MeHg, PCB, or PCB + MeHg during Gestation and Lactation

	PND 0	PND 2	PND 7	PND 14	PND 21
Control	6.4 ± 0.1	8.3 ± 0.2	17.4 ± 0.2	32.6 ± 0.6	50.9 ± 0.8
MeHg	6.6 ± 0.1	8.5 ± 0.1	17.2 ± 0.2	32.6 ± 0.5	51.7 ± 0.6
PCB	6.2 ± 0.1	7.8 ± 0.2*	15.5 ± 0.4**	28.9 ± 0.6***	44.5 ± 1.0***
PCB + MeHg	6.0 ± 0.1^{+}	7.4 ± 0.2**	14.5 ± 0.4***	28.3 ± 0.5***	43.3 ± 0.8***

Note. Body weights are in grams.
*Group differs from controls at $p < 0.1$.
**Group differs from controls at $p < 0.01$.
***Group differs from controls at $p < 0.001$.

Inter-rod distances changed across days. On average, the rats made fewer slips on day 2. The inter-rod distance used on day 1 was rather narrow, causing difficulties especially in the larger males, while on days 3 and 4 the inter-rod distances were rather wide for all animals. Also, there was a significant SEX × DAY interaction ($F_{3,96} = 4.443$, $p = 0.006$). Males made more hind-limb slips than females on day 1, probably because of the narrowness of the inter-rod distance and their larger size.

For traverse time on the parallel bars, there were no significant effects of EXPOSURE (Fig. 4). There was a significant effect of DAY ($F_{3,96} = 30.673$, $p < 0.001$). Rats took the longest time to cross the bars on day 4, when the inter-rod distance was the widest (Fig. 4).

Rotating Rod. There was a significant EXPOSURE main effect for the number of slips made on the rotating rod ($F_{3,27} = 4.576$, $p = 0.010$). The PCB + MeHg rats made significantly more slips than the controls (Fig. 5A). There was also a significant EXPOSURE × RPM interaction ($F_{12,108} = 2.919$, $p = 0.002$). *Post hoc* analyses revealed that the PCB + MeHg rats made significantly more slips than the controls at both 25 and 30 rpm (Fig. 5B). Neither the EXPOSURE × DAY nor the EXPOSURE × SEX interaction was significant, so the data are presented collapsed across DAY and SEX (Fig. 5). No other interactions with EXPOSURE were significant.

There was a marginal SEX effect ($F_{1,27} = 4.109$, $p = 0.053$), with females making fewer slips than males (0.91 ± 0.10 and 1.15 ± 0.11 slips, respectively). There was also a significant DAY effect ($F_{2,54} = 31.276$, $p < 0.001$). The rats made on average the least number of slips on the rough-surface rod on day 1 (0.71 ± 0.08 slips) and the most number of slips on day 2 with the foam hurdle rod (1.44 ± 0.13 slips). On the completely smooth rod on day 3, the rats made an intermediate number (0.94 ± 0.11) of slips. There was a significant RPM effect ($F_{4,108} = 171.330$, $p < 0.001$). As expected, the rats made more slips as the rpm increased. The SEX × RPM interaction was also significant ($F_{4,108} = 2.936$, $p = 0.024$). Males made more slips than females at the higher rpms. Lastly, there was a significant DAY × RPM interaction ($F_{8,216} = 14.802$, $p < 0.001$), reflecting the increased slips made at 15, 25, and 30 rpm on day 2, when the rod with foam hurdles was used.

DISCUSSION

Combined PCB and MeHg exposure caused significant impairments on the rotating rod, while neither chemical alone caused a significant increase in the number of slips. In contrast, combined exposure to PCBs and MeHg did not cause any significant differences in performance on the rope-climb or parallel-bars tests compared to the controls. MeHg females were slightly impaired on the rope climb task, while MeHg males made less hind-limb slips on the last two days of testing on the parallel bars. PCBs alone did not alter performance on either the rope climb or the parallel bars.

TABLE 3
Average (± SE) Organ:Body Weight Ratios (g) on PND 21 for Pups Exposed to MeHg, PCB, or PCB + MeHg during Gestation and Lactation

	Brain:body weight ratio	Liver:body weight ratio	Thymus:body weight ratio
Control	0.0295 ± 0.0004	0.0367 ± 0.0037	0.0046 ± 0.0005
MeHg	0.0296 ± 0.0006	0.0353 ± 0.0012	0.0042 ± 0.0004
PCB	0.0325 ± 0.0008*	0.0643 ± 0.0025***	0.0034 ± 0.0005***
PCB + MeHg	0.0344 ± 0.0010***	0.0656 ± 0.0040***	0.0035 ± 0.0005***

*Group differs from controls at $p < 0.05$.
***Group differs from controls at $p \leq 0.001$.

