# MOTOR FUNCTION FOLLOWING DEVELOPMENTAL EXPOSURE TO PCBS AND/OR MEHG*

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**Abstract**

Recent studies raise concern for combined exposure to polychlorinated biphenyls (PCBs) and methylmercury (MeHg), two environmental contaminants that are found in fish and seafood. Past accidental poisonings in humans show that exposure to high levels of either contaminant is associated with motor impairments, including alterations in cerebellar functions such as balance and coordination. Epidemiological studies of lower level exposures suggest some neuromotor impairment in exposed children, but the majority of these studies have focused on cognitive endpoints rather than examining a full-range of motor function. In particular, the cerebellum could be a sensitive target for combined PCB and MeHg toxicity. MeHg is known to damage the cerebellum, but PCBs may also cause cerebellar damage via thyroid hormone disruption during development. In addition, *in vitro* studies report interactive effects of PCBs and MeHg on ryanodine-sensitive calcium signaling. Ryanodine receptors are found especially within the cerebellum, and alterations in calcium signaling within the cerebellum could impair long-term depression and subsequent motor learning. This article reviews the motor impairments reported in humans and laboratory animals following exposure to PCBs and/or MeHg during development. There is need for a better understanding of the interactive effects of PCBs and MeHg, especially in regard to motor function.

**Keywords (3-6 words):** Polychlorinated biphenyls (PCBs), methylmercury (MeHg), motor, development, humans, rats

## 1. Introduction

Polychlorinated biphenyls (PCBs) and methylmercury (MeHg) are two of the most ubiquitous environmental contaminants (1, 2), and both are neurotoxic (3). Exposure to PCBs and MeHg can occur simultaneously since the two contaminants are found in the same food sources, especially fish, seafood, and marine mammals (4, 5). Although there is very little research investigating the effects of combined exposure to PCBs and MeHg, several recent studies suggest there may be cause for concern. Interactive effects of PCBs and MeHg have been reported on neuronal calcium regulation and dopamine function *in vitro* (6, 7), and we recently reported an additive effect of exposure to the two chemicals *in vivo* on the rotating rod, a cerebellar motor task (8). In contrast, there was no evidence of an interaction between the two chemicals on a spatial learning task (9). Apparent interactive effects of PCBs and MeHg have also been observed in epidemiological studies (10, 11).

PCBs are persistent environmental contaminants that were commercially produced from 1929 until the late 1970s in the United States and also in the former USSR, Germany, Italy, France, and Japan (2). Their primary use was as dielectric fluids for transformers and capacitors, but PCBs were also used as hydraulic lubricants, heat-transfer fluids, flame-retardants, sealants, plasticizers, pesticide extenders, and in carbonless copy paper (12). The PCB molecule consists of a biphenyl ring structure with 10 positions available for chlorine substitution. There are 209 different PCB congeners with different biological activities and toxicity depending on the number and position of chlorine atoms on the molecule. The different congeners are classified into two main classes--coplanar and *ortho*-substituted. Coplanar PCBs are named for their planar conformation that results from chlorines in the *para* and *meta* positions on the phenyl rings and no chlorines in the *ortho* positions. Thus, coplanar

PCBs have a similar structure to dioxins and a similar mechanism of toxicity via their binding to the aryl hydrocarbon receptor (AhR). Congeners with two or more *ortho*-chlorines are referred to as *ortho*-substituted PCBs. The *ortho*-chlorines repel each other making a coplanar configuration impossible. Thus, *ortho*-substituted PCBs have a much lower affinity for the AhR and different, and less understood, mechanisms of toxicity. Due to their high lipophilicity and low biodegradability, PCBs from the environment bioaccumulate in the adipose tissue of living organisms, including humans. Human exposure to PCBs occurs primarily through low-level food contamination, and consumption of contaminated fish is an important source (13).

Mercury in the environment originates from both natural and anthropogenic sources. In nature, mercury can be released from mineral deposits, volcanoes, forest fires, oceanic emission, and crust degassing. Human activities such as mining, mineral processing, chloroalkali production, and combustion of fossil fuels also release mercury into the environment (1). Inorganic mercury compounds can then enter waterways where they undergo a process of methylation via microorganisms to produce methylmercury (MeHg). MeHg has a high affinity for protein sulfhydryl groups causing it to bioaccumulate in organisms (14). However, terrestrial food is a negligible source of MeHg exposure for humans (1). Like PCBs, the primary sources of MeHg for humans are contaminated fish and seafood. MeHg is found in virtually all species of fish (15), and fish and sea mammals near the top of the food chain contain the largest quantities due to biomagnification.

The developing organism is often more vulnerable to toxic insult than the adult organism, and this is true for both PCBs and MeHg. Both PCBs and MeHg cross the placenta, and PCBs are also present in breast milk (16-19). There are often negative consequences for the developing fetus even when the mother is asymptomatic. In two separate MeHg poisoning

episodes in Japan and Iraq, exposed children had severe disabilities, while most of their mothers exhibited only mild symptoms during pregnancy such as minor visual disturbances and paraesthesias (20-22). ~~no symptoms of MeHg exposure [3,99].~~ Likewise, in PCB poisonings in Japan and Taiwan, children were severely impaired while the mothers were not (23, 24).

This review focuses on the effects of developmental exposure to PCBs and/or MeHg on motor function. We first present evidence to show that the cerebellum, which is critical for balance and coordination, could be a target for PCB and MeHg toxicity, resulting in subsequent motor impairments. Next, we review the accidental poisoning incidents in which the neurotoxicity of PCBs and MeHg was first realized, followed by the later epidemiological studies undertaken to examine the neurodevelopmental toxicity of the individual contaminants, and finally the studies of the individual contaminants conducted in laboratory animals. We end with a discussion of directions for future research.

## 2. Cerebellum as a Possible Target for PCB and MeHg Toxicity

The cerebellum has an important role in mediating certain aspects of motor function, including balance and coordination. PCB exposure results in similar PCB levels in the cerebellum as the frontal cortex (25), while the striatum had marginally less PCB accumulation than the other two brain areas. Other studies have found no major regional brain differences in PCB accumulation (26, 27) or have reported higher PCB levels in fiber tracts throughout the brain (28), likely due to their higher fat content. Very few studies have focused on the effects of PCBs on the cerebellum, but there is some evidence that PCBs could target the cerebellum. PCBs are known to alter intracellular calcium signaling (29, 30), and the cerebellum appears to

be more sensitive than the frontal cortex or striatum to PCB-induced alterations in mitochondrial $Ca^{+2}$ buffering and protein kinase C activity (25).

PCBs also reduce circulating thyroxine concentrations and could selectively damage the cerebellum via a thyroid hormone dependent mechanism. Thyroid hormones are important for brain development (31), and in particular, for cerebellar development for various reasons, including the late developmental timeline of the cerebellum compared to other brain regions (32), its high expression of thyroid hormone receptors (33), and its sensitivity of gene expression to alterations in thyroid hormone status (33). Several studies to date have demonstrated the complex effect PCBs have on brain ~~cerebellar~~ development, possibly via thyroid hormone alterations. Developmental PCB exposure was shown to increase calcineurin in the cerebellum of adult female but not male rats (34). Calcineurin is a calmodulin-regulated phosphatase that increases with prenatal hypothyroidism (35). Thus, it appears that PCB exposure caused a hypothyroid-like effect on calcineurin in the cerebellum of female rats. Conversely, another study found that developmental PCB exposure may have a thyromimetic effect in the brain, despite significant reductions in circulating thyroxine (36). This study observed thyroid hormone-like elevations in the expression of RC3/neurogranin and myelin basic protein, two key thyroid hormone responsive genes in the developing brain (36). In contrast, other chemical goitrogens, such as propylthiouracil and methimazole, reduce the expression of these same genes (37, 38). PCB exposure increased RC3/neurogranin expression within the piriform and retinosplenial granular cortices, illustrating that PCBs' alterations in thyroid-hormone action may also disrupt motor functions at the cortical level. The authors theorize that at higher doses, specific PCB congeners may become concentrated in brain tissues to the extent that they overcome or reverse the effects of increasingly severe

hypothyroxinemia, producing a thyromimetic effect. However, the mechanism for this effect is not clear since PCBs do not appear to bind to the thyroid hormone receptor (39). Lastly, it was reported in abstract form that developmental PCB exposure reduced Purkinje cell branching area on PND22 but there was recovery by PND60 (40). Interestingly, either neonatal hypo- or hyperthyroidism can reduce Purkinje cell branching area (41, 42). Thus, PCBs may adversely affect these cells regardless of whether they are acting by blocking or mimicking thyroid hormone.

