INTERACTIVE EFFECTS OF DEVELOPMENTAL PCB EXPOSURE AND RELEVANT ENVIRONMENTAL RISKS ON THE AUDITORY SYSTEM

BY

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DISERTATION

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ABSTRACT

Polychlorinated biphenyls (PCBs) are environmental contaminants that can cross the mammalian placenta and mobilize from fat stores into breast milk to affect the developing offspring. Developmental PCB exposure has been shown to cause auditory deficits that last through adulthood. We assessed the interaction of PCBs with other environmental risks to examine if there were additive or interactive effects on auditory function. Rats were exposed developmentally to a unique environmental PCB mixture, the Fox River PCB mixture (FRM), and/or to molar equivalent doses of a commercial polybrominated diphenyl ether (PBDE) mixture, DE-71, that represents the PBDEs found in humans to assess the potential for additive effects of PCBs and PBDEs on cochlear function. In adulthood, the cochlear integrity of the offspring was tested by measuring distortion product otoacoustic emissions (DPOAEs). DPOAE amplitudes were decreased and thresholds were elevated in the 6 mg/kg PCB group. Exposure to PBDEs alone did not cause DPOAE deficits. Importantly, there was an interactive effect from combined exposure such that the individual low doses of PCBs and PBDEs did not result in DPOAE deficits, but the two combined produced a deficit similar to that of the high dose PCB group. These effects were accompanied by sharp reductions in thyroxine (T4) concentrations measured in littermates at weaning.

We then assessed the interaction of developmental PCB exposure and noise exposure during adulthood to evaluate whether early PCB exposure increased the susceptibility to noise-induced hearing loss (NIHL). Since PCBs are known to cause hearing deficits via outer hair cell (OHC) damage, and the OHCs protect the inner ear from noise-induced damage, it was hypothesized that PCB-exposed rats would have
more severe NIHL after intense noise exposure. Half of the rats developmentally exposed to PCBs were exposed to 97dB octave-band noise centered at 8 kHz for 4 hours a day for 5 consecutive days. Baseline DPOAEs were decreased in the PCB groups with the clearest effect in the 6 mg/kg PCB group. Rats in the 0, 1 and 3 mg/kg PCB groups all showed the same amount of temporary hearing loss 1 day after noise exposure, with partial recovery by 28 days after noise exposure. The PCB-exposed groups did not show any evidence of an increased susceptibility to the noise.

Surprisingly, many of the rats in the 6 mg/kg PCB group exhibited behavior characteristic of the first stage of audiogenic seizures (AGS), known as wild running near the onset of noise exposure and had to be excluded from this study. This led to a study to examine whether developmental PCB exposure increases the susceptibility to AGS in adulthood. Once rats completed the NIHL study, they were subjected to loud noise for 2 min, starting at 100dB, and they were re-exposed after 24-48 to hours at 105dB and then at 110dB if they did not exhibit clonus seizures at the lower noise intensities. Female rats exposed to 3 or 6 mg/kg PCBs had significantly higher incidences of AGS compared to controls. Male rats exposed to 6 mg/kg PCBs had higher incidences of AGS compared to noise-naïve (NN) controls, but not compared to controls used in the earlier NIHL study. These effects were accompanied by sharp reductions in T4 concentrations measured at weaning in the PCB animals.

These series of studies not only confirmed that developmental PCB exposure causes long term deficits in the auditory system that are still present in adulthood, but also demonstrated that PCBs can interact with other environmental risks to worsen the effects seen with PCBs alone.
Dedicated to my father, Patrick Poon.

He has never given up on anything in life.
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CHAPTER 1

LITERATURE REVIEW

INTRODUCTION

Traditional toxicological methods test one compound at a time to investigate mode of action and toxic effects on specific systems, but compounds do not exist individually in the environment. While it is important to understand how individual toxicants act, it is equally important to understand the effects of mixtures of toxicants as well as the effects of toxicants together with other environmental risk factors in order to realistically understand the risks to human health. It has been shown in many animal studies that developmental exposure to polychlorinated biphenyls (PCBs) causes long-lasting hearing deficits that persist into adulthood (Crofton, 1998; Crofton et al., 2000a; Goldey et al., 1995; Goldey and Lasky et al., 2002; Lilienthal et al., 2011; Powers et al., 2006; Powers et al., 2009). Recently in human studies, environmental PCB exposure also was associated with hearing loss (Trnovec et al., 2008; Trnovec et al., 2010). PCBs can co-exist with other risk factors for hearing loss. These include exposures to other chemicals such as polybrominated diphenyl ethers (PBDEs) and also exposure to loud noise. PBDEs are structurally similar to PCBs and have been reported to have similar toxic effects, therefore exposure to both PCBs and PBDEs may have additive or interactive effects on auditory function. Loud noise can cause noise-induced hearing loss (NIHL), and co-exposure to PCBs and noise could potentially exacerbate the hearing loss caused by either noise or PCBs alone. Exposure to PCBs during cochlear development may also insult the auditory system, causing it to be more sensitive to
noise and increasing the susceptibility to audiogenic seizures (AGS). The studies described herein will address the gap in our knowledge of the potential for PCBs to interact with other risk factors in the environment that may exacerbate hearing loss and/or increase susceptibility to audiogenic seizures.

POLYCHLORINATED BIPHENYLS

Polychlorinated biphenyls (PCBs) were manufactured compounds used in industry as dielectric fluids in capacitors and transformers, as sealants, and in carbonless copy paper (Crinnion, 2011). They were banned from production in the 1970s, but due to their chemical stability and long half-lives, they continue to persist in our environment. They have bio-accumulated and bio-magnified up the food chain and the main route of exposure to humans is through the consumption of contaminated fish and seafood (Crinnion, 2011). A main cause for concern is that PCBs can cross the mammalian placenta and affect the developing fetus as well as enter breast milk and affect the developing infant (Jacobson et al., 1984).

The PCB molecule is made up of two phenyl rings which can be substituted with anywhere from 1-10 chlorines resulting in 209 different PCB congeners (as reviewed in Crinnion, 2011). The 209 different congeners have varying toxic effects depending on the number and position of chlorines on the molecule. Coplanar PCBs are congeners with two para-chlorines, two or more meta-chlorines, and no ortho-chlorines (Safe, 1993). Coplanar PCBs, as the name suggests, can assume a planar configuration, and can activate the aryl-hydrocarbon receptor, exerting toxic effects similar to those of dioxins. Non-coplanar PCBs have one or more ortho-chlorines, which prevent the
molecule from assuming a planar conformation (Safe, 1993). These PCBs have less affinity for the aryl-hydrocarbon receptor, but are known to produce neurochemical and endocrine changes. Commercial PCB mixtures, known as Aroclors, were complex mixtures of mostly ortho-substituted congeners.

**NEUROTOXIC AND ENDOCRINE EFFECTS OF PCBS**

PCBs have been shown to alter various aspects of cognitive processes. Deficits in inhibitory control (the inability to withhold responding when not appropriate) and working memory were reported following developmental PCB exposure in rodents and monkeys (as reviewed in Eubig *et al.*, 2010), and prenatally exposed children (as reviewed Boucher *et al.*, 2009). PCBs have also been shown to cause neurochemical alterations such as depletion of dopamine (DA) in the frontal cortex of rats and these changes could account for the behavioral changes (Seegal *et al.*, 1997).