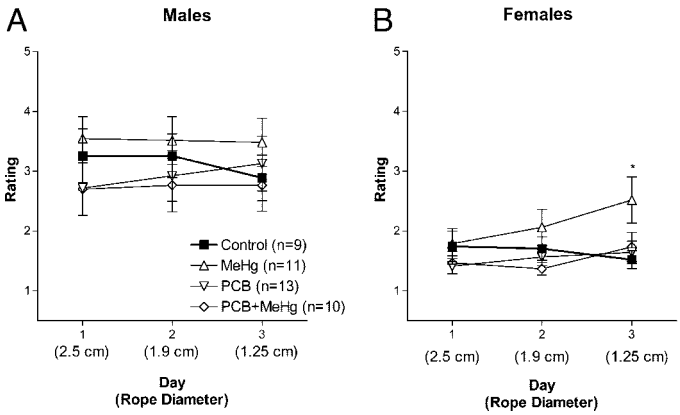

FIG. 1. Rope climb. Mean rating scores (± SE) for the rope-climb test are graphed for each exposure group separately for males (A) and females (B). Rope diameter changed across days as indicated in the parentheses. MeHg females received significantly worse rating scores than control females on the third day of testing with the thinnest rope. However, when the females were analyzed separately with body weight as a covariate, there were no longer any significant effects of exposure. *Denotes a group that differs from controls at $p < 0.05$.

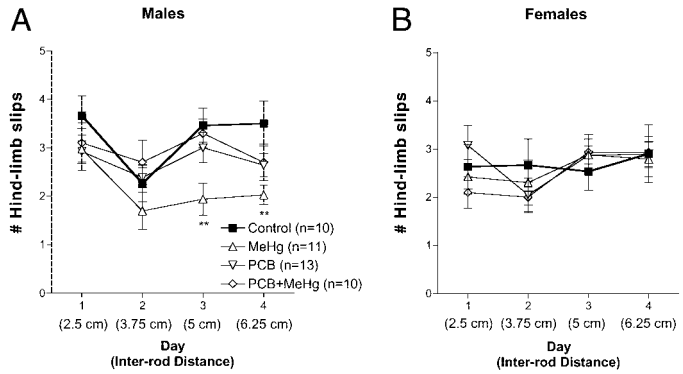

FIG. 3. Parallel bars. Mean number of hind-limb slips (± SE) for the parallel-bars test is graphed for each exposure group separately for males (A) and females (B). Inter-rod distance changed across days as indicated in the parentheses. MeHg males made fewer slips than control males on days 3 and 4 of parallel-bar testing. **Denotes a group that differs from controls at $p < 0.01$.

Rotating Rod

The principal motor deficit observed was on the rotating rod task. Combined PCB and MeHg exposure caused a significant increase in the number of slips at the highest rpms (25 and 30 rpm) across all three days of testing. This deficit was present in both males and females. PCBs and MeHg seemed to have an additive effect on rotating-rod performance, since neither chemical alone caused a significant increase in slips. However, comparison of the lines on the graph (Fig. 5) for each individual chemical suggests that PCB exposure contributed more to the deficit than did MeHg. It is unknown if this would be the case if a higher dose of MeHg were used. The dose of MeHg used in this study was significantly lower than doses used in previous motor studies. The total dose of MeHg in our study was approximately 1.20 mg, compared with 2–18 mg used in previous studies of motor abilities. For example, Sakamoto *et al*. (2002) found rotarod deficits using a dose of MeHg 10-fold higher than the dose employed in our study. The rotarod task is different from the rotating-rod task used here in that, in our study, the rats were required to not only stay on the moving rod but also to cross it lengthwise. Deficits on the rotating rod in the combined PCB and MeHg rats may be indicative of cerebellar damage. Morphological damage to the cerebellum caused by early postnatal X-irradiation of the rat cerebellum has been shown to impair rotating-rod performance (Brunner