Unlike PCBs, there is clear evidence that MeHg damages ~~targets~~ the cerebellum. Autopsy studies in MeHg-exposed humans found the cerebellum to have the highest levels of total mercury in the brain (43, 44), and cerebellar damage is often reported following MeHg exposure in humans. In general, MeHg damage in humans is thought to be less localized with fetal exposure than with infant or adult cases (45, 46). Autopsy studies from fetal MeHg poisonings have documented a number of cerebellar changes, including reduction in size, atrophy of the folia, underdevelopment of white matter, no demyelination areas but poor myelination, abnormal cell migration, and heterotopic cells within the white matter (22, 45, 47, 48). Lower MeHg exposures may still result in heterotopic cells within the white matter (43). Within the granule cell and molecular layers, there is narrowing of both layers, degeneration and loss of granule and Purkinje cells, thickened PC dendrites, increases in microglia and oligodendroglia, and damaged basket cells and parallel fibers (22, 47). ~~Reports include general tissue loss, in particular granule cell loss, and abnormal Purkinje cell migration [18,30,41,42]. MeHg has been shown to selectively target the cerebellum in animal studies [i.e. 88], and~~ ~~n~~Numerous studies in rodents have found morphological changes in the cerebellum following MeHg exposure. Prenatal exposure in mice causes simplified folial pattern (49), degeneration

of , ~~including~~ Purkinje and granule cells (50) ~~degeneration and loss,~~, and reduction or absence of synaptic densities (51). Early postnatal exposure in mice causes~~,~~ decreased Purkinje cell dendritic arborization (48) and ~~,~~reduced granular and molecular layer thicknesses (52)~~s~~. In rats, prenatal or early postnatal MeHg exposure caused no discernible neuronal damage within the cerebellar cortex under the light microscope (53-55), but extended postnatal exposure starting on PND1 and continuing to at least PND30 caused marked reduction and degeneration in granule cells (56). ~~, and simplified folial patterns [68].~~It should also be noted that developmental MeHg exposure damages other parts of the CNS, including widespread cortical damage which could contribute to motor symptoms.

One common mechanism of neurotoxicity for both PCBs (57) and MeHg (58) is disruption of intracellular calcium signaling. In fact, it has been recently demonstrated that PCBs and MeHg have interactive effects on intracellular calcium signaling in cerebellar granule cells (6). Lower concentrations of PCBs (5 μM of 2,2' dichlorobiphenyl, PCB 4) and MeHg (1.5 μM) synergistically increased calcium release, whereas at higher concentrations (10 or 20 μM of 2,2' dichlorobiphenyl and 2.0 μM MeHg) or with longer exposure times (> 10 minutes), the two chemicals antagonized each other's effects. These effects may be due to interactions at a common site, the ryanodine receptor (RyR). Previous work by Pessah and colleagues showed that *ortho*-substituted PCBs interact with the immunophilin FKBP12/RyR complex to alter RyR $Ca^{+2}$ signaling (reviewed in (59)). It is believed that PCBs stabilize the open conductance state of the RyR, while MeHg destabilizes the closed channel via either oxidative metabolism and/or binding directly to the channel and alteration of RyR $Ca^{+2}$ affinity. Antagonism may occur at higher concentrations or longer exposure times because higher concentrations of PCBs seem to facilitate MeHg-induced inactivation of calcium release

channels. Specifically, PCBs may enhance the open state of RyR allowing MeHg to more readily gain access to thiol groups of the RyR needed for inactivation. The lag time of the inactivation process may be due to the slower access of MeHg to these sites, as compared to the sites of activation. In addition, 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 (60). LTD has been proposed as the possible underlying physiological basis for motor learning (61). We have recently shown that developmental PCB exposure causes RyR dysregulation and motor impairments in the adult rats (62). 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 function.

It should be mentioned that although the cerebellum is traditionally assigned the role of balance and coordination of voluntary movement, it is now believed that the cerebellum may play a much larger integrative role in the CNS than previously believed. In addition to ataxia, cerebellar disease can also cause intellectual impairments and aberrant behavior in patients (63). Further, there is evidence that cerebellar damage may contribute to many psychiatric disorders (63). The recently defined cerebellar cognitive affective syndrome includes impairments in executive, visual-spatial, and linguistic abilities, and there can be affective disturbances ranging from emotional blunting and depression, to disinhibition and psychotic features (64). So, it is possible that some of the cognitive impairments reported with PCB and/or MeHg exposure may involve cerebellar dysfunction.

## 3. Accidental Poisonings

*3-1. PCB Poisonings.* The neurotoxicity of both PCBs and MeHg was first realized through accidental poisoning incidents. Human PCB poisonings occurred in Japan in 1968 and Taiwan in 1979. In both poisonings, people became ill after ingesting rice oil that had been contaminated with PCBs during the manufacturing process. The poisonings became known as Yusho ("rice oil disease") in Japan and YuCheng in Taiwan. The disease was characterized by its dermal abnormalities—acneform lesions, brown pigmentation of the skin, and ocular swelling, but many patients also reported headaches, memory loss, numbness, hypoesthesia, and neuralgia of the limbs (65, 66). Pregnant women who suffered from the poisonings gave birth to babies that were smaller and had dark brown pigmentation of the skin (67). A subset of Yusho children were examined, and a number of abnormalities were reported, including ~~finding a number of abnormalities, such as~~ growth impairment, slowness, lack of endurance, hypotonia, jerkiness, clumsy movement, apathy, and IQs averaging around 70 (23) (Table 1). The children of YuCheng were followed more closely, and a number of adverse outcomes were associated with the PCB poisoning, including lower body weight and height, hyperpigmentation of the skin, hypertrophy of the gums, deformities of the nails, and increased frequency of bronchitis (24). YuCheng infants were also found to have lower scores on the Bayley Scales of Infant Development psychomotor index, which assesses the degree of body control, large muscle coordination, fine manipulatory skills of the hands and fingers and dynamic movement. They were also delayed compared to unexposed infants on 32 of 33 developmental milestones including motor milestones such as turning pages, holding pencils, imitating drawn circles, and catching a ball (24) (Table 1). In addition, YuCheng children scored on average about 5 points lower on standardized intelligence tests (68) and had a higher

frequency of behavioral problems and higher activity levels, as measured by the Rutter's Child Behavior Scale A and a modified Werry-Weiss-Peters Activity Scale (69).

*3-2. MeHg Poisonings.* Like PCBs, the concern over human exposure to MeHg was first realized following acute outbreaks of high-dose MeHg poisoning. T~~t~~he first MeHg outbreaks were~~occurr~~ in Japan ~~ing~~ at Minamata ~~Bay~~and Niigata, and a later outbreak occurred in ~~Japan, and the second in~~ Iraq. During the 1950s, MeHg was released into Minamata Bay from industrial pollution, and more than 2000 people were poisoned after consuming the fish from the polluted bay (70). The syndrome induced from MeHg poisoning was termed Minamata disease and included symptoms such as narrowing of the visual fields, paraesthesias, and impaired speech and hearing. Notably, motor abilities were also affected causing loss of coordination and impaired gait (Table 2). Children exposed *in utero* were particularly vulnerable to MeHg. They had severe disabilities including mental retardation, cerebral palsy, and seizures, while more than half of their mothers had no symptoms of MeHg exposure (21).

The second outbreak of MeHg poisoning took place in Iraq in the winter of 1971-1972. Rural villagers in Iraq consumed homemade bread made from seed grain that was intended for planting and had been treated with a methylmercurial fungicide. The earliest symptom reported by exposed adults was paresthesia~~s~~, while ataxia was the earliest clinical finding (71). Severely affected individuals reported visual effects such as blurred vision and constriction of the visual field leading to blindness in the most severe cases. Slurred speech and hearing difficulties were also reported. Children exposed to MeHg *in utero* had delayed motor development, which was defined by failure to sit without support by 12 months, to pull to standing position by 18 months, or to walk two steps without support by 2 years of age (20) (Table 2). Increased limb tone and deep tendon reflexes (hyper-reflexia) with persisting

extensor plantar responses (Babinski reflex) were reported in the exposed children (20) (Table 2). Ataxia, hypotonia, and athetoid movements were also observed (72) (Table 2).

Autopsy studies from Japan and Iraq document cerebellar damage following fetal and postnatal MeHg exposure (22, 45, 73). However, it must be acknowledged that other areas of the CNS are also damaged, including diffuse cortical damage and abnormal cytoarchitecture (22). In addition, there is incomplete myelination of nerve fibers and hypoplasia of the corpus callosum (22, 47). CNS damage outside of the cerebellum may also contribute to motor impairments.