PCBs have also been shown to alter endocrine function, and, in particular to reduce circulating thyroid hormone concentrations in animal models, which could contribute to both the behavioral and neurochemical changes (Brouwer *et al.*, 1998; Goldey *et al.*, 1995; Hedge *et al.*, 2009; Morse *et al.*, 1996). Repeated administration of PCB mixtures, coplanar PCBs and non-coplanar PCBs, over a period of 7 days in male rats produced significant reductions in total and free thyroxine (T4) (Martin and Klaassen, 2010). The mechanism by which this occurs is not fully understood, but the molecular structure of PCBs and their metabolites are similar to that of thyroid hormone (TH) (Fonnum and Mariussen, 2009). It has been demonstrated that PCBs can bind to TH receptors and TH-transport proteins (Brouwer *et al.*, 1998). PCBs can displace
endogenous TH from transport proteins, making it more available for metabolism. PCBs can also induce TH metabolism by activation of UDP-glucuronyl transferases (Brouwer et al., 1998). The animal studies also indicate that although circulating T4 is reduced after maternal PCB exposure, there is usually little to no reduction in triiodothyrinine (T3) levels (Morse et al., 1996). Human studies have been less definite on the effects of PCBs on the thyroid system with adult PCB concentrations being inversely correlated with T3, T4, and thyroid stimulating hormone (TSH) levels, positively associated with TSH levels, or no association (Crinnion, 2011; Salay and Garabrant, 2009). Human studies on the effect of developmental PCB exposure on the thyroid system also yielded mixed results with PCBs measured in the breast milk, maternal serum or cord blood serum being associated with higher TSH levels, lowered total T4 levels, or no changes (Hagmar, 2003; Sauer et al., 1994; Wilhelm et al., 2008).

**Thyroid Hormone and Hearing:** The role of thyroid hormone in the normal development of the brain and auditory system is well established. TH regulates gene expression related to the proliferation, migration, synaptogenesis, and myelination of neurons (Howdeshell, 2002; reviewed in Porterfield, 2000).

Clinical hypothyroidism during auditory system development causes profound morphological changes in the cochlea and functional deficits in hearing (Uziel et al., 1985; Uziel, 1986). Research has shown that both maternal and fetal TH are necessary to regulate genes that encode proteins involved in structural components and physiological processes in the inner ear. Several mouse models have been used to study hearing deficits due to abnormalities of the thyroid system, including the Tshr<sup>hvt</sup>
strain, a thyroid receptor (TR) knockout, the Prop1\textsuperscript{df} and Pit1\textsuperscript{dw} strains, lacking TSH producing cells, and the Cga\textsuperscript{tm1Sac} strain, which lacks the alpha subunit of TSH (Karolyi et al., 2007). A study using the hypothyroid Pit1dw mutant mouse showed that this mutant had abnormal structural changes in the tectorial membrane (TM) at postnatal day (PND) 21, and a reduction of the potassium channel genes KCNQ4 in the OHCs and KCNJ10 in the stria vascularis, which can lead to OHC death and lowered endo-cochlear potentials (Mustapha et al., 2009). TRβ\textsuperscript{-/-} mutant mice also showed a thickening and enlargement of the TM (Winter et al., 2009), abnormalities that could have accounted for the hearing deficits also measured in these animals. Another approach has been to induce hypothyroidism during development by exposing dams to the goitrogen methimazole (MMI). Studies in this model showed that TH is responsible for myelin gene expression of the VIIIth cranial nerve before the onset of hearing (Knipper et al., 1998). The immature myelination of the nerve leaves the cochlea incapable of conveying normal nerve signals during the onset of hearing. Together, these various approaches have demonstrated the importance of thyroid hormones in the development and functional integrity of the cochlea.

**PCBS AND HEARING LOSS**

**Animal Models:** Most studies have shown that developmental exposure to PCBs leads to long-lasting hearing deficits in the rodent model. Maternal exposure of Long Evans rats to Aroclor 1254 (A1254) resulted in increased auditory thresholds in the offspring as measured using reflex modification audiometry. However, increased thresholds were only observed at low frequencies (Goldey et al., 1995). The same
exposure resulted in sharp reductions in circulating T4 concentrations in littermates at weaning, and it was hypothesized that PCB-induced hypothyroidism during development of the auditory system could be responsible for the hearing loss. This hypothesis was tested by giving postnatal thyroxine replacement to PCB-exposed rat pups. Thyroxine treatment attenuated the low-frequency hearing loss, suggesting that reduced T4 concentrations were at least partially responsible for the hearing deficit (Goldey and Crofton, 1998). Further research showed that postnatal exposure to A1254 during the first three weeks of life was the critical period for ototoxicity. Rat dams exposed to A1254 had their litters cross-fostered to control dams (prenatal PCB exposure group) or to other A1254 dams (pre- and postnatal PCB exposure group) on the day of birth and, conversely, control pups were fostered to A1254 dams (postnatal PCB exposure group) or to other control dams (no exposure) at birth. Hearing deficits were observed in both groups that had postnatal PCB exposure via lactation (Crofton et al., 2000b). Later the authors reviewed all of their previous work and concluded that at least a 60% reduction in T4 levels during the postnatal period was needed to cause a statistically significant hearing loss (Crofton, 2004; Crofton and Zoeller, 2005).

The same authors also showed that the PCB-induced hearing loss was correlated with the loss of outer hair cells in the region of the cochlea responsible for low-frequency hearing (Crofton et al., 2000a). There were no alterations in other cochlear structures, including the inner hair cells. The cochlea was further confirmed as a site of action for PCBs in our lab using distortion product otoacoustic emissions (DPOAEs), which are a direct measure the functional integrity of the outer hair cells in the cochlea. Exposure to A1254 during the perinatal period resulted in reduced DPOAE
amplitudes and increased thresholds (Lasky et al., 2002). Our lab later extended the evidence that PCBs disrupt cochlear function by showing that developmental exposure to an environmentally relevant PCB mixture (Fox River PCB Mixture-FRM) resulted in reduced DPOAE amplitudes and increased DPOAE thresholds across a range of frequencies (Powers et al., 2006; Powers et al., 2009). More recently, another research group has shown that developmental exposure to individual non-dioxin like PCB congeners resulted in prolonged wave II and IV latencies during brainstem auditory evoked potentials (BEAPs). Because the latencies of wave II and IV were prolonged to the same degree relative to waves II and IV in controls, it was concluded that this indicated a cochlear site of action rather than a site of action along the ascending auditory pathway (Lilienthal et al., 2011). One rat study did not see hearing loss as measured by auditory brainstem responses (ABRs) but did find that developmental exposure to PCB 95 (a non-coplanar PCB) resulted in an irregularly shaped and topographically abnormal primary auditory cortex (Kenet et al., 2007).

**Human Studies:** Less evidence about the impact of developmental exposure to PCBs on hearing loss exists in the human literature. Seven-year old children in the Faroe Islands with higher than normal PCB exposure due to consumption of PCB-contaminated whale blubber were reported to have lower auditory thresholds using standard auditometry, but only at frequencies of 250 and 12,000 Hz, and only in the left ear (Grandjean et al., 2001). Longnecker et al. (2004) also found an association between higher maternal PCB exposure during pregnancy and a slight increase in hearing thresholds in 8-year old children, but increased thresholds were only present at
2 kHz in the left ear and 4 kHz in the right ear. More recently, a longitudinal study in a highly PCB-contaminated region of Slovakia found that, in 8-year old children, higher serum levels of PCBs were associated with increased hearing thresholds at low frequencies using pure tone audiometry and with lower transient otoacoustic emission (TEOAE) amplitudes, which can measure the integrity of the cochlea (Trnovec et al., 2008). The same children were tested again at age 12 and decreased DPOAE and TEOAE amplitudes were associated with greater PCB exposure, especially at low frequencies, indicating a cochlear site of action for PCBs in humans (Trnovec et al., 2010).

POLYBROMINATED DIPHENYL ETHERS

Polybrominated diphenyl ethers (PBDEs) are manufactured compounds used as flame retardants in electronic equipment, furniture, textiles, and building materials (Birnbaum and Staskal, 2004; Costa and Giordano, 2007). They are additives and are not chemically bound to the products, therefore they can readily leach out into the environment. They are structurally stable and can bio-accumulate and bio-magnify in the food chain (Costa and Giordano, 2007). PBDEs are a cause for concern because they have increased exponentially in biota and in human tissue over the past 20 years, and because, like PCBs, they can cross the mammalian placenta and enter into breast milk (Fischer et al., 2006; Meironyte et al., 1999).