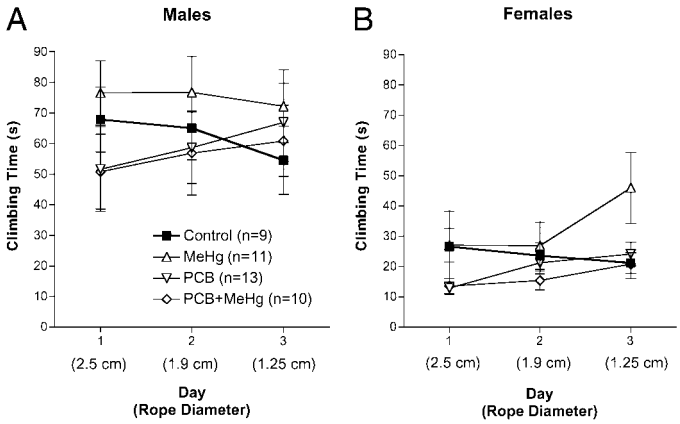

FIG. 2. Rope climb. Mean climbing time (± SE) is graphed for each exposure group separately for males (A) and females (B). Rope diameter changed across days as indicated in the parentheses. There were no significant differences among the exposure groups.

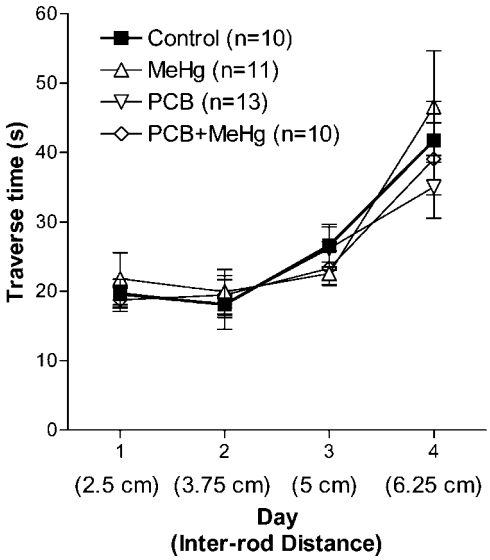

FIG. 4. Parallel bars. Mean traverse time (± SE) across the parallel bars is graphed by exposure group. Inter-rod distance changed across days as indicated in the parentheses. There were no differences among exposure groups in traverse time on the parallel bars.

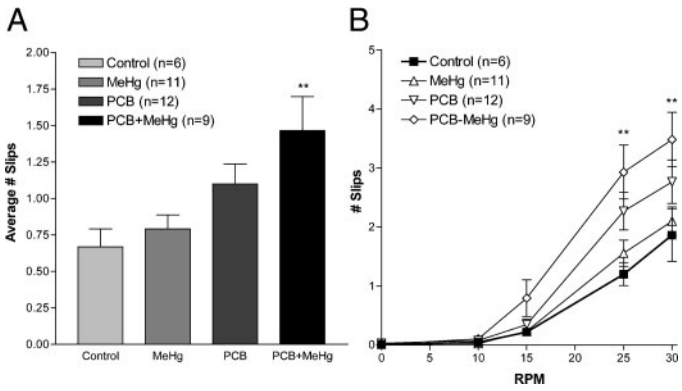

FIG. 5. Rotating rod. Mean number of slips (± SE) on the rotating rod is graphed by exposure group. Neither the exposure by sex nor the exposure by day interaction was significant, so the data have been averaged across sex and the three days of testing. (A) The number of slips averaged across rpm is shown. PCB + MeHg rats made significantly more slips than controls on the rotating rod. (B) The mean number of slips is graphed by rpm. PCB + MeHg rats made significantly more slips than controls at 25 and 30 rpms. **Denotes a group that differs from controls at $p < 0.01$.

and Altman, 1973; Pellegrino and Altman, 1979). More recently, postnatal alcohol exposure was found to cause both rotating-rod deficits and cerebellar damage (Klintsova *et al*., 1998).