*3-3. Summary of PCB and MeHg Poisonings.* Motor impairments were prevalent in the accidental PCB and MeHg poisonings. Children suffering from PCB poisoning were reported to have clumsy movements (23). Loss of coordination and changes in gait were observed at Minamata Bay in children and adults suffering from MeHg poisoning. Ataxia, hypotonia, and athethoid movements were reported following MeHg exposure in Iraq, along with a delay in motor development, including learning to walk. Both chemical poisonings point toward impairments of balance and coordination, which may be indicative of cerebellar damage. Yet, later epidemiological studies of the two chemicals have placed very little emphasis on assessments of motor function.

**4. Epidemiological Studies Assessing Low-Level Environmental Exposures.**

*4-1. Epidemiological Studies of PCBs.* In the years following the Yusho and YuCheng poisonings, several epidemiological studies were initiated to investigate the impact of lower level environmental exposure to PCBs. Prenatal PCB exposure has been associated with decreased birth weight and growth in some studies (74-76). PCBs also have been associated with diminished immune function and subtle changes in circulating thyroid hormones in

infants and children exposed during development (reviewed in (77) and (31), respectively). However, some of the most striking effects following developmental exposure to PCBs have been on the nervous system.

Seven longitudinal studies of PCB neurotoxicity in children have been completed or are ongoing. The first study followed the children of women who regularly consumed PCB-contaminated fish from Lake Michigan. At birth, PCB exposure was associated with poorer performance on the Brazelton Neonatal Behavioral Assessment Scale (NBAS). The findings included motoric immaturity, greater amount of startle, and more abnormally weak (hypoactive) reflexes (78) (Table 1). At 7 months of age, PCB exposure was related to poorer visual recognition memory, as assessed on the Fagan Test of Infant Intelligence (79). At 4 yrs of age, there were significant impairments on the McCarthy General Cognitive Index, but there were no significant effects on the McCarthy motor scale, which measures both fine and gross motor function, or on the Beery Test of Visual-Motor Integration, which uses design copying to measure the child's ability to integrate and coordinate visual perception and motor output (80) (Table 1). Cognitive and memory deficits, including lower IQ scores, greater impulsivity, poorer concentration and working memory deficits, were still present at 11 years of age, the last age tested (81, 82). No assessments of motor function were reported at 11 years of age.

A second longitudinal study was conducted in North Carolina to examine children whose mothers were exposed to background levels of PCBs. In North Carolina, neurologic abnormalities similar to those observed in Michigan were observed at birth, including hypotonicity and hyporeflexia on the NBAS (83) (Table 1). The Bayley Scales of Infant Development were given at 6, 12, 18, and 24 months, and prenatal exposure to PCBs was associated with decreased scores on the psychomotor index at all time points tested (84, 85)

(Table 1). The authors report that the observed motor effects in infancy were consistent with those seen with anterior horn cell dysfunction or mild hypothyroidism (84). However, the Bayley measures a wide range of gross and fine motor skills, and the published reports do not provide any information regarding which aspects of psychomotor function were impacted by PCB exposure. When the children were older (3, 4, and 5 years of age), motor impairments were not observed on the McCarthy motor scale (86) (Table 1). As stated in the study (86), the McCarthy motor scale is not an exact analogue of the Bayley Psychomotor scale. Alternatively, the children may have recovered or growth may have diluted their PCB body burden reducing the motor effects (86). Whatever the explanation, the results are consistent with those of the Michigan study, which also did not see any effects on the McCarthy Motor scale.

A third study in the Netherlands examined children following background maternal exposure to PCBs, polychlorinated dibenzodioxins, and polychlorinated dibenzofurans. They found poorer neurologic condition at birth and a higher incidence of hypotonia associated with PCB exposure (87) (Table 1). They also found lower psychomotor scores on the Bayley scales at 3 and 7 months but not at 18 months (88) (Table 1). As in the North Carolina study, no details were provided about which aspects of motor function were affected. At 18 months, transplacental PCB exposure was negatively related to neurological condition. The neurological examination given at that age included observations of motor functions in the areas of grasping, sitting, crawling, standing, and walking (89). Later neurological examinations at 3.5 years focused on similar motor functions (prehension, sitting, crawling, standing, and walking) as well as fluency of movements, but PCB exposure was no longer related to neurological condition at this age (90) (Table 1). However, at 3.5 years, the same

children did score lower on all subscales of the Kaufman Assessment Battery for Children (Kaufman ABC), including the sequential processing scale, which includes subtests for ~~average~~ hand movements and gross and fine motor skills (91). Again, it is unclear whether the children scored lower on these specific subtests. Interestingly, at 7 years of age, there was an association between PCB exposure and scores on McCarthy Motor subscale when parental and home characteristics were less optimal (92) (Table 1). At 9 years of age, higher prenatal PCB levels were associated with longer and more variable reaction times on a simple reaction time test and also with lower scores on the Tower of London, a test of executive function (93). No tests of motor function were given at the 9-year examination.

Another cohort of children was followed in Oswego, New York, in which the mothers consumed sport-caught fish from Lake Ontario. The NBAS was given 25-48 hours after birth, and a significant relationship between heavily chlorinated PCBs and impairments on the Habituation and Autonomic clusters was observed (94). PCB-exposed infants had more abnormal reflexes, startles, and tremors (Table 1), and they were also over reactive to stimulation failing to habituate to repeated auditory, tactile and visual stimulation (94). Like the earlier Great Lakes study, the Oswego study again found a dose-dependent relationship between PCB levels and poorer performance on the Fagan Test of Infant Intelligence, a test of short-term memory (95). Again replicating the earlier Michigan study (80), the Oswego study found that PCB exposure was significantly related to poorer performance on the McCarthy Scales of Children's Abilities at 3 years of age (11), but the motor subscale was not significantly associated with PCB exposure (Table 1). Further analysis found an especially strong relationship between PCB exposure and impaired performance on the Block Building, Word Knowledge, and Draw-a-Design subtests (11) (Table 1). MeHg exposure was also

measured in this cohort, and there appeared to be an interaction between the two chemicals such that when levels of PCBs were high, MeHg exposure was related to poorer McCarthy performance (11). Retesting at 4.5 years of age found that the more highly PCB-exposed children had caught up to the least exposed children on the McCarthy scales (11) (Table 1). However, additional testing at 4.5 years of age showed that children with higher PCB levels had poorer response inhibition on a continuous performance test (96). The splenium of the corpus callosum, a pathway implicated in the regulation of response inhibition, was measured via magnetic resonance imaging in a subset of this cohort. When the splenium was smaller, there was a larger association between PCBs and impaired response inhibition (96).

A fifth cohort exposed to background levels of PCBs has been followed in Germany. In Germany, they failed to confirm the effects of PCBs that the two Great Lakes cohorts observed on visual recognition memory via the Fagan Test of Infant Intelligence, but the German study reported a low retest-/interrater-reliability on the Fagan Test given at 7 months of age (97). However, the German study did report an association between PCB exposure and cognition as assessed by both the Bayley Mental Development index and the Kaufman ABC (98). They also observed deficits up to 3.5 years of age on the Bayley psychomotor scales, which assesses both fine and gross motor skills (98) (Table 1).

Data concerning developmental PCB exposure has recently been reported from the Collaborative Perinatal Project that followed pregnant women in 11 U.S. cities from 1959 to 1965 (99). Overall, there was not an association between prenatal PCB exposure and Bayley Mental or Psychomotor scores (99). However, results from some cities (New Orleans and Baltimore) indicated adverse effects of PCBs on the psychomotor scores, while results from other cities (Richmond and Providence) showed the opposite trend (99) (Table 1).

Lastly, investigations ~~of another PCB cohort~~ are currently underway following Inuit children from Canada. A recent publication reports the effects of exposure to a number of neurotoxicants, including PCBs, chlorinated pesticides, mercury, and lead, on neuromotor function in preschool children (100). Their assessments included a neurological exam that measured posture and passive tone at resting, reflexes and postural reactions. Gross motor function was also assessed with 10 motor tasks, including walking on toes and on heels, walking on a line forward and backward, hopping on one foot, simple and complex tapping reproduction tasks, remaining motionless for 1 minute, hand coordination, and downward arm drift. Lastly, they assessed postural hand tremor, reaction time, standing postural sway oscillations, rapid pointing movements and rapid alternating movements. Current PCB exposure in the children was related to larger transversal sway oscillations in the balance condition in which children were standing in the tandem position with one foot in front of the other (heel to toes) (100) (Table 1). There were no clear PCB effects on any of the other endpoints assessed (100) (Table 1). There were significant associations between blood lead concentration at testing time and neuromotor deficits, consistent with other reports (101, 102).