PBDEs are chemically similar to PCBs, but consist of two phenyl rings connected by an ether bond and substituted with bromines rather than chlorines. Like PCBs, there are 209 different congeners. Commercially used PBDEs were marketed as three
different mixtures (penta-, octa-, and deca-brominated BDEs), but these mixtures contain fewer congeners than PCB mixtures therefore the number of PBDE congeners dispersed in the environment is less than for PCBs. Both the penta- and octa- BDEs have either been voluntarily taken out of production in the United States or banned in the European Union, while the decaBDE is still used in other parts of the world (Costa and Giordano, 2007).

PBDEs and PCBs have been shown to exert similar toxicological effects. PBDEs have been shown to affect behavior in rodents by increasing locomotor activity, and impairing sustained attention and inhibitory control (Birnbaum and Staskal, 2004; Driscoll et al., 2009; Fonnum and Mariussen, 2009). Developmental PBDE exposure also results in hypothyroxinemia. Perinatal exposure in rats to DE-71 (a commercial PBDE mixture containing mostly penta-brominated ethers) resulted in lowered serum T4 concentrations, with no change in T3, and induction of UDP-glucuronyl transferases (Zhou et al., 2002), changes that are very similar to those observed with PCB exposure. In humans, children with higher concentrations of PBDEs scored lower on tests of cognitive development at 24, 36, 48 and 72 months of age (Herbstman et al., 2010). Similar to PCBs, the data on the effects of PBDEs on thyroid levels in humans are inconsistent. Studies have concluded that maternal and fetal blood PBDE levels showed no correlation with T4 levels (Mazdai et al., 2003), adult PBDE levels were associated with an increase in T4 in sport fishermen (Turyk et al., 2008), and higher PBDE serum levels were associated with lowered TSH, but no change in T4 in pregnant women (Chevrier et al., 2010).
COMBINED EXPOSURE TO PCBS AND PBDES

Although PBDEs and PCBs have similar toxic effects and similar routes of exposure, to date there are very few studies assessing combined exposure to PCBs and PBDEs to determine if they have additive or interactive effects. In one study, 10-day old mice were exposed to a single dose of PCBs and/or PBDEs and spontaneous activity was tested at 4 and 6 months of age (Eriksson et al., 2006). An interactive effect of PCBs and PBDEs was evident, with the primary effect being a failure to habituate across the testing session, resulting in hyperactivity near the end of the session in the combined exposure groups compared to the groups exposed to either chemical alone. Striatal synaptosomes isolated from LE rats co-exposed to PCBs (FRM) and PBDEs (DE-71) at PND 7, 14, and 21 also revealed additive effects, elevating DA in the medium and reducing synaptosomal DA concentrations when compared to the FRM alone (Dreiem et al., 2010). Likewise, co-exposure to the FRM and DE-71 at equimolar doses from gestational day 6 to PND 21 in rat dams had additive effects in lowering T4 levels in the offspring (Miller et al., 2012). To date, there has been no research focusing on the effects of PBDEs on hearing, or the potential interactive effects of combined exposure to PCBs and PBDEs on hearing.

NOISE-INDUCED HEARING LOSS (NIHL)

Noise-induced hearing loss (NIHL) is an environmental risk factor for a lower quality of life and can be caused by either an acute exposure to loud sound or chronic exposure to moderately loud sound. The National Institute of Occupational Safety and Health (NIOSH) has reported that approximately 30 million Americans are exposed to
daily noise levels that can lead to hearing loss (Franks et al., 1996). In recent years, the prevalence of noise-induced hearing loss has risen, especially in children and young adults (Daniel, 2007). These younger populations are unknowingly exposing themselves to levels of noise that put them at risk for NIHL through leisure activities such as concerts and listening to digital music players.

NIHL can result from a temporary threshold shift (TTS) from which hearing can recover, or a permanent threshold shift (PTS) in which the hearing loss is nonreversible because the cochlea is unable to repair itself after the trauma (Konings et al., 2009). The louder the noise, the more damage there will be to the inner ear. The intensity of noise is measured as the sound pressure level in decibels (dB SPL). According to the NIOSH recommendations (1998), noise is potentially harmful starting at 85 dB over an 8-hour work day. Chronic exposure to noise at 90 to 130 dB results in metabolic decomposition of the hair cells including swollen nuclei and stereocilia disruption (Konings et al., 2009). Exposure to noise greater than 130 dB results in mechanical destruction that is not time dependent, such as the OHCs becoming detached from the basilar membrane (Lim, 1986).

MECHANISMS FOR NIHL

Outer hair cell (OHC) loss is thought to be one of the main factors underlying NIHL. This hair cell loss may be due to the production of free radicals or reactive oxygen species (ROS) that damage DNA and induce apoptosis. ROS are produced by the mitochondria after intense noise exposure, due to increases in metabolic activity (Le Prell et al., 2007). This increase in metabolic activity coincides with a decrease in blood
flow to the cochlea (Quirk et al., 1992). A decrease in blood flow, lasting 10 minutes or
more after noise exposure (110 dB), may lead to ischemia and further ROS formation
(Le Prell et al., 2007). An increase in hydroxyl radicals (OH) in the perilymph has been
seen following acute noise exposure that causes PTS (Ohlemiller et al., 1999). Hydroxyl
radicals are known to damage DNA as well as induce lipid peroxidation. An increase in
lipid peroxidation was seen in the OHCs following noise exposure (Ohinata et al., 2000).
Free radicals also build up in the inner ear after noise exposure, with peak ROS levels
observed 7-10 days after a noise exposure that was associated with an increase in
severity of OHC loss, but no significant loss of inner hair cells (Yamashita et al., 2004).

Noise exposure can also increase the calcium concentration in hair cells
(Fridberger and Ulfendahl, 1996) and cause glutamate excitotoxicity (Le Prell et al.,
2007). An increase in calcium concentration can activate calcineurin and calpains, both
of which were increased after noise trauma (Le Prell et al., 2007). Downstream events
from calcineurin activation include the activation of the pro-apoptotic bcl-2 protein, Bad,
and caspase-dependent cell death pathways, leading to apoptosis.

**NIHL AND OTOTOXICITY**

Co-exposure to noise and drugs has been reported to increase the risk for and
degree of hearing loss. For example, smoking can interact with loud noise to make one
more susceptible to NIHL. Smoking alone has been suggested to increase hearing
thresholds in populations free from significant noise exposure (Cruickshanks et al.,
1998). Smokers who worked in factories with daily exposures to loud noise had more
hearing loss than nonsmokers exposed to the same level of noise (Pouryaghoub et al.,
This interactive effect of smoking and loud noise exposure is probably due to the toxic effects of cigarette smoke, such ischemia of the cochlea along with the damaging mechanical/metabolic effects of acoustic trauma (Wild et al., 2005).

