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MOTOR IMPAIRMENT IN RATS EXPOSED TO PCBs AND METHYLMERCURY DURING EARLY DEVELOPMENT

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RUNNING HEAD: MOTOR IMPAIRMENT AND PCB+MEHG EXPOSURE

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ABSTRACT

Epidemiological and laboratory studies indicate that PCBs and 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 PND60, 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 controls on a task requiring them to traverse a rotating rod. Rats exposed to PCBs alone were also somewhat impaired relative to controls, whereas MeHg-exposed rats were not significantly different from controls. There were no statistically significant deficits related to PCB or MeHg exposure on a vertical rope-climbing test or a parallel bars 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
INTRODUCTION

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, 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. 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 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 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 function, 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 (Sakamoto et al., 1993; Inouye et al., 1985; Kobayshi et al., 1981; Magos et al., 1985), 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 PND21 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 PND22 that recovered by PND60.

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 PND21 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 wks 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.

**METHODS**

*Animals and Exposure*. Female Long-Evans rats were purchased from Harlan Sprague Dawley (Madison, WI) in 3 cohorts spaced 6 mo 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 1
male and 1 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
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 hrs of pairing to prevent MeHg exposure to the breeding males. 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. Average MeHg exposure was 41.90 µg/kg/d prior to mating and 58.90 µg/kg/d 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 one week. Breeding sessions were from 11 am to 8pm, which was during the dark period of their diurnal cycle. Pregnancy was determined by the appearance of a sperm plug, and pregnant females were then removed and housed individually. On PND2, litters were reduced to 10 pups (5 males and 5 females where possible), cross-fostering with extra pups from the same treatment group as needed. Pups were weaned on PND21. One male and one female from each litter were randomly selected on PND28 for motor testing. Other littermates were selected for cognitive testing and hearing assessments (Widholm et al., in preparation), and for organ weight assessment at weaning (see below). Pups were housed in same-exposure, single-sex pairs or triplets after PND28.
Motor testing. 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 four weeks. During the first week, rats were tested on the rope climb task. 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 was 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. Rats were trained on four consecutive days to climb 2ft-long vertical ropes leading to a square horizontal platform (15 cm²) mounted 1 m above the floor. On the first day, 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, rats were tested using a 2.5 cm rope followed by decreasing rope diameters of 1.9 cm and 1.25 cm on Days 2 and 3, respectively. 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 cm²) 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. Rats were given three consecutive trials on each day of testing. A trial started by placing the rat on the starting platform. 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 rats stopped moving forward, testers would gently prod them from behind and cue the rat to move forward by lightly tapping the rods in front of the rat. 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 3-sided plywood structure supported the rod and had 4 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 rats were trained to cross the 2m-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 4 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, rats were guided by the tester’s hands to move forward. If necessary, 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 5 slips or falls in one trial. For analysis, unfinished trials were assigned the maximum score of 5 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, 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 4-way ANOVAs (EXPOSURE(4) x COHORT(3) x SEX(2) x DAY(3)). For parallel bars, the traverse time and number of hind-limb slips were also analyzed via separate 4-way ANOVAs (EXPOSURE(4) x COHORT(3) x SEX(2) x DAY(4)). For the rotating rod, the number of slips was analyzed via a 5-way ANOVA (EXPOSURE(4) x COHORT(3) x SEX(2) x DAY(3) x 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. Dams in all exposure groups had gestational lengths, gestational weight gains, liver weights, litter sizes, percent male pups, and percent live births similar to controls (Table 1). The birth weight of pups exposed to PCB+MeHg was on average slightly decreased relative to controls (p = 0.062), while no other groups differed from controls (Table 2). On PND2, average weights of PCB+MeHg pups were significantly decreased (p < 0.01) compared to controls, while PCB pups were also slightly smaller on average than control pups (p = 0.088) (Table 2). The average pup weights for the PCB and PCB+MeHg groups were consistently significantly smaller than controls on PND7, 14, and 21 (Table 2). The body weights for MeHg-exposed pups did not differ from 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 PND21 (see Table 2), 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 PND21 in the PCB alone and PCB+MeHg exposure groups (Table 3), which is an effect 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 PND21 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 controls on brain, liver, or thymus to body weight ratios on PND21 (Table 3).

Body weights postweaning. The body weights of rats exposed to PCBs or PCB+MeHg were also significantly reduced postweaning ($F_{3,12} = 6.901, p = 0.006$). Body weights were significantly reduced by 5-11% for PCB-exposed rats and by 11-15% for PCB+MeHg-exposed rats up to PND63. In contrast, the body weights of the MeHg-exposed rats were not significantly different from controls up to PND63, but MeHg-exposed rats weighed on average 7% less than controls during testing which occurred between PND62 and 102. During testing, PCB rats weighed on average 8% less and PCB+MeHg rats on average 11% less than 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 control females on day 3 (Figure 1B). A similar pattern of effects was seen for climbing time (Figure 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 x SEX x 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 control males on testing days 3 and 4 (Figure 3A). There were no significant differences in the number of hind-limb slips among the females (Figure 3B). The two-way EXPOSURE x SEX interaction was also significant ($F_{3,32} = 3.710, p = 0.021$), while neither the EXPOSURE x 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 ($F_{3,96} = 6.843, p < 0.001$). Inter-rod distances changed across days. On average, 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 x 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 (Figure 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 (Figure 4).