Although most of these studies focused primarily on cognitive outcomes, there are some indications that motor functions were impacted by PCB exposure. Four studies assessed newborns and all found abnormalities, including hypotonia and abnormal reflexes. The North Carolina, Dutch, and German studies all employed the Bayley Scales, and all observed an association between PCB exposure and lower scores on the Bayley Psychomotor index. However, the Bayley results from the Collaborative Perinatal Project were more equivocal finding two cities with lower scores, two with higher scores, and seven cities with no effect. The Dutch study observed deficits on the McCarthy Motor scale but the Michigan, Oswego

and North Carolina studies did not. As discussed earlier, the McCarthy Motor scale assesses a broad range of motor functions including large muscle coordination and fine motor skills. Unfortunately, these reports do not provide any detail about which specific aspects of motor function were affected in these children. However, the Inuit study has focused on neuromotor function and recently reported an association between current PCB exposure and impaired balance.

*4-2. Epidemiological Studies of MeHg.* After the Minamata and Iraq MeHg poisonings, other populations with low-level environmental MeHg exposure were studied. Cree Indian children aged 12 to 30 months from northern Quebec were followed (103). They were given physical and neurological exams along with the Denver Developmental Screening Test (DDST), which has four major function sectors: gross motor, fine motor, language, and personal-social. There were no impairments associated with MeHg exposure in the girls. In fact, there was a marginally significant improvement in their ratings of coordination on the neurologic examination (103). However, in the boys, abnormal muscle tone and reflexes on the neurologic examination were associated with higher MeHg exposure (103) (Table 2). A more recent cross-sectional study in French Guiana confirms the increased deep tendon reflexes, especially in the MeHg-exposed boys, and the study also reports poorer leg coordination associated with current maternal hair mercury levels (104) (Table 2).

A study in New Zealand also used the DDST and reported an association between increasing prenatal MeHg exposure and poorer scores (105). Approximately 50% of the high MeHg children had abnormal or questionable DDST test results compared to only 17% of the matched controls (Table 2). However, all developmental delays were on the fine motor or language sectors with the exception of one MeHg child and one matched control that were

delayed on the personal-social sector. None of the children were delayed on the gross motor sector. Of the fine motor tests, children were most commonly classified as "delayed" because of failure to copy a circle, imitate a bridge with cubes, or build a tower of 8 cubes. There was a trend for delayed milestones including sitting up, walking and talking, as reported by the mothers (105) (Table 2). There was also a small but non-significant deficit of the MeHg-exposed children on vision tests (105). Lastly, there were more MeHg-exposed children than their matched controls that either did not understand or failed a somatosensory test assessing touch and temperature (105).

Children in the Seychelles Islands in the western Indian Ocean have been examined for MeHg neurotoxicity due to the populations' relatively high consumption of MeHg-contaminated ocean fish. Studies of Seychellois children have failed to find any reliable neurological deficits associated with prenatal mercury exposure (106-109). In an initial pilot study, experimenters gave the DDST to allow comparison to the New Zealand study. As seen in the New Zealand study, MeHg exposure was associated with increased reports of questionable or abnormal responses on the DDST (110) (Table 2). The effect in the Seychelles Islands was smaller than that observed in New Zealand. At 9 years of age, no differences were observed on a battery of cognitive tests nor on a finger-tapping test (108) (Table 2). However, boys actually had improved scores with increasing maternal hair MeHg exposure on the grooved pegboard, a test of manipulative dexterity, and the Beery-Buktenica Visual Motor Integration tasks, a test requiring the subjects to copy simple to complex geometric figures (108) (Table 2). For females, poorer performance on the grooved pegboard task was associated with increasing prenatal MeHg exposure (Table 2), but the authors attributed this effect to a single influential data point (108).

In the main study in the Seychelles Islands, increasing prenatal MeHg exposure was associated with decreasing activity levels in males as assessed in the Infant Behavior Record from the Bayley Scales of Infant Development given at 29 months (107) (Table 2). In contrast to the pilot study, there were no associations of MeHg exposure and DDST outcomes (111) (Table 2). At 5.5 years of age, no adverse outcomes were associated with MeHg exposure, but no tests specific to motor function were given (106). At 9 years of age, MeHg exposure was associated with a decreased tendency for hyperactivity as rated on the Conner's teacher rating scale (109) (Table 2). Also at 9 years of age, increased MeHg exposure was associated with decreased performance in the Grooved Pegboard for the non-dominant hand in males only, which is opposite of the improved Grooved Pegboard performance observed in the pilot study males (109). The authors interpreted both effects as likely being due to chance (109). No associations were observed on other motor tests, including finger tapping, portions of the Bruininks-Oseretsky test of motor proficiency, or tests of visual motor integration (109) (Table 2).

In contrast, in children in the Faroe Islands, whose mothers consumed pilot whale meat and blubber contaminated with both MeHg and PCBs, MeHg exposure was associated with decreased height and body weight in children up to 3.5 years of age (112). These children were also found to have language, attention, and memory deficits, along with a trend for visuospatial and motor dysfunctions (113). Physical examination at 7 years of age found no effects on reciprocal motor coordination or simultaneous finger movements, but children with questionable or deficient performance on the finger opposition test had higher MeHg levels (113) (Table 2). There were slight negative associations on the test of postural sway actually indicating less body sway in children with higher MeHg exposure (113) (Table 2). There were

four conditions for the postural sway testing that included standing with eyes open or closed on a platform with and without foam underneath, and the only marginally significant association ($p = 0.09$) was for the eyes closed, no foam condition, which presumably is not the most challenging condition. No effect of MeHg was observed on the Tactual Performance Test, which measures tactile processing by blindfolding the child and asking them to put 6 geometrical shapes into their appropriate places on a foam board. However, there were failures to complete this test, especially in the younger children, which may mask exposure effects (113). The trend for visuospatial deficits included increased errors in the copying of complex figures in the Bender Gestalt test (113) (Table 2). In the Faroe Islands, MeHg exposure was associated with poorer performance on the finger-tapping task (preferred hand) (Table 2) and slower reaction times on continuous performance tasks, the latter of which may or may not be motor-related (113). The deficit on the finger-tapping test, particularly in the boys, was confirmed in a later reanalysis of the data using a case-control design (114). A motor deficit was also suggested in a hand-eye coordination test in the case-control design (114), but this deficit may have been muted by a floor effect since this test was reported to be too difficult for many of the children (113).

The recently published study of Inuit children described the previous section examined the neuromotor effects following mercury exposure in addition to exposure to PCBs and other neurotoxicants (100). They found that blood mercury concentrations in the children at the time of testing were significantly associated with higher action tremor amplitude as the child alternated between touching proximal and distal (arm almost fully extended) targets with a hand-held stylus (100) (Table 2). No other measures of neuromotor function were significantly associated with the mercury exposure (Table 2).

Lastly, Grandjean and colleagues who conducted the Faroe Island study have also studied MeHg exposure in children in two cross-sectional studies. One study in the Madeira Islands found that concurrent maternal hair mercury was not associated with children's performance on finger tapping, hand-eye coordination, or continuous performance tests (115) (Table 2). Another cross-sectional study in the Brazilian Amazon found that the children's current hair MeHg levels were associated with fine motor deficits on the Santa Ana pegboard test but not on the finger-tapping test (116) (Table 2).

Three of the studies—Cree Indian, New Zealand, and Seychelles—examined infants using the DDST, and all three studies reported adverse outcomes associated with MeHg exposure. The Faroe Island study has only reported findings beginning at 7 years of age. A number of deficits were observed in the Faroe Island children, including cognitive endpoints and endpoints involving motor systems. In contrast, the Seychelles Islands study, which tested another large cohort of children, has not shown any consistent deficits associated with MeHg. Most of the motor deficits observed in these epidemiological studies are in 7 or 9 yr-olds and are restricted predominantly to fine motor skills: finger tapping, pegboard, hand-eye coordination, figure copying, and slower reaction times (113). With the exception of the Inuit study and the tests of postural sway in the Faroe Islands, measures of balance and coordination were not generally conducted. The only common motor-related task in both the Faroe and Seychelles Islands was finger tapping, which was associated with MeHg exposure in the Faroe Island study but not in the Seychelles study.

There are a number of differences among these studies, including the tests given, the time of assessment, different measurements of mercury exposure (maternal hair in the Seychelles vs. cord blood in the Faroe Islands), and exposures to additional contaminants (i.e.