Animal studies have demonstrated that chemicals such as organic solvents can also interact with noise to increase the risk of NIHL (Fechter, 2004). Adult rats that had simultaneous exposure to toluene and noise at 92 dB for 6 hours/day, 5 days/week, for 4 weeks had higher auditory thresholds as measured by BAEPs than rats exposed to either toluene or noise alone (Lataye and Campo, 1997). Combined exposure to styrene, an organic solvent used in reinforced plastics, and noise at 97 dB for 6 hours/day, 5 days/week, for 4 weeks resulted in elevated auditory thresholds and OHC loss that exceeded the effects seen with either styrene or noise alone (Lataye et al., 2000). Acrylonitrile, an industrial chemical used to make fibers, plastics and rubbers, in combination with moderate noise (95 or 97 dB 4 hours/day for 5 days) caused permanent threshold shifts as measured by compound action potentials (CAP), decreased DPOAE amplitudes, and OHC loss (Pouyatos et al., 2005). Exposure to either the acrylonitrile or the noise alone did not result in any permanent hearing damage or OHC loss. Finally, chronic exposure to JP-8 jet fuel plus noise at 105 dB once for 4 hours resulted in greater impairment of auditory function as measured by DPOAEs, CAP and OHC loss than the noise alone, and exposure to jet fuel plus a chronic exposure to 102 dB of noise for 4 hours/day, for 5 consecutive days led to lowered DPOAE amplitudes compared to individual exposures to noise or jet fuel alone (Fechter et al., 2007).
COMBINED EXPOSURE TO PCBS AND NOISE

As reviewed above, previous research has shown adult rats exposed to chemicals in combination with moderate noise have increased susceptibility to hearing loss. However, very little is known about exposure to chemicals during cochlear development, and whether this can promote greater sensitivity to NIHL later in life. Although previous research has demonstrated that developmental exposure to PCBs leads to long-lasting cochlear dysfunction (Powers et al., 2006; Powers et al., 2009), and Powers et al. (2009) did investigate the interactive effects of developmental PCB and methylmercury exposure, to date there are no studies assessing whether developmental PCB exposure combined with later noise exposure results in greater hearing loss than is seen with either exposure by itself.

AUDIOGENIC SEIZURES

Audiogenic seizures (AGS) are a type of generalized seizures elicited by intense sound. They are considered to be generalized seizures because they do not arise from specific cortical or subcortical locations in the brain. AGS are also known as brainstem seizures as they require activation of the auditory brainstem, mainly in the inferior colliculus (Coleman et al., 1999; Eells et al., 2004; Faingold, 2002; Ishida et al., 1995; Ross and Coleman, 2000). Measures of c-fos mRNA expression after induced AGS showed that c-fos mRNA is mainly expressed the inferior colliculus, with weak hippocampal expression (Ishida et al., 1995). On the other hand, induction of forebrain-type seizures via electroshock or pentylentetrazole (PTZ) administration induced Fos gene expression in forebrain areas such as the cerebral cortex and piriform cortex, and
in the amygdala (Eells et al., 2004). Increased Fos gene expression in the cortex, hippocampus, medial hypothalamus, and amygdala is seen after kindling of AGS, suggesting that AGS can eventually recruit forebrain structures (Simler et al., 1994).

The progression of AGS can be divided into different phases (reviewed in Ross and Coleman, 2000). The first reliable component of AGS is wild running or a running fit. During this phase, the animal runs full speed, uncontrollably. A second phase of wild running may occur after a few seconds of inactivity, and according to the Jobe Audiogenic Reponse Score (ARS) (Jobe et al., 1973), one running fit is more severe than two running fits. After the wild running phase, the animal may progress to clonic convulsions characterized by full-body muscle spasms, rocking motions, and jumping. Clonus may lead to tonic seizures, the most severe stage of AGS, characterized by extension of the dorsal surface of the animal, although this stage has only been reported in genetically susceptible rodent strains such as the genetically epileptic prone rat (GEPR) and Wistar rats. Finally, when noise stimulation stops, the animal goes into post-ictal recovery, which is characterized by immobilization with full recovery after 15 min.

Rodents that are susceptible to AGS have either genetic susceptibility, like the GEPRs and Wistar Audiogenic Rats (WAR), or have been made susceptible by insulting the auditory system during the critical period of auditory development, which is postnatal in the rodent. When Wistar rats were exposed to intense sound on PND 14, AGS was observed at PND 28 (Pierson and Swann, 1991). Mice that were sound-deprived by having their tympanic membranes destroyed on PND 14 were more susceptible to AGS (Chen et al., 1973). Postnatal exposure to the aminoglycoside antibiotic, kanamycin,
intraperitoneally in rats resulted in greater susceptibility to AGS (Pierson and Swann, 1988). A hypothesis explaining why an insult during cochlear development would increase the susceptibility to AGS is that short-term hearing loss during the maturation of the auditory system leads the animal to be hypersensitive to intense stimulation later in life (reviewed in Ross and Coleman, 2000). Although Long-Evans (LE) rats are more resistant to seizures, they can be made susceptible to AGS if primed by loud tone bursts postnatally, during cochlear development (Ross and Coleman, 1999). Therefore, LE rats can be made more susceptible to AGS via an insult to cochlear development, which also occurs with early PCB exposure.

**PCBs AND AUDIOGENIC SEIZURES**

It is possible that PCB exposure may increase the susceptibility to AGS based on previous studies showing that PCB exposure may lower the threshold for cortical seizures. When perinatally PCB-exposed rats (PCB 95 or Aroclor 1254) were challenged with picrotoxin, after-discharges (epileptic waveforms) in the evoked potentials were observed (Kim and Pessah, 2011). Also, rats developmentally exposed to PCB 95 at 1 mg/kg/day had small, but significantly reductions in the latency to myoclonus and the onset of tonic-clonic seizures when exposed to flurothyl, a convulsive drug, and also kindled seizures slightly, but significantly faster when exposed to pentylenetetrazole (PTZ) (Lein et al., 2010).

PCBs could also increase susceptibility to AGS via lowered thyroid hormone during development. Previous studies have shown that developmental hypothyroidism can increase susceptibility to audiogenic seizures. Rat pups treated with propylthiouracil...
(PTU) during PND 0-19 had higher incidences of AGS at 4 months of age (Kato et al., 1996). Similarly, rat dams fed low iodine diets pregnancy and throughout lactation had hypothyroid offspring with higher incidences of AGS (Van Middlesworth, 1977).

CONCLUSION

In conclusion, while there are toxicological studies researching the effects of individual chemicals or individual risk factors (drugs, loud noise) on hearing loss, there are very few studies that address the potential for interactive effects of chemical exposures and other environmental risk factors on hearing and seizure susceptibility. These studies help to fill this significant gap in our knowledge by elucidating whether exposure to either another chemical with a similar mechanism of action (PBDEs) or to moderate noise will result in greater cochlear dysfunction than is seen with PCBs alone, and if developmental PCB exposure will interact with loud noise to increase susceptibility to audiogenic seizures.

REFERENCES


CHAPTER 2

SPECIFIC AIMS

As reviewed in Chapter One, evidence in both animal models and humans suggests that exposure to PCBs can lead to hearing loss with a site of action at the level of the cochlea (Crofton et al., 2000; Powers et al., 2006; Trnovec et al., 2010). However, there is almost no published research to date addressing the potential for interactive effects of PCBs and other chemical or environmental risk factors on cochlear function. Despite the similar chemical and toxicological properties of PCBs and PBDEs, there have been no studies examining the potential for additive effects of PCBs and PBDEs on hearing. Likewise, despite the fact that both PCBs and loud noise target the OHCs, there are no studies assessing the potential for interactive effects of PCB exposure and noise on the auditory system. The goal of this thesis is to address these critical gaps in knowledge using a well-established rodent model of developmental PCB exposure (Kostyniak et al., 2005; Powers et al., 2006).

AIM 1: Examine the potential for interactive effects of developmental exposure to PCBs and PBDEs on auditory function. PBDEs and PCBs are both environmental contaminants that cross the mammalian placenta and are present in breast milk. PBDEs are structurally similar to PCBs and have similar toxic effects, including reduction of circulating thyroxine concentrations. Importantly, thyroid hormones are critical to the development of the cochlea. To date, there are no studies examining whether developmental exposure to PBDEs can cause long lasting hearing loss similar to that produced by PCBs or whether combined exposure to PBDEs and PCBs can produce an
additive or interactive effect on cochlear function. It is hypothesized that developmental exposure to PBDEs will cause long lasting cochlear dysfunction and that co-exposure to PCBs and PBDEs will exacerbate the PCB-induced deficit in cochlear function. To address this aim, female Long-Evans rats will be orally exposed to corn oil vehicle, PCBs (3, or 6 mg/kg), molar equivalent doses of PBDEs (5.7 or 11.4 mg/kg), 3 mg/kg PCBs + 5.7 mg/kg PBDEs, or 6 mg/kg PCBs + 11.4 mg/kg PBDEs throughout gestation and lactation. Pups will then be tested for cochlear dysfunction as young adults at approximately postnatal day (PND) 100 using DPOAEs.