**Rotating Rod.** There was a significant EXPOSURE main effect for the number of slips made on the rotating rod \( (F_{3,27} = 4.576, p = 0.010) \). PCB+MeHg rats made significantly more slips than controls (Figure 5A). There was also a significant EXPOSURE x RPM interaction \( (F_{12,108} = 2.919, p = 0.002) \). Post-hoc analyses revealed that PCB+MeHg rats made significantly more slips than controls at both 25 and 30 rpms (Figure 5B). Neither the EXPOSURE x DAY nor the EXPOSURE x SEX interaction was significant, so the data are presented collapsed across DAY and SEX (Figure 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 \( (0.91 \pm 0.10 \text{ and } 1.15 \pm 0.11 \text{ slips, respectively}) \). There was also a significant DAY effect \( (F_{2,54} = 31.276, p < 0.001) \). Rats made on average the least number of slips on the rough surface rod on day 1 \( (0.71 \pm 0.08 \text{ slips}) \) and the most number of slips on day 2 with the foam hurdle rod \( (1.44 \pm 0.13 \text{ slips}) \). On the completely smooth rod on day 3, rats made an intermediate number \( (0.94 \pm 0.11 \text{ of slips}) \). There was a significant RPM effect \( (F_{4,108} = 171.330, p < 0.001) \). As expected, rats made more slips as the RPM increased. The SEX x 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 x RPM interaction \( (F_{8,216} = 14.802, p < 0.001) \), reflecting the increased slips made at 15, 25 and 30 RPMs 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 bar tests compared to 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.

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 rpms) 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 to 2-18 mg used in previous studies of motor abilities. For example, Sakamoto et al. (2002) found rotarod deficits using a dose of MeHg tenfold 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 rats were required to not only stay on the moving rod but also 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 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 control females on the third day of testing, which used the 
thinnest rope. MeHg females also tended to take longer than 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 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 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 climb performance.

Parallel Bars. None of the exposed groups showed deficits relative to controls on the 
parallel bars. However, MeHg exposure unexpectedly improved parallel bar performance in
male rats. MeHg males made significantly fewer hind-limb slips than control males on days 3 and 4 of testing when the inter-rod distances were the widest. 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 transverse 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, 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 have damage to the somatosensory or vestibular systems contributing 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 (T₃ and T₄) when measured on PND21 (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 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
Motor Impairment and PCB+MEHG Exposure

(Reviewed in Brouwer et al., 1998; Kolaja and Klaassen, 1998). In addition, PCB metabolites have been shown to compete with circulating T₄ 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 cause 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). Bemis and Seegal (2000) 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 in 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 inactivation of 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 that we used caused a decrease in Purkinje cell branching area on PND22 that had recovered by PND60. 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.

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REFERENCES


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### Table 1.
Reproductive outcomes of dams exposed to MeHg, PCB, or PCB + MeHg during gestation and lactation. Means (± SE) are given below.

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

### Table 2.
Average body weights (± SE) for pups born to dams exposed to MeHg, PCB, or PCB + MeHg during gestation and lactation. Body weights are in grams.

<table>
<thead>
<tr>
<th></th>
<th>PND0</th>
<th>PND2</th>
<th>PND7</th>
<th>PND14</th>
<th>PND21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.4 ± 0.1</td>
<td>8.3 ± 0.2</td>
<td>17.4 ± 0.2</td>
<td>32.6 ± 0.6</td>
<td>50.9 ± 0.8</td>
</tr>
<tr>
<td>MeHg</td>
<td>6.6 ± 0.1</td>
<td>8.5 ± 0.1</td>
<td>17.2 ± 0.2</td>
<td>32.6 ± 0.5</td>
<td>51.7 ± 0.6</td>
</tr>
<tr>
<td>PCB</td>
<td>6.2 ± 0.1</td>
<td>7.8 ± 0.2</td>
<td>15.5 ± 0.4</td>
<td>28.9 ± 0.6</td>
<td>44.5 ± 1.0</td>
</tr>
<tr>
<td>PCB + MeHg</td>
<td>6.0 ± 0.1</td>
<td>7.4 ± 0.2</td>
<td>14.5 ± 0.4</td>
<td>23.3 ± 0.5</td>
<td>43.3 ± 0.8</td>
</tr>
</tbody>
</table>

+ group differs from controls at $p < 0.1$
** group differs from controls at $p < 0.01$
*** group differs from controls at $p < 0.001$

### Table 3.
Average (± SE) organ:body weight ratios (g) on PND21 for pups exposed to MeHg, PCB, or PCB + MeHg during gestation and lactation.

<table>
<thead>
<tr>
<th></th>
<th>Brain:Body Weight Ratio</th>
<th>Liver:Body Weight Ratio</th>
<th>Thymus:Body Weight Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.0295 ± 0.0004</td>
<td>0.0367 ± 0.0037</td>
<td>0.0046 ± 0.0005</td>
</tr>
<tr>
<td>MeHg</td>
<td>0.0296 ± 0.0006</td>
<td>0.0353 ± 0.0012</td>
<td>0.0042 ± 0.0004</td>
</tr>
<tr>
<td>PCB</td>
<td>0.0325 ± 0.0008*</td>
<td>0.0643 ± 0.0025***</td>
<td>0.0034 ± 0.0005***</td>
</tr>
<tr>
<td>PCB + MeHg</td>
<td>0.0344 ± 0.0010***</td>
<td>0.0656 ± 0.0040***</td>
<td>0.0035 ± 0.0005***</td>
</tr>
</tbody>
</table>

* group differs from controls at $p < 0.05$
*** group differs from controls at $p \leq 0.001$
**Figure 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 $p < 0.05$. 
Figure 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.
Figure 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 $p < 0.01$
**Figure 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.
Figure 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 $p < 0.01$.