PCBs). Perhaps one of the most important differences between the Faroe and Seychelles Islands studies is the additional exposure to PCBs in the Faroe Islands. Analyses of the children's blood in the Seychelles found no detectable exposure to PCBs (106), but the level of PCB exposure in the Faroe Islands was quite high--3-4-fold higher than in most other PCB studies to date, including those described above (117). In addition, the total mercury level in the maternal hair samples was actually higher in the Seychelles, where no deficits were observed, than in the Faroe Islands (6.8 vs. 4.27 ppm, respectively) (106, 113). 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 (10).

In conclusion, the majority~~only~~ of motor deficits observed in the MeHg epidemiological studies ~~have~~ involve~~d~~ fine motor skills and hand tremor. Excluding the recently published Inuit study, other studies have not fully evaluated many aspects of motor function. The Faroe Islands study reported slightly less body sway in the postural test, but other aspects of motor coordination and balance that require the cerebellum were not assessed. The Inuit study, which reports mercury levels comparable to the Faroe Islands study, found no association between MeHg exposure and postural sway (100). A common symptom of the poisonings in Minamata and Iraq was ataxia. Thus, it is surprising that the potential for lower level MeHg exposures to affect balance and coordination has not been more fully investigated.

*4-3. Summary of Epidemiological Studies of PCBs and MeHg.* Epidemiological studies of developmental PCB or MeHg exposure have mainly focused on cognitive outcomes, but some motor functions were assessed. PCBs have been associated with decreased motor scores on various tests given from 6 months to 6.5 years of age. In contrast, MeHg has been associated

with fine motor deficits that were present at 7-9 years of age. The full range of motor functions has not been adequately assessed with either contaminant. In particular, there have been few tests of cerebellar-mediated functions, including balance and coordination, despite the fact that the poisoning episodes for both PCBs and MeHg suggested the cerebellum as a site of action.

**5. Studies in Laboratory Animals.**

*5-1. PCB Studies in Laboratory Animals.* The neurotoxicity of PCBs has been demonstrated in numerous animal studies. Several studies have found that developmental PCB exposure impairs cognition, especially learning and memory, in both primates (118-123) and rodents (9, 124-130). PCBs have also been shown to impair hearing in developmentally-exposed rats (131-135) possibly due to damage to the outer hair cells of the cochlea via a thyroid hormone dependent mechanism (131, 134).

A few studies have examined the effects of perinatal exposure to PCBs on reflex development in rats. All but one of the studies have examined the surface-righting reflex, in which the rat rights itself after being placed on a surface on its back. Three studies reported ~~,~~ ~~and found~~ no delay in surface-righting reflex development (125, 136, 137). One study (138) reported slower surface righting times on PND3-6 with PCB exposure, but another study with a slightly lower dose (139) found no impaired surface-righting ability when tested on PND17 (139) (Table 3). One study (140) found that PCB exposure delayed the ontogeny of the air-righting reflex, in which the rat is dropped from a short distance with its back toward the ground and must right itself in order to land on its feet, while other study (139) found no changes in air-righting ability on PND 28, 43, or 65 (Table 3). Three~~wo~~ of the studies also investigated negative geotaxis, in which the rat is placed head-down on an inclined board and the latency to turns its body to a head-up position is recorded. One (140) reported a delay in

the ontogeny of negative geotaxis, and another (138) reported slower times to turn upright on PND5 and 6. However, the third study (125)~~but the other~~ observed no effects on negative geotaxis (Table 3). PCB exposure slowed development of cliff avoidance (125, 136) ~~development of cliff avoidance~~ and swimming ability (125) (Table 3). PCB-exposure resulted in prolonged swimming in circles rather than straight lines.

The effects of PCBs on overall locomotor activity levels are difficult to interpret because of differences between studies on factors such as the PCB mixture or congener studied, dose, timing and duration of exposure, age at assessment, and method for assessing activity levels. For example, depending on the study, coplanar PCB 77 has increased (141, 142), decreased (143), or had no effect (144) on locomotor activity (Table 4). PCB95, a di-ortho-substituted PCB, decreased locomotor activity in adult rats that were exposed *in utero* (145) (Table 4). Mice exposed to Aroclor 1254 (a commercial PCB mixture) pre- and postnatally were found to be hyperactive (129) (Table 4). However, rats exposed to the same mixture pre- and postnatally exhibited either a transient reduction in motor activity (134, 135) or no~~t~~ alternations at all (139) (Table 4). Exposure of rats to Chlophen A30, another commercial mixture of PCBs, during gestation and lactation caused a transient hyperactivity (146) (Table 4). Exposure to Fenclor 42 (yet another commercial PCB mixture) either pre- or postnatally caused hypoactivity (125) (Table 4). Eriksson and colleagues have studied single PCB congeners in mice exposed on PND10. They found no changes in activity levels with several mono-*ortho*-substituted PCB congeners, including PCB 105 (147) and PCBs 118 and 156 (148) (Table 4). In contrast, they found that coplanar PCB 126 (147) or *ortho*-substituted PCB 28 and 52 (148, 149) each altered spontaneous activity by causing a disruption of habituation (Table 4). Similar to the PCB 118-exposed mice, PCB 118-exposed rats initially

showed no change in activity levels when tested on PND30-34 (136) but were hyperactive when retested on PND70-74, illustrating the importance of the timing of assessment.

Studies in animal models investigating the effects of PCBs on balance and coordination are not extensive or conclusive. In an early study (142), high doses of a single coplanar PCB congener (PCB 77) in mice produced a bizarre "spinning" syndrome characterized by intermittent stereotypic circling, head bobbing, and hyperactivity (Table 5). PCB spinner mice also showed decreased muscular strength on a forelimb grip strength test and impairment on a visual placement test in which the mouse was required to stretch its forelimbs outward to grasp a ring (Table 5). All of the PCB-exposed mice, even these not displaying the spinning syndrome, were impaired in their ability to traverse a wire rod (Table 5). In contrast to the decreased forelimb grip strength in Tilson's mice, Aroclor 1254-exposed rats showed no changes in grip strength (139) or in duration of forepaw suspension from a taut wire (140) (Table 5). In addition, there were no changes in gait or coordination as observed by the experimenter or in landing foot splay (139) (Table 5). Exposure to a single dose of the mono-ortho PCB118 on GD6 caused no changes in forelimb grasp in the offspring, but postweaning PCB-exposed female rats developed the ability to stay on the rotating rod for 3 minutes a few days sooner than the control females (136) (Table 5). Aroclor 1254 exposure impaired rotorod performance on PND12, especially in the PCB-exposed males (138).

We recently exposed rats to PCBs pre- and postnatally and tested them on three motor tasks: 1) climbing up a 2 ft-long vertical rope, 2) traversing sets of parallel bars with varying inter-rod distances, and 3) crossing a 2-m long rotating rod suspended 1 m above ground with the rotating speed increasing from 0 to 30 rpms over a series of trials. PCBs had no effect on the rope climb or parallel bars, but there was a slight impairment on the rotating rod task (8)

(Table 5). The rotating rod task was designed by Altman to assess cerebellar-mediated motor function (150). X-irradiation of the cerebellum on PND4 and 5 causes structural disorganization of the cerebellar cortex and cell loss, and these rats displayed impaired rotating rod performance but no change in swimming speed (151). In addition, rats neonatally exposed to alcohol were impaired as adults on the rotating rod (152) and had significant cerebellar damage, including decreases in Purkinje cell number, molecular layer volume and total volume within the paramedian lobule (153). Thus, although very few animal studies have measured balance and coordination, our results suggest that PCBs may impair these cerebellar-mediated motor functions.

*5-2. MeHg Studies in Laboratory Animals.* Neurotoxic effects of developmental MeHg exposure have been documented in animal studies. MeHg exposure in primates impairs memory and discrimination (154) and performance in operant reward tasks (155, 156). MeHg-exposed rats were impaired on delayed spatial alternation, a task of spatial working memory (9), and MeHg mice have shown impaired avoidance learning (157). The effects of developmental MeHg exposure on the sensory systems have been extensively investigated in monkeys, finding hearing deficits (121, 158), impaired spatial and temporal visual function (159, 160), and impaired vibration sensitivity (161). MeHg may also accelerate the normal age-related declines in the somatosensory (162) and auditory (163) systems.