Rope Climb

The only deleterious effect of MeHg alone on motor function was observed in the MeHg-exposed females on the rope climb. The MeHg females received inferior rating scores compared to the control females on the third day of testing, which used the thinnest rope. The MeHg females also tended to take longer than the control females to climb the rope on the third day. The MeHg males showed the worst performance on the rope climb among the males, although they were not significantly worse than the control males. This suggests that MeHg might cause a deficit in rope-climbing ability, even at the low dose used in this study. PCB exposure did not affect vertical rope-climbing ability, nor did the combined PCB + MeHg-exposure group differ from the controls. It is unclear why MeHg in combination with PCBs did not cause deficits similar to those seen with MeHg alone on the rope-climb task. However, body weight affected rope-climbing abilities. Lower body weights were significantly correlated with superior rope-climb rating scores. Therefore, it is possible that any MeHg-induced climbing deficit was offset by the reduced body weight in the combined exposure group. In fact, when we reanalyzed the data using body weight as a covariate, the exposure effects were no longer significant, suggesting that any effect of MeHg on rope-climbing ability is subtle. In addition, reanalysis with body weight as a covariate also removed any significant effects of sex on rope-climbing performance.

Parallel Bars

None of the exposed groups showed deficits relative to the controls on the parallel bars. However, MeHg exposure unexpectedly improved parallel-bar performance in the male rats. The MeHg males made significantly fewer hind-limb slips than the control males on days 3 and 4 of testing, when the inter-rod distances were the widest. The MeHg females were not improved on the parallel-bars task, indicating a sex-specific improvement that has no precedent in the literature. In fact, several studies report hind-limb dysfunction in rodents exposed to MeHg, including hind-limb crossing and flexion when the animal is held upside down by its tail (Inouye *et al*., 1985; Kobayashi *et al*., 1981; MacDonald and Harbison, 1977; Magos *et al*., 1985; Sakamoto *et al*., 1993; Spyker, 1975). MacDonald and Harbison (1977) reported motor incoordination and hind-limb paralysis in their heavily exposed adult male mice (10 and 50 μg/ml in drinking water). An improvement on a test of hind-limb function was not expected with these MeHg-exposed males and is difficult to explain. However, closer examination of the rotating-rod data also indicates a trend for improved performance in the MeHg males. This low dose of MeHg might have improved some aspect of motor function in the males that was evident on the parallel bars and rotating rod but not on the rope climb.

The clearest deficits were observed on the rotating rod in the combined PCB and MeHg–exposure group, while similar deficits were not observed on the rope climb or parallel bars. The rotating rod is a more complex motor skill task than the rope climb or parallel bars. It requires both fine motor coordination and precise postural control (Altman and Bayer, 1997). In order to successfully traverse the rotating rod, the rats must detect the speed of rotation of the rod and adapt, while both the rope climb and parallel bars were static surfaces. In fact, the PCB + MeHg rats were not impaired on the rotating rod when it was stationary or moving slowly. Impairments were only observed at the highest speeds of rotation. Thus, the rotating rod has a greater sensory component, both somatosensory and vestibular, than the rope-climb or parallel-bars tests. At this time, we cannot rule out the possibility that the PCB + MeHg-exposed rats had damage to the somatosensory or vestibular systems that contributed to their impaired performance on the rotating rod.

Thyroid hormones regulate the development of the vestibular system (Bhatia *et al*., 1977; Dechesne *et al*., 1984; Dememes *et al*., 1986; Sato *et al*., 1987). PCBs have been shown to disrupt thyroid hormone function (reviewed in Brouwer *et al*., 1998). In this study, exposure to PCBs alone or in combination with MeHg caused a significant reduction in circulating thyroid hormone concentrations (T_3 and T_4) when measured on PND 21 (data not shown), and this is in agreement with previous reports of PCB exposure (i.e., Crofton *et al*., 2000b). Previous research has shown that PCB-induced reductions in thyroid hormone levels appear to cause hearing impairments

via loss of outer hair cells within the organ of Corti (Crofton *et al*., 2000a). Thus, it is plausible that PCBs could similarly damage the vestibular apparatus, which is also dependent on thyroid hormones for proper development, and this could lead to deficits in balance and coordination. However, the PCB-alone group, while impaired relative to the controls, was not as dramatically impaired as the combined exposure group. Therefore, the possibility that PCB exposure damages some component(s) of the vestibular system is only a partial explanation for the selective deficits on the rotating-rod task.