**AIM 2: Determine whether developmental PCB exposure increases susceptibility to noise-induced hearing loss later in life.** Noise-induced hearing loss is relevant to children and young adults as they are exposed to loud or moderate sound via music concerts, digital media players, and other leisure activities such as watching movies in theaters and playing with loud toys. Loud or moderate noise exposure can cause temporary or permanent hearing loss depending on the sound level and the length of exposure. Stiffening of the stereocilia on the OHCs functions to protect the cochlea from noise-induced damage by reducing the amount of shearing force of the hair cells that leads to cellular metabolic overload (Lim, 1986). It is hypothesized that because developmental PCB exposure causes cochlear dysfunction via OHC damage, exposure to PCBs during the period of cochlear development will increase the susceptibility to noise-induced hearing loss when the rats are adults. To address this aim, female Long-Evans rats will be orally exposed to corn oil vehicle or PCBs (1, 3, or 6 mg/kg/day) throughout gestation and lactation. Baseline auditory function will be assessed in two male and two female pups per litter at approximately PND 200 using
DPOAEs. After baseline recordings, half of the pups from each litter will be exposed to noise at 97dB for 4 hours/day for 5 consecutive days and their DPOAEs will be measured 1 day after and 4 weeks after noise exposure to reveal if the PCB and noise exposures lead to temporary or permanent hearing loss. The other pups from each litter will not undergo any noise exposures and their DPOAEs will be tested at the same time points as the noise-exposed pups.

**AIM 3: Determine whether developmental PCB exposure increases susceptibility to audiogenic seizures later in life.** Unexpectedly some PCB-exposed rats tested in AIM 2 exhibited seizure-like behaviors when exposed to loud noise. Rats from that study were developmentally exposed to PCBs and subjected to 97dB of noise for 4 hours a day, for 5 consecutive days in adulthood. Before each testing day, rats were habituated to the noise by gradually increasing the noise intensity from 75 to 97dB over a period of 15 mins. On the first day of testing, during this initial 15 min of noise exposure many of the rats in the highest dose group (6 mg/kg PCB) exhibited bouts of wild running followed by a period when they were unresponsive to stimuli. **Due to this observation, it is hypothesized that developmental PCB exposure during the period of cochlear development will increase the susceptibility to audiogenic seizures when the rats are adults.** To address this aim, rats tested in AIM 2, will then be exposed to 2 minutes of octave-band noise, 100 db centered at 8 kHz,, and re-exposed after 24-48 to hours at 105dB and then at 110dB if they do not exhibit clonus at the lower noise intensities. The incidence of audiogenic seizure behaviors (wild running and wild running progressing into clonus) will be recorded.
REFERENCES


CHAPTER 3

EFFECTS OF DEVELOPMENTAL EXPOSURE TO POLYCHLORINATED BIPHENYLs AND/OR POLYBROMINATED DIPHENYL ETHERS ON COCHLEAR FUNCTION*

ABSTRACT

Developmental exposure to polychlorinated biphenyls (PCBs) causes hearing loss that may be due to reduced thyroxine during cochlear development. Polybrominated diphenyl ethers (PBDEs) are structurally similar to PCBs and reduce thyroxine. This study utilized an environmental PCB mixture, and a commercial PBDE mixture, DE-71, that represents the PBDEs found in humans to assess the potential for additive effects of PCBs and PBDEs on cochlear function. Female Long-Evans rats were dosed with corn oil vehicle, PCBs (3, or 6 mg/kg), molar equivalent doses of PBDEs (5.7 or 11.4 mg/kg), 3 mg/kg PCBs + 5.7 mg/kg PBDEs, or 6 mg/kg PCBs + 11.4 mg/kg PBDEs throughout gestation and lactation. At weaning, pup blood was taken to assess thyroxine concentrations. One male and one female from each litter were maintained until adulthood for distortion product otoacoustic emission (DPOAE) measurements of cochlear function. DPOAE amplitudes were decreased and thresholds were elevated in the 6 mg/kg PCB group. Exposure to PBDEs did not cause DPOAE deficits. There was an interactive effect from combined exposure such that the individual low doses of PCBs and PBDEs did not result in DPOAE deficits, but the two combined produced a

deficit similar to that in the high dose PCB group. Serum thyroxine concentrations of all groups were reduced compared to controls, but PBDEs produced a less dramatic reduction than PCBs, which could explain the lack of DPOAE effects. Importantly, there was evidence that the co-exposure to sub-threshold doses of PCBs and PBDEs can have an additive effect on cochlear function.

Key words: PCBs, PBDEs, auditory system, DPOAE.
INTRODUCTION

Polychlorinated biphenyls (PCBs) have long been recognized as a public health concern, but polybrominated diphenyl ethers (PBDEs) have more recently emerged as widespread environmental contaminants. PBDEs are used as flame retardants in consumer products and because their chemical structure is similar to that of PCBs, they may have similar actions (Birnbaum and Staskal, 2004). Both chemicals cross the placenta and are transferred to the neonate via lactation (Jacobson et al., 1984; Meironyte et al., 1999). Human and animal studies have revealed adverse outcomes from developmental PCB exposure including cognitive, motor, sensory, and neurochemical deficits (Eubig et al., 2010; Sable and Schantz, 2006). Less evidence is available for PBDEs, but human and animal studies conducted to date have shown that developmental exposure to PBDEs produces deficits similar to those produced by PCBs (Costa and Giordano, 2007; Eriksson et al., 2006; Gascon et al., 2011; Herbstman et al., 2010; Kodavanti et al., 2010; Zhou et al., 2002) and that exposure to both chemicals can produce interactive effects (Eriksson et al., 2006).

Rodent studies provided the first evidence for auditory deficits after developmental PCB exposure. Developmental exposure to a commercial PCB mixture, Aroclor 1254 (A1254), resulted in long-lasting, low-frequency hearing loss (Goldey et al., 1995). Thyroid hormone is necessary for normal cochlear development (Uziel, 1986), and A1254 is known to markedly reduce serum thyroxine concentrations (Goldey et al., 1995; Morse et al., 1996). Not surprisingly, further studies demonstrated that thyroxine replacement could partially ameliorate the hearing loss caused by developmental A1254 exposure (Goldey and Crofton, 1998). The cochlea was
specifically indicated as the likely site of action in a study that found loss of outer hair cells in perinatally PCB-exposed rats (Crofton et al., 2000a). This was later confirmed when our group used distortion product otoacoustic emissions (DPOAEs)—a method that directly measures the functional integrity of the outer hair cells—to assess hearing in perinatally PCB-exposed rats (Lasky et al., 2002). Developmental exposure to A1254 reduced DPOAE amplitudes in response to low frequency tones (Lasky et al., 2002). Later we demonstrated that developmental exposure to an environmental mixture of PCBs led to attenuated DPOAE amplitudes and elevated DPOAE “thresholds”, across a wide range of frequencies, with DPOAE threshold defined as the sound level required to measure a DPOAE 6 dB or more above the noise floor (Powers et al., 2006; Powers et al., 2009). More recently, exposure to individual, non-dioxin-like PCB congeners was reported to elevate brain auditory evoked potential thresholds, as well as prolong the latencies of waves II and IV (Lilienthal et al., 2011). The authors speculated that because the latencies of waves II and IV were prolonged to the same degree relative to waves II and IV in controls, this indicates a cochlear site of action rather than along the level of the ascending auditory pathway.