Unlike PCBs, a number of studies have investigated motor function in MeHg-exposed animals. The development of various reflexes is impaired following high-dose MeHg exposure. Delays in surface righting have been observed in some studies following prenatal exposure (164-166) but not in others (167, 168) (Table 6). Mice exposed to MeHg prenatally showed righting difficulty after 6 mos of age (51) (Table 6). Abnormalities in the air-righting

reflex also have been observed in prenatally exposed mice (49) and rats (169) (Table 6). Prenatally exposed rats have shown less pivoting (movement of the forelimbs which rotate the body about a hindlimb pivot point) (164) (Table 6). Two studies observed no change in negative geotaxis in rats (167, 170), whereas a third study (165) reported an acceleration of negative geotaxis following the same exposure paradigm (Table 6). In another study, a slightly higher dose of MeHg caused more falling on the negative geotaxis test (164) (Table 6). Similarly, mice exposed to MeHg prenatally had difficulties on inclined plane and vertical grid tasks (51) (Table 6). Lastly, MeHg caused retardation in walking (49, 166), and in the development of swimming abilities in mice (171, 172) and rats (164, 165, 169, 173) (Table 6).

As with PCBs, the effects of MeHg on locomotor activity are unclear. Hyperactivity has been observed following prenatal exposure to MeHg but only when rats were tested before PND22 (174) (Table 7). In another study, prenatal MeHg exposure resulted in a trend towards hyperactivity in male rats (170) (Table 7). Other studies have found no change in activity levels following prenatal MeHg exposure in mice (157, 166) and rats (175) (Table 7). Hypoactivity has also been observed following prenatal MeHg exposure in rats (165, 167, 176, 177) and mice (49) (Table 7). Lastly, another study (178) exposed rats to MeHg perinatally and observed hypoactivity in the males with no changes in activity in the females (Table 7).

Early postnatal exposure to MeHg impaired rotarod performance in rats (179) (Table 8). Mice exposed to MeHg in adulthood were also impaired on the rotarod (180-182). Abnormal walking ability has been observed in mice exposed to MeHg during gestation (49, 166) (Table 8), and MeHg exposure has been shown to cause hind-limb dysfunction (49, 51, 179, 181, 183, 184). Severe movement and postural disorders, including hypertonicity of limb

flexors, flexion deformities, hyperreflexia, and epileptiform seizures with myoclonic jerking of the hindlimbs, have been observed following postnatal MeHg exposure in rats (185) (Table 8).

We recently exposed rats to MeHg pre- and postnatally and tested them on three motor tasks, which were described in the previous section. MeHg caused a slight impairment in vertical rope climbing in female rats, no impairments on the parallel bars or rotating rod task (8) (Table 8). However, it must be noted that the MeHg dose used in this study was approximately 2-10-fold less than those used in other rodent studies.

*5-3. Studies of Combined PCB and MeHg Exposure in Laboratory Animals.* Despite the fact that humans are often exposed to PCBs and MeHg in combination, combined exposure to these two chemicals has not been well studied in animal models. Prior to our current research, only one other group had investigated neurobehavioral effects following the combined treatment of animals with PCBs and MeHg (172). This study employed 6 groups of mice to examine the effects of combined exposure to PCBs and MeHg. Dams from 3 of the 6 groups were fed a diet containing 500 ppm of PCBs (Kanechlor 500, a commercial mixture) from gestation day (GD) 0 to postnatal day (PND) 21. Females in these PCB groups were also given methylmercuric chloride (0, 0.4 or 4 mg/kg) orally from GD15 to PND21. Two more groups received MeHg alone at either 0.4 or 4 mg/kg, and a control group received neither PCBs nor MeHg.

The study compared the groups across a number of behavioral tests. There were no significant differences among groups on preweaning tests of negative geotaxis, surface righting, or visual cliff. Females exposed to 0.4 mg/kg MeHg + PCBs were significantly slower crawling away from the cliff edge on a cliff avoidance task. PCB exposure alone has been shown to delay cliff avoidance (125). As has been reported with either PCB (125) or

MeHg exposure alone (164, 165, 169, 171, 173), retarded swimming ability was present in all groups, especially in the PCB-exposed groups. When tested in an open field, MeHg caused an increase in activity (increase in # squares traversed and # of rearings) on PND21 and at 10 weeks, while high dose MeHg + PCBs or PCBs alone caused a decrease in # of squares traversed when tested at 10 weeks. The open field activity effects were apparently transitory since no differences were observed when animals were retested at 32-36 weeks. PCB-exposed mice were significantly impaired on a hind-limb support task, with the greatest deficit being observed in the PCB group receiving the highest dose of MeHg. This finding is in agreement with other studies finding hind-limb dysfunctions following high MeHg doses (49, 51, 179, 181, 183, 184). In addition, the high dose MeHg + PCB group also received lower scores on a visual placing test (when suspended by the tail and lowered over a solid object, the mouse should raise its head and extend its forelimbs). Similarly, PCB-exposed mice have previously been shown to have impairments on the visual placement task (142). It seems that the deficits on the hind-limb support task and the visual placement task may have resulted from the additive effects of PCB and MeHg, since the greatest hind-limb impairments were observed in the PCB + high MeHg group. Similarly, only that group was impaired on the visual placing test.

We recently investigated the combined effects of PCB and MeHg exposure in rats. Dams were divided into four groups (controls, PCBs alone, MeHg alone, and PCB+MeHg) with exposure beginning 4 weeks prior to breeding and continuing to PND16. The PCB dose was 6 mg/kg/d of Aroclor 1254, a commercial PCB mixture, diluted in corn oil, pipetted onto vanilla wafers, and fed to the dams. The MeHg-exposed rats received methylmercuric chloride dissolved at a concentration of 0.5 µg/ml (0.5 ppm) in their drinking water. The resulting

offspring were tested after PND60 on three tasks involving the cerebellum: vertical rope climb, traversing parallel bars, and crossing a 2-m long rotating rod. We observed no effects of combined PCB and MeHg exposure on the vertical rope climb or traversing the parallel bars. However, we found an additive effect of PCBs and MeHg on the rotating rod task. PCBs or MeHg alone non-significantly impaired performance on the rotating rod, while combined exposure caused a significant impairment when compared to control rats (8). The effect seemed to be driven more by the PCB exposure, but again we used a very low dose of MeHg.

*5-4. Summary of PCB and MeHg Studies in Laboratory Animals.* Both PCBs and MeHg cause cognitive and sensory deficits in animal models. In addition, both contaminants cause delays in reflex development in rodents. A wider range of reflexes has been examined following MeHg exposure, but both chemicals delay air righting, the development of swimming abilities, and possibly negative geotaxis. Locomotor activity results for both PCBs and MeHg are rather inconclusive, and this is likely due to variations in study design, including the timing of exposure, the timing of testing, the methodology used to assess activity, and in the case of PCBs, the type of PCBs used—mixtures vs. single coplanar vs. *ortho*-substituted congeners. Again, cerebellar-mediated motor function has been assessed more thoroughly in the MeHg studies, and impairments on rotarod, walking abnormalities, hind-limb dysfunctions, and severe movement and postural disorders have been noted. The only comparable effects with PCB exposure are impairments traversing a wire rod or rotating rod. The two studies of combined PCB and MeHg exposure indicate that two contaminants may have additive effects on hind-limb support, visual placing, and rotating rod tasks.

## 6. Conclusions and Directions for Future Research

Higher doses of PCBs or MeHg, as seen in the poisonings, have been associated with impairments in motor function, particularly functions involving the cerebellum (23, 70-72). MeHg is known to damage the cerebellum in both human (45, 46, 73, 186) and laboratory animal exposures (48-52, 183, 187), whereas the effects of PCBs on the cerebellum are relatively unstudied. In the epidemiological studies investigating lower-level exposures to PCBs or MeHg, there have been some indications of motor impairments (11, 85, 92, 98, 109, 113), but the primary focus has been on cognitive function and the full range of motor functions has not been assessed, particularly functions of a cerebellar nature.

MeHg exposure in animals has been shown to impair cerebellar-mediated motor functions, including walking ability (49, 166), hind-limb dysfunction (49, 51, 179, 181, 183, 184), and rotarod performance (179), and can result in severe movement and postural disorders (185), but the threshold for these effects is not known. Most of the studies to date have used relatively high doses of MeHg. Very few animal studies have assessed motor function following PCB exposure, but there are some findings suggesting that PCBs impair cerebellar-mediated motor function, such as traversing a wire rod (142), or a rotating rod (8, 138).