Alterations in thyroid hormones following PCB exposure could also damage cerebellar development. Although it is known that developmental PCB exposure reduces circulating thyroid hormones, the effect of PCBs on the thyroid system is complex. Despite profound hypothyroxinemia, PCBs do not consistently increase thyroid-stimulating hormone (reviewed in Brouwer *et al*., 1998; Kolaja and Klaassen, 1998). In addition, PCB metabolites have been shown to compete with circulating T_4 for binding to the carrier protein transthyretin (Brouwer and van den Berg, 1986; Darnerud *et al*., 1996; Meerts *et al*., 2002). Currently, there is no convincing evidence for PCB binding to the thyroid receptor. Nevertheless, Zoeller *et al*. (2000) have reported that perinatal exposure to PCBs causes thyroid hormone–like elevations in the expression of the thyroid hormone responsive genes RC3/neurogranin and myelin basic protein. Thus, PCBs may be acting as a thyroid mimic in the brain, and it may be prudent to consider both neonatal hypo- and hyperthyroidism as possible models of abnormal cerebellar development and function.

Since rotating-rod impairments were observed only in the combined exposure group, it seems possible that MeHg exposure may have caused some independent damage to the cerebellar motor system. Damage to other motor systems cannot be ruled out, but MeHg is known to target the cerebellum (Eto, 1997; Leyshon and Morgan, 1991). Higher doses of MeHg are known to cause motor deficits in laboratory animals, including impaired rotarod performance (Sakamoto *et al*., 1993, 2002), and prenatal exposure to higher doses of MeHg also causes morphological changes in the cerebellum (Chang *et al*., 1977; Choi *et al*., 1981; Sager *et al*., 1984). Thus, there is a possibility that MeHg-induced cerebellar damage contributes to the motor deficits observed following MeHg exposure, but damage to other neural systems may also occur. Although the rotating-rod deficits seem to be primarily PCB-driven, MeHg exposure appears to have contributed to the effect. This behavioral effect does mimic the *in vitro* dopamine effect observed by Bemis and Seegal (1999), in which low doses of MeHg had no effect on dopamine when given alone but accentuated the effects of PCBs on dopamine when the two compounds were given together.

Another possible mechanism underlying the rotating-rod deficit in the combined PCB and MeHg-exposure group is an interactive effect on the ryanodine receptor (RyR), as suggested by Bemis and Seegal (2000), who hypothesized that the synergistic and antagonistic effects on calcium release *in vitro* were due to interactions at the RyR. PCBs are known to stabilize the open conductance state of the RyR (Wong and Pessah, 1997), and it is suggested that this open RyR state allows MeHg access to thiol groups in the RyR, resulting in MeHg-induced inactivation of the calcium release channel. Two of the three RyR isoforms are found in the cerebellum. RyR1 is found in Purkinje cells of the cerebellum, while RyR2 is found throughout neurons in the brain but especially within the cerebellum (reviewed in Ogawa, 1994). A study in cultured Purkinje cells suggests that Ca^{+2} release from internal stores, particularly from ryanodine-sensitive stores, is necessary for the induction of long-term depression (LTD) within the cerebellum (Kohda *et al*., 1995). LTD has been proposed as a possible cellular substrate of motor learning (Ito, 1989). Thus, if combined PCB and MeHg-exposure results in the inactivation of the RyR calcium release channel, then this would likely impair LTD induction within the cerebellum as well as motor learning, including rotating-rod performance.

In conclusion, combined PCB and MeHg exposure during development caused impairments on the rotating-rod task in adulthood. Two possible scenarios would explain the impairment in the combined exposure group. The first scenario is that PCBs and MeHg have independent mechanisms of toxicity, and it is the combinatory effect of the two chemicals that results in the rotating-rod deficit. The second scenario is that PCBs and MeHg participate in the same mechanism of toxicity, such as a combined effect on the RyR.

Recently, Mervis *et al*. (2002) reported that a dose of PCBs similar to the one we used caused a decrease in Purkinje cell branching area on PND 22 that had recovered by PND 60. Future studies should examine the effects of PCBs on Purkinje cells and the cerebellum. Examinations of the vestibular system following PCB exposure may also be warranted.

ACKNOWLEDGMENTS

The authors would like to thank Jeff Gilmor, Luke Hamann, Melissa Jorgensen, Chris Lowry, Daniel Watts, and Adam Young for their assistance in testing the animals in this study. We would also like to acknowledge Dr. John Widholm, who assisted in the dosing of these animals. This research was supported by EPA R-828 95301, EPA R-82939001, NIH AA09838 and AG10154, and NIEHS PO1 ES11263. In addition,V.C.W. was supported on NIEHS T32 ES07326 during conduct of the research and preparation of the manuscript.

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