There are limited human studies assessing the impact of PCB exposure on auditory function, but several studies suggest that prenatal exposure may lead to subtle auditory deficits. A study in the Faroe Islands found higher prenatal PCB exposure was associated with increased auditory thresholds at 250 and 12,000 Hz, but only in the left ear (Grandjean et al., 2001). In another study higher PCB concentrations in maternal serum were associated with slightly increased hearing thresholds, but only at 2,000 Hz in the left ear and 4,000 Hz in the right ear (Longnecker et al., 2004). More recently,
hearing of a cohort of 8-9 year-old Slovakian children with higher serum PCB concentrations than the children assessed previously was measured using transient otoacoustic emissions (TEOAEs), a technique that specifically assessed the integrity of the outer hair cells in the cochlea. In these children, higher serum PCB concentrations were associated with low frequency hearing loss as indicated by reduced TEOAE amplitudes and increased TEOAE thresholds (Trnovec et al., 2008). These same children were tested again at age 12 using both TEOAEs and DPOAEs and an association between higher PCB concentrations and reduced TEOAE and DPOAE amplitudes was observed. Again, this effect was observed primarily at low frequencies (Trnovec et al., 2010).

Exposure to PBDEs has been shown to reduce circulating thyroid hormone concentrations in humans and rodents (Turyk et al., 2008; Zhou et al., 2002). However, to date, there are no published human or animal studies assessing the impact of developmental PBDE exposure on cochlear function. The present study used a rat model to investigate developmental exposure to PBDEs either alone or in combination with PCBs as a possible cause of cochlear dysfunction. PBDE doses were the molar equivalents of PCB doses previously demonstrated to produce DPOAE deficits (Powers et al., 2006). The goal was to determine if developmental exposure to PBDEs could cause cochlear dysfunction and if the combination of the two compounds would have an interactive effect on outer hair cell integrity.
MATERIALS AND METHODS

Animals. One hundred seven primiparous female Long-Evans rats, approximately 60 days of age, were purchased from Harlan (Indianapolis, IN) in three cohorts spaced approximately 6 months apart. The animals were maintained in facilities accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care. Specifically, rats were individually housed in standard polycarbonate plastic shoebox cages with corn-cob bedding, in a temperature- and humidity-controlled room (22°C, 40–55% humidity) on a 12/12-hr reverse light/dark cycle (lights off at 0830 hr). Food and water were available ad libitum. Rats were fed Harlan Teklad rodent diet (W) 8604. All procedures were approved by the Institutional Animal Care and Use Committee at the University of Illinois at Urbana-Champaign and were in accordance with the guidelines of the National Institutes of Health (2002) and National Research Council (2003).

Exposure. After 1 week of adaptation to the vivarium, the rats in each cohort were assigned to exposure groups (distributed evenly by body weight) and given one of seven treatments consisting of PCBs and/or PBDEs in corn oil, or corn oil vehicle only. Each exposure group was represented in each cohort. Exposure began 28 days before breeding and continued until pups were weaned on postnatal day (PND) 21. The PCB mixture (Fox River PCB mixture) was formulated to mimic the congener profile found in walleye, a popular sport-caught fish, taken from the Fox River in northeast Wisconsin. The mixture consisted of 35% Aroclor 1242 (Monsanto lot KB 05-415; St. Louis, MO) 35% Aroclor 1248 (AccuStandards lot F-110; New Haven, CT), 15% Aroclor 1254 (Monsanto lot KB 05-612), and 15% Aroclor 1260 (AccuStandards lot 021-020)
(Kostyniak et al., 2005). The doses of the PCB mixture (3 and 6 mg/kg/day) were selected based on the results of earlier studies assessing the \textit{in vivo} developmental toxicity and auditory toxicity of the mixture in rats (see Kostyniak et al. 2005; Powers et al. 2006). The PBDE mixture was DE-71 (Cambridge Isotope Lab lot I1-9570; Andover, MA), a discontinued technical commercial PBDE mixture mainly consisting of 42-44% BDE-99 and 36% BDE-47, the two PBDEs that are most prevalent in human tissue. The PBDE doses were 5.7 and 11.4 mg/kg/day, the molar equivalents to the 3 and 6 mg/kg/day PCB doses. The low combined dose was 3 mg/kg/day PCB plus 5.7 mg/kg/day PBDE and the high combined dose was 6 mg/kg/day PCB plus 11.4 mg/kg/day PBDE. The PCB and PBDE mixtures were diluted in corn oil (Mazola) and pipetted onto one-half of a vanilla wafer cookie at a volume of 0.4 mL/kg body weight of the rat (Keebler Golden Vanilla Wafers). To arrive at a volume of 0.4 mL/kg body weight, the Fox River mix and DE-71 were diluted in corn oil to achieve concentrations of 7.5 mg/mL and 15 mg/mL for the PCB doses of 3 mg/kg and 6 mg/kg, and 14.25 mg/mL and 28.5 mg/mL for the PBDEs doses of 5.7 mg/kg and 11.4 mg/kg respectively. The PCB and/or PBDE-treated cookies were fed to the female rats daily at approximately 11:00 hr. The amount of dosing solution applied to the cookies was adjusted daily to account for weight gain. Corn oil vehicle alone was pipetted onto cookies for rats in the vehicle control group.

\textit{Breeding, pregnancy, and weaning.} Four weeks after the initiation of exposure, each female was paired with an unexposed male Long-Evans rat (Harlan, Indianapolis, IN) in a hanging wire cage for 8 consecutive days. The breeding cages contained standard rat
chow and standard tap water (*ad libitum*). The females were returned to their home cages each day for PCB and/or PBDE dosing. Consumption of the cookie was confirmed before the females were returned to the breeding cages. The females were monitored twice daily for the presence of a sperm plug in order to establish gestational day 0. Females who did not give birth were retained and their uteri examined for implantation sites.

On the day of parturition (PND 0), the pups were examined for gross abnormalities, sexed and weighed, and the number of stillbirths was noted. On PND 2, the litters were reduced to 10 pups (five males and five females when possible), and extra pups were cross-fostered to litters with at least 7 pups from the same treatment group to bring them to 8–10 pups. Cross-fostered pups were marked by ear clip and were not used for auditory testing. The day of eye opening was recorded. Pups were weighed weekly. There were 83 successful litters. Of the remaining females, 10 were not pregnant and 14 had litters that were too small (≤7 pups) to be included in this study. Overall, the non-pregnant females and small litters were relatively evenly distributed across treatment groups.

Dosing continued until the pups were weaned on PND 21. On PND 21, the dams were euthanized and the liver weight and number of uterine implantation sites were recorded. Two pups per litter, one male and one female, were retained for the auditory testing. Organ weights (brain, liver, and thymus) were obtained from one male and female per litter and serum was collected for measurement of total thyroxine (T4) concentration. Pups retained on the day of weaning were housed in same-exposure, same-sex pairs or triplets with food and water available *ad libitum*. Auditory testing
began at approximately PND 100 to determine if developmental exposures to PCBs and/or PBDEs resulted in permanent hearing deficits.