Mechanistically, there are reasons to suspect that the cerebellum may be especially vulnerable to combined PCB and MeHg exposure. PCBs have complex effects on thyroid hormones, reducing circulating thyroxine concentrations, but at the same time showing evidence of thyromimetic effects on gene expression in the brain. The cerebellum is particularly vulnerable to thyroid hormone alterations during development. MeHg is known to target the cerebellum, damaging its structure (45, 46, 187). Further, PCBs and MeHg have a common site of action at the ryanodine receptor, altering intracellular calcium signaling. As recently reported (6), combined PCB and MeHg exposure *in vitro* synergistically increased

calcium release, but at higher concentrations or longer durations, the two chemicals antagonized each other. PCBs stabilize the open conductance state of the RyR (59), and it is speculated that this allows MeHg to more readily gain access to thiol groups of the RyR and inactivate the receptor. In particular this is important for the cerebellum because RyRs are found throughout the brain but especially within the cerebellum (188). Further, alterations in ryanodine-sensitive calcium signaling within the cerebellum could impair long-term depression and subsequent motor learning.

Recent studies raise concern for combined PCB and MeHg exposure. As discussed, interactive effects of PCBs and MeHg have been reported on neuronal calcium regulation and dopamine function *in vitro* (6, 7). We recently reported an additive effect of the two chemicals *in vivo* on the rotating rod, a cerebellar motor task (8). Lastly, interactive effects of PCBs and MeHg have also been observed in epidemiological studies. PCB-associated deficits were found within the highest tertile of MeHg exposure in the Faroe Island Study (10), and conversely, when levels of PCBs were high in the Oswego study, MeHg exposure was related to poorer McCarthy performance (11). We believe that these findings illustrate the importance of understanding the implications of combined exposure, and future studies are needed at all levels, *in vitro, in vivo*, and epidemiological to understand this potential human health risk.

There are a few areas that should be addressed in future research. First, studies of the effects of PCBs on motor function in both humans and animal models are lacking. Additional experiments in laboratory animals are needed to clarify the effects of PCBs on various motor functions, including balance and coordination. These studies should employ environmentally relevant mixtures of PCBs in order to provide insights as to what aspects of motor function should be evaluated in epidemiological studies of exposed children. Second, ongoing

epidemiological studies of both PCBs and MeHg should incorporate more tests of motor function and motor learning into their batteries. Since the cerebellum is a potential site of action, the batteries should include tests with a major cerebellar component, such as balance beam, postural sway, or eye-blink conditioning. Lastly, we need to better understand the possible interactive effects of PCBs and MeHg on the nervous system so we can advise people, especially pregnant women or women planning to become pregnant, on these potential neurotoxic consequences.

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**Table 1.**
Reported motor impairments in human studies of PCBs.

| Study | Age(s) Assessed | Motor Outcomes | Reference |
|---|---|---|---|
| ***Accidental PCB Poisonings*** | | | |
| Yusho, Japan | not specified | clumsy movement, hypotonia, slowness, jerkiness | (23) |
| YuCheng, Taiwan | 2.5 yrs | lower scores Bayley psychomotor index<br>delayed on 32 of 33 developmental milestones | (24) |
| ***Epidemiological Studies of PCBs*** | | | |
| Michigan | birth | motoric immaturity, hyporeflexia | (78) |
| | 4 years | no changes McCarthy motor scale or<br>Beery test of Visual-Motor integration | (80) |
| North Carolina | birth | hyporeflexia, hypotonicity | (83) |
| | 6,12,18, 24 mos | lower scores Bayley psychomotor index | (84, 85) |
| | 3, 4, and 5 yrs | no change McCarthy motor scale | (86) |
| Dutch | birth | poorer neurological condition, hypotonia | (87) |
| | 3 and 7 mos | lower scores Bayley psychomotor index | (88) |
| | 18 mos | no changes Bayley psychomotor index | (88) |
| | 18 mos | poorer neurological condition | (89) |
| | 3.5 yrs | no changes neurological condition | (90) |
| | 7 yrs | lower scores McCarthy motor scale, especially with less optimal parental & home environments | (92) |
| Oswego | birth | more abnormal reflexes, tremors | (94) |
| | 3 yrs | no changes McCarthy motor scale, but significant impairments on Block Building & Draw-a-Design | (11) |
| | 4.5 yrs | no changes McCarthy scales | (11) |
| German | 7, 18, and 30 mos | lower scores Bayley psychomotor index | (98) |
| Collaborative Perinatal Project | 8 mos | Bayley psychomotor scores lower in New Orleans & Baltimore, higher in Richmond & Providence, & no changes in other 7 cities | (99) |
| Inuit | 4-6 yrs | larger transverse postural sway during balance conditions<br>no changes neurological condition<br>no changes gross motor | (100) |

**Table 2.**
Reported motor impairments in human studies of MeHg.

| Study | Age(s) Assessed | Motor Outcomes | Reference |
|---|---|---|---|
| ***Accidental MeHg Poisonings*** | | | |
| Minamata Bay | not specified | loss of coordination, impaired gait | (70) |
| Iraq | 1-2 yrs | delayed motor development | (20) |
| | not specified | increased limb tone & deep tendon reflexes, ataxia, hypotonia, athetoid movements | (20) (72) |
| ***Epidemiological Studies of MeHg*** | | | |
| Cree Indians | 12-30 mos | abnormal muscle tone & reflexes in boys | (103) |
| French Guiana | 2-6 yrs | increased tendon reflexes, especially in boys | (104) |
| | 5-12 yrs | poorer leg coordination more so in boys<br>no changes in finger tapping | |
| New Zealand | 4 yrs | more abnormal or questionable DDST results<br>delayed milestones (i.e. sitting up, walking) | (105) |
| Seychelles Islands | | | |
| Pilot Study | 5-109 wks | more abnormal or questionable DDST results | (110) |
| | 9 yrs | no change in finger tapping<br>MeHg boys improved on grooved pegboard & on Visual Motor Integration tasks<br>MeHg girls impaired on grooved pegboard | (108) |
| Main Study | 29 mos | decreased active levels in MeHg males | (107) |
| | 6.5 mos | no changes DDST | (111) |
| | 9 yrs | decreased tendency for hyperactivity<br>decreased grooved pegboard in boys<br>no changes finger-tapping, motor proficiency, or visual motor integration tests | (109) |
| Faroe Islands | 7 yrs | questionable or deficient finger opposition performance<br>slightly less postural sway 1 of 4 testing conditions<br>increased errors copying complex figures<br>poorer performance finger-tapping task<br>slight deficit hand-eye coordination task | (113) |
| Inuit | 4-6 yrs | increased hand action tremor amplitudes<br>no changes neurological condition or gross motor | (100) |
| Madeira Islands | 7 yrs | no changes finger tapping or hand-eye coordination | (115) |
| Brazilian Amazon | 7-12 yrs | fine motor deficits Santa Ana pegboard,<br>no changes finger tapping | (116) |

**Table 3.**
Developmental reflexes in PCB-exposed laboratory animals.

| Species | Dose<br>PCB used | Exposure Period | Motor Outcomes | Reference |
|---|---|---|---|---|
| Long-Evans rats | 0.25 or 1.0 µg/kg/d<br>PCB126<br>(coplanar) | 5 weeks prior to breeding through to lactation | no delay in surface righting | (137) |
| Fischer 344 | 5-10 mg/kg/d<br><br>2-4 mg/kg/d<br>1-2 mg/kg/d<br>Fenclor 42 | 5 daily doses 2 weeks prior to breeding<br>GD6-15<br>PND1-21 | no delay in surface righting<br>no delay in negative geotaxis<br>slowed development of swimming abilities<br>slowed development of cliff avoidance | (125) |
| Sprague-Dawley rats | 375 µg/kg<br>PCB 118<br>(mono-ortho) | GD6 | no delay in surface righting<br>delayed development of cliff avoidance | (136) |
| Wistar rats | 26 ppm<br>Aroclor 1254 | mating to weaning | delay in air-righting reflex<br>delay in negative geotaxis | (140) |
| Long-Evans rats | 1 or 6 mg/kg/d<br>Aroclor 1254 | GD6-PND21 | no impairment in righting ability | (139) |
| Sprague-Dawley rats | 10 mg/kg/d<br>Aroclor 1254 | GD11-PND21 | impairment in surface righting<br>impairment in negative geotaxis | (138) |