**Total Thyroxine Radioimmunoassay.** Serum T4 concentration was measured by radioimmunoassay modified for rat serum as reportedly previously by Eltom et al., (1992) with further modifications to improve sensitivity according to the method of Schneider et al., (2006). The assay was conducted over 5 days. On the first day, 10 µl of serum was added to 200 µl of GAB buffer (0.2M Glycine, 0.13M Sodium acetate trihydrate, 0.02% bovine serum albumin BSA, pH 8.6.) L-T4 standards equivalent to 10,000 for nonspecific binding, or 620, 310, 155, 77.5, 38.8, 19.4, 9.7, 4.85, 2.43, 1.22, 0.61 and 0 pg were included to create the standard curve. Next, 100 µl of GAB containing 2mg/ml of 8-anilino-1-napthalene-sulfonic acid (ANS, Sigma) was added. The primary antibody used was a polyclonal rabbit anti-T4 antibody (Cat#20-TR40, Fitzgerald Industries International, Concord, MA). Approximately 0.006 µCi of [\(^{125}\text{I}\)]-T4 was added on the third day. On the fifth day, 50 µl of a 200 µg/ml (10 µg) solution of rabbit immunoglobulin (Cat# I5006, Sigma) was added, followed by 100 µl of a GAR secondary antibody solution (Cat#R0881, Sigma) prepared at 60% of the manufacturers recommended volume for a final dilution of approximately 1:8. Tubes were incubated at room temperature for 30 minutes before addition of 1 ml of a 25% wt/vol solution of polyethylene glycol (PEG) in phosphate-buffered saline (0.01 M NaCl, 0.01 M NaH\(_2\)PO\(_4\), pH 7.5). Tubes were then centrifuged, aspirated, and counted in a gamma counter (Packard Cobra Autogamma II). Data were linearized by log-logit regression. All samples in this study were run in a single assay. The serum T4 assay had a limit of
detection of 1.0 nmol/liter with 10 µl of serum, or 0.8 µg per tube. The intraassay CV at 46.3 nmol/liter (36 µg/tube) was 10.5%.

**Distortion product otoacoustic emissions.** DPOAEs are acoustic responses generated when the cochlea is stimulated by two pure tones (called \( f_1 \) and \( f_2 \) primaries). Loss of outer hair cells or impairment of outer hair cell function results in DPOAE deficits. DPOAE testing was conducted in a sound-attenuated chamber, lined with sound-absorbing foam, within a quiet, isolated laboratory. Before testing, rats were sedated with 0.5 mL/kg ketamine/xylazine (87:13) intraperitoneally. Once sedated, rats were placed on a thermo-regulating heating pad (no. 50-7053-R, Harvard Apparatus, Holliston, MA) to maintain body temperature. Rats were placed on their sides and the DPOAE probe was positioned in the left ear canal.

DPOAEs were recorded using Tucker Davis Technologies (TDT; Alachua, FL) System 2/System 3 digital signal processing hardware and software. Stimuli were directed into the ear canal through a single ear probe. The probe contained two earphones and a microphone and had a soft rubber tip that sealed the ear canal from external noise. All DPOAE stimuli were created using TDT SigGen software and recordings were made using TDT BioSig software. Details regarding the instrumentation can be found in Powers et al. (2006).

The DPOAEs were generated by simultaneously presenting two sinusoids, \( f_1 \) and \( f_2 \) (\( f_2/f_1 = 1.2 \)), into the sealed ear canal of the rat. The sound levels for the \( f_1 \) and \( f_2 \) primaries were calibrated to 60 dB sound pressure level (SPL) and 50 dB SPL (0 dB = 20 µPa), respectively, using a pressure field microphone (no. 4192, Brue and Kjaer,
Norcross, GA) in a 2-cc calibration coupler (no. 4946, Bruel and Kjaer). The amplitude of the distortion product at the frequency defined by $2f_1 - f_2$ was then measured by recording the pressure in the sealed ear canal. In mammals, the $2f_1 - f_2$ distortion product is the most robust and is commonly measured as a reliable indicator of outer hair cell function (Lonsbury-Martin and Martin, 1990). Six stimulus pairs were selected for DPOAE testing ($f_2 = 2, 3, 4, 6, 8, \text{and} 12$ kHz). Figure 3.1 shows an example of recorded DPOAE output of the $f_1$ and $f_2$ primaries as well as the $2f_1 - f_2$ distortion product.

DPOAE testing consisted of measuring suprathreshold DPOAE amplitudes followed by DPOAE thresholds at each of the six stimulus pairs, beginning with 2 kHz. Each distortion product was the average response of 100 stimulus pair presentations (presentation rate = 6/sec). The final distortion product represented an average of two trials (each having 100 separate stimulus presentations). DPOAE amplitudes were calculated by subtracting surrounding noise from the $2f_1 - f_2$ distortion product. The noise was defined as the average of the 10 neighboring frequencies (five above and five below the $2f_1 - f_2$ distortion product). After the suprathreshold amplitudes were measured, DPOAE thresholds were then determined by reducing the $f_1$ and $f_2$ primaries in 5-dB steps. Thresholds were defined as the lowest $f_2$ dB level at which the $2f_1 - f_2$ distortion product was $> 6$ dB above the surrounding noise.

Statistical analysis. All statistical analyses were conducted using SPSS for MS Windows (version 15.0; SPSS Inc., Chicago, IL) with statistical significance set at $p < 0.05$. In some cases of repeated measures factors, a sphericity violation occurred. In
such cases, a Greenhouse-Geisser correction was used to reduce the risk of a Type I error if $\epsilon$ was $<0.75$ and a Huynh-Feldt correction was used when $\epsilon$ was $>0.75$. DPOAE amplitudes and DPOAE thresholds were analyzed via separate three-way analyses of variance with treatment as a between-subjects variable, frequency as a repeated measure, litter as the unit of variance, and sex nested within litter. Post hoc comparisons were conducted using Tukey’s test to examine the nature of significant treatment effects obtained from the overall analyses. Reproductive data included litter size, percent male births, percent live births, percent gestational weight gain, percent lactational weight gain, ratio of liver:body weight, and uterine implantation sites in the dam at weaning. For each dependent variable, ANOVA was conducted using treatment as a between-subjects factor. When significant treatment effects were obtained, post hoc Tukey’s tests were done to allow comparisons between treatment groups and the control group as well as comparisons between low and high dose treatment groups and between single and combined treatment groups.

Developmental data included average day of eye opening, postnatal weight gain, and organ:body weight ratios of the pups. Postnatal weight gain was determined by body weights on PND 0, 7, 14, and 21. These data were analyzed via mixed ANOVA with treatment as a between-subjects factor, sex (nested within litter) and age as repeated measures factor. Organ:body weight ratios for the brain, liver, and thymus were measured at the day of weaning and analyzed via mixed ANOVA with treatment as a between-subjects factor, and litter as a unit of variance with sex nested within litter. Post hoc Tukey’s tests were conducted to examine treatment effects. Thyroid hormone
data (T4) was analyzed via ANOVA with treatment as a between-subjects factor and sex nested within litter.

RESULTS

Reproductive/developmental endpoints. No clinical signs of overt toxicity were noted in the dams from any of the treatment groups and all results seen here were similar to the findings in previous studies in our lab using similar doses of the Fox River PCB mixture. There were no significant treatment effects on percent gestational weight gain, percent lactational weight gain, dam liver weight to body weight ratio, litter size, percent male births, percent live births per litter, or implantation sites. There was a main effect of treatment \[F(6,76)=2.654, p=0.022\] on day of eye opening in pups. Tukey’s test revealed that the 6 mg/kg PCB group opened their eyes significantly earlier than the 5.7 mg/kg PBDE group (\(p=0.04\)) and the 11.4 mg/kg PBDE group (\(p=0.012\)), but none of the treatment groups differed significantly from the control group. There was no significant treatment X sex interaction.