**Table 4.**
Locomotor activity in PCB-exposed laboratory animals.

| Species | Dose<br>PCB used | Exposure Period | Motor Outcomes | Reference |
|---|---|---|---|---|
| CD-1 mice | 32 mg/kg/d<br>PCB77<br>(coplanar) | GD10-16 | increased activity | (141, 142) |
| Long Evans rats | 0.5 or 1.5 mg/kg/d<br>PCB77 (coplanar),<br>1.5 mg/kg/d<br>PCB47 (di-ortho), or<br>0.5 mg/kg/d PCB77+<br>1.0 mg/kg/d PCB47 | GD7-18 | increased activity on PND340 | (144) |
| NMR1 male mice | 0.41 or 41 mg/kg<br>PCB77<br>(coplanar) | PND10 | initial decrease in activity<br>followed by disrupted habituation | (143) |
| NMR1 male mice | 0.046, 0.46 mg/kg<br>PCB126<br>(coplanar) | PND10 | initial decrease in activity<br>followed by disrupted habituation | (147) |
| NMR1 male mice | 0.23, 0.46, 4.6<br>mg/kg PCB105<br>(mono-ortho,<br>coplanar-like) | PND10 | no change in activity | (147) |
| NMR1 male mice | 0.23, 0.46, 4.6<br>mg/kg PCB118<br>(ortho)<br>0.25, 0.51, 5.1<br>mg/kg PCB156<br>(ortho) | PND10 | no change in activity | (148) |
| Sprague Dawley rats | 375 µg/kg<br>PCB 118<br>(mono-ortho) | GD6 | no change in activity PND30-34<br>hyperactive PND70-74 | (136) |
| NMR1 male mice | 0.18, 0.36, 3.6<br>mg/kg PCB28<br>(ortho)<br>0.20, 0.41, 4.1<br>mg/kg PCB52<br>(ortho) | PND10 | initial decrease in activity<br>followed by disrupted habituation | (148, 149) |
| Sprague Dawley rats | 8 or 32 mg/kg/d<br>PCB95<br>(ortho) | GD10-16 | no changes on PND35<br>decreased activity on PND100 | (145) |

**Table 4 (continued).**
Locomotor activity in PCB-exposed laboratory animals.

| Species | Dose<br>PCB used | Exposure Period | Motor Outcomes | Reference |
|---|---|---|---|---|
| ICR mice | 11 or 82 ppm (diet)<br>Aroclor 1254 | 3 days prior to mating to PND21 | increased activity | (129) |
| Long Evans rats | 4 or 8 mg/kg/d<br>Aroclor 1254 | GD6-PND21 | transient decrease in activity on PND15 | (134, 135) |
| Long Evans rats | 1 or 6 mg/kg/d<br>Aroclor 1254 | GD6-PND21 | no change in activity | (139) |
| Wistar rats | 30 mg/kg (diet)<br>Clophen A30 | 60 days prior to breeding to adulthood | transient increase in activity | (146) |
| Fischer 344 rats | 5-10 mg/kg/d | 5 daily doses 2 weeks prior to breeding | decreased activity PND14 & 21 | (125) |
| | 2-4 mg/kg/d | GD6-15 | no change in activity | |
| | 1-2 mg/kg/d<br>Fenclor 42 | PND1-21 | decreased activity on PND14 but not 21 | |

**Table 5.**
Complex motor effects ~~Balance and coordination effects~~ in PCB-exposed laboratory animals.

| Species | Dose<br>PCB used | Exposure Period | Motor Outcomes | Reference |
|---|---|---|---|---|
| CD-1 mice | 32 mg/kg/d<br>PCB77<br>(coplanar) | GD10-16 | spinning syndrome in some mice<br>decreased forelimb grip strength<br>impaired visual placement<br>impaired traversing of wire rod | (142) |
| Wistar rats | 26 ppm<br>Aroclor 1254 | mating to weaning | no change in forepaw suspension | (140) |
| Long Evans | 1 or 6 mg/kg/d<br>Aroclor 1254 | GD6-PND21 | no change in grip strength, gait, coordination, or landing foot splay | (139) |
| Sprague Dawley rats | 375 µg/kg<br>PCB 118<br>(mono-ortho) | GD6 | no change in development of forelimb grasp<br>postweaning PCB-exposed females developed the ability to stay 3 min on the rotating rod a few days sooner than control females | (136) |
| Long Evans rats | 6 mg/kg/d<br>Aroclor 1254 | 4 wks prior to breeding to weaning | slight impairment on rotating rod | (8) |
| Sprague-Dawley rats | 10 mg/kg/d<br>Aroclor 1254 | GD11-PND21 | impairment on rotorod | (138) |

**Table 6.**
Developmental reflexes in MeHg-exposed laboratory animals.

| Species | MeHg Dose | Exposure Period | Motor Outcomes | Reference |
|---|---|---|---|---|
| Sprague Dawley rats | 0.25 mg/kg/d<br>1.25 or 2.5 mg/kg/d<br>2.5 mg/kg/d | GD6-15 | less pivoting<br>retarded swimming development<br>delayed surface righting, more falling on negative geotaxis | (164) |
| Sprague Dawley rats | 6 mg/kg/d<br>2 mg/kg/d | GD6-9 | delayed surface righting<br>accelerated negative geotaxis<br>retarded swimming development | (165) |
| Mice | 0.5, 1, 2, 4 or 8 mg/kg | GD7, 9, 12, or 13 | delayed surface righting<br>difficulties on inclined plane and vertical grid tasks | (51) |
| ICR mice | 3 mg/kg/d<br>5 mg/kg | GD12-14<br>GD12 only | delayed surface righting<br>retarded walking development | (166) |
| Charles River rats | 0.08, 0.4, or 2 mg/kg/d | GD6-15 | no change in surface righting | (168) |
| Sprague Dawley rats | 2 mg/kg/d | GD6-9 | no change in surface righting,<br>no change in negative geotaxis | (167) |
| Inbred C3H/ HeN mice | 20 mg/kg | GD13, 14, 15, 16, or 17 | delayed air-righting reflex<br>retarded walking development | (49) |
| Holtzman rats | 2 ppm dietary | G0 to end of study | delayed air-righting reflex<br>retarded swimming development | (169) |
| Rats | 2 or 6 mg/kg/d | GD6-9 | no change in negative geotaxis | (170) |
| JCL-ICR female mice | 0.4 or 4 mg/kg/d | GD15 to weaning | retarded swimming development | (172) |
| Mice | 8 mg/kg/d | GD7-9 | retarded swimming development | (171) |
| Kfm:WIST Rats | 0.5 mg/kg/d | GD6-9 | retarded swimming development | (173) |

**Table 7.**
Locomotor activity in MeHg-exposed laboratory animals.

| Species | MeHg Dose | Exposure Period | Motor Outcomes | Reference |
|---|---|---|---|---|
| Long Evans rats | 5 or 8 mg/kg | GD8 or 15 | increased activity before PND22 | (174) |
| Rats | 2 or 6 mg/kg | GD6-9 | trend for increased activity in males only | (170) |
| ICR mice | 3 mg/kg/d<br>5 mg/kg | GD12-14<br>GD12 only | no change in activity | (166) |
| CFW mice | 1, 2, 3, 5, or 10 mg/kg | GD8 | no change in activity | (157) |
| Sprague Dawley rats | 8 mg/kg | GD8 | no change in activity | (175) |
| Sprague Dawley rats | 2 or 6 mg/kg/d | GD6-9 | decreased activity | (167, 176) |
| Sprague Dawley rats | 10 mg/kg | GD4 | decreased activity | (177) |
| Sprague Dawley rats | 6 mg/kg/d | GD6-9 | decreased activity | (165) |
| Inbred C3H/ HeN mice | 20 mg/kg | GD13, 14, 15, 16 or 17 | decreased activity | (49) |
| Sprague Dawley rats | 0.5 mg/kg/d in dams' drinking water | GD7-PND7 | decreased activity in males, no changes in activity in females | (178) |

**Table 8.**
Complex motor effects in MeHg-exposed laboratory animals.

| Species | MeHg Dose | Exposure Period | Motor Outcomes | Reference |
|---|---|---|---|---|
| Wistar rats | 2.6-10 mg/kg/d<br>7.14 or 10 mg/kg/d | PND14-24<br>PND14-24 or PND35-45 | impaired rotarod performance<br>hind-limb dysfunction | (179) |
| ICR mice | 3 mg/kg/d<br>5 mg/kg | GD12-14 or<br>GD12 only | abnormal walking | (166) |
| Inbred C3H/ HeN mice | 20 mg/kg | GD 13, 14, 15, 16 or 17 | abnormal walking<br>hind-limb dysfunction | (49) |
| Mice | 0.5, 1, 2, 4, or 8 mg/kg/d<br>8 mg/kg/d | GD7, 9, 12, or 13 | hind-limb dysfunction<br>incoordination & ataxia | (51) |
| Sprague Dawley rats | 5 mg/kg/d | PND5 to approx. PND24 | severe movement & postural disorders | (185) |
| Long Evans rats | 0.5 ppm in dams' drinking water | 4 weeks prior to breeding to PND16 | slight rope climb impairment in females<br>slight parallel bar improvement in males | (8) |