Analysis of pup postnatal weight gain revealed a main effect of treatment \[F(6,76)=13.218, p<0.001\] and a significant treatment X time interaction \[F(8.318, 105.363)=11.486, p<0.001\]. Simple ANOVA revealed a significant effect of treatment at each recorded time-point (PND 0, 7, 14, and 21). Significant differences at each time-point were driven primarily by lower body weights in the 6 mg/kg PCB and the high combined treatment groups compared to other treatment groups. Rats in these groups weighed about 18% less than those in the control group at weaning.
Analysis of brain:body weight ratio revealed a main effect of treatment \(F(6,76)=8.948, p<0.001\) and Tukey's tests revealed that this difference was driven by higher brain:BW ratios in the 6 mg/kg PCB and high combined groups. Analysis of liver:body weight ratio also revealed a main effect of treatment \(F(6,76)=114.151, p<0.001\), and Tukey's test revealed that all treatment groups had significantly higher liver:body weight ratios than the control group. Finally, analysis of thymus: body weight revealed a main effect of treatment \(F(6,76)=15.562, p<0.001\), and Tukey's test revealed all exposure groups that received PCBs had significantly lower thymus:body weight ratios than controls.

**DPOAES.** The numbers of litters used in the analysis of DPOAEs were 10, 10, 13, 11, 12, 11, and 10 for the control, 3 mg/kg PCB, 6 mg/kg PCB, 5.7 mg/kg PBDE, 11.4 mg/kg PBDE, low combined, and high combined groups. Figure 3.2 illustrates DPOAE amplitudes at \(f_2\) frequencies of 2, 3, 4, 6, 8, and 12 kHz. There was a significant main effect of treatment \(F(6,70)=20.035, p<0.001\), a significant main effect of frequency \(F(2.892,202.409)=126.97, p<0.001\), and a sex X frequency interaction \(F(3.683,257.809)=4.67, p=0.002\). Comparisons of the sexes revealed that DPOAE amplitudes of male rats were lower than females at 12 kHz (graph not shown). There was no significant main effect of sex and no treatment X sex interactions. Tukey's tests revealed that DPOAE amplitudes were significantly lower in the 6 mg/kg PCB \(p<0.001\), low combined \(p=0.001\) and high combined \(p<0.001\) groups compared to control.

Neither of the PBDE alone groups differed significantly from the control group.
Figure 3.3 illustrates DPOAE thresholds at $f_2$ frequencies of 2, 3, 4, 6, 8, and 12 kHz. There was a significant main effect of treatment [$F(6,70)=15.342$, $p<0.001$] and a significant main effect of frequency [$F(4.465,312.535)=297.915$, $p<0.001$]. There was no significant main effect of sex and no sex X treatment or frequency X treatment interactions. Tukey’s tests revealed that DPOAE thresholds were significantly higher in the 6 mg/kg PCB ($p<0.001$), low combined ($p=0.05$) and high combined ($p=0.002$) groups compared to control. Thresholds of the PBDE alone groups did not differ significantly from the control group.

**Thyroxine Concentrations.** Figure 3.4 illustrates serum total T4 concentrations measured in littermates of the rats that underwent DPOAE testing. T4 was measured at weaning (PND 21). There was a significant effect of treatment [$F(6,61)=89.222$, $p<0.001$]. Tukey’s tests revealed that all treatment groups had significantly lower T4 levels than the control group ($p<0.001$), but the 5.7 mg/kg PBDE and the 11.4 mg/kg PBDE groups had significantly higher T4 levels than the PCB alone and combined groups ($p<0.05$).

**DISCUSSION**

This study confirmed that developmental exposure to the Fox River PCB mixture can result in deficits in cochlear function that last into adulthood. Decreased DPOAE amplitudes and increased thresholds were present across a range of frequencies, consistent with the results of previous studies in our lab (Powers et al., 2006; Powers et
al., 2009). On the other hand, developmental exposure to the PBDE mixture, DE-71 at molar equivalent doses did not produce any DPOAE deficits. Interestingly, combined exposure to subthreshold doses of PCBs and PBDEs did produce significant hearing deficits, suggesting an additive effect.

Thyroid hormones are very important in the development of the cochlea and hypothyroidism during the developmental period can lead to permanent hearing deficits (Uziel, 1986). It is well established that exposure to PCBs, especially during the postnatal or lactational period when rodent cochlear development is occurring causes auditory deficits that persist into adulthood (Crofton et al., 2000b). Previous data suggest that a 60% decrease in T4 levels during the critical period for cochlear development is needed to result in hearing deficits (Crofton, 2004; Crofton and Zoeller, 2005). In this study the Fox River PCB mixture was shown to reduce serum total T4 concentrations more than 60% at weaning compared to the control group, while molar equivalent doses of DE-71 produced a smaller effect on T4 and did not reduce the concentrations by more than 60%. Developmental exposure to PBDEs alone did not produce any DPOAE deficits. This is consistent with a previous study that exposed rodents to PBDEs (Bromkal 70-5 DE or DE-47) and isomolar equivalent PCB doses (Aroclor 1254 or PCB 105) and found that PCBs were more potent in reducing T4 concentrations than PBDEs (Hallgren et al., 2001).

Although reduced T4 concentrations may play a role in the cochlear dysfunction we observed, the sharp delineation between no effect in the DE-71 groups and clear cochlear dysfunction in the 6 mg/kg PCB or combined PCB/PBDE groups, with a relatively modest further decrease in T4 concentrations, suggests that reductions in T4
may only be part of the story. In addition, T4 concentrations differed only slightly in the 3 and 6 mg/kg PCB-exposed groups, yet the 6 mg/kg dose group showed a clear deficit in cochlear function whereas the 3 mg/kg group did not.

PCBs have been shown to bind to the ryanodine-receptor (RyR) and stabilize the high-affinity binding conformation of the receptor, thereby increasing intracellular calcium concentrations (Wong and Pessah, 1997). In particular, the Fox River Mix has been shown to have RyR activity (Kostyniak et al., 2005) and enhance the release of calcium from intracellular stores (Pessah et al., 2006). RyR has been identified in the rat cochlea and its localization includes the inner and outer hair cells (Beurg et al., 2005; Morton-Jones et al., 2006). An increase in intracellular calcium concentrations through activation of the RyR may regulate the motion of the outer hair cells (Dallos and Harris, 1978), and, thus, increased RyR activation could potentially contribute to the observed cochlear deficits. Studies have demonstrated that administration of drugs (caffeine and ryanodine) that induce calcium release from RyR stores into the perilymph reversibly suppressed DPOAEs (Bobbin, 2002). Interestingly developmental exposure to PCB-95, a potent activator of the RyR with only a modest effect on thyroxine concentrations, severely disrupted the tonotopic map of the auditory cortex, but did not result in hearing loss as measured by the auditory brain stem response (ABR) (Kenet et al., 2007).

PCBs and PBDEs can also disrupt neurotransmitter systems and induce oxidative stress and these effects could also play a role in cochlear dysfunction. Non-coplanar PCBs can inhibit plasma membrane uptake of glutamate (Mariussen and Fonnum, 2001), which plays important roles in the cochlea, while the penta-BDEs were generally a poor plasma membrane uptake inhibitor (Mariussen and Fonnum, 2003).
More exogenous glutamate excitotoxicity during cochlear development can lead to elevated hearing thresholds in rats (Janssen et al., 1991). PCBs have also been shown to increase reactive oxygen species (ROS) formation in vitro, thereby inducing cellular death (Mariussen et al., 2002).

The 3 mg/kg PCB dose did not result in any DPOAE deficits, whereas previous studies in our lab using the 3 mg/kg dose did find significant DPOAE deficits (Powers et al., 2006; Powers et al., 2009). This apparent discrepancy may be due to the fact that the rats in these previous studies were tested at an older age—approximately 200 days, while in this study rats were tested at about 100 days of age. The rats in those studies had 100 more days of exposure to daily noise (e.g. room ventilation, lab and care staff personnel entering and exiting the room, and cage cleanings), and exposure to moderate noise over long periods can lead to damage of the outer hair cells and noise induced hearing loss (Konings et al., 2009). Early exposure to this lower dose of PCBs may prime the outer hair cells to be more susceptible to the moderate noise experienced in everyday life, leading to a hearing loss that emerges as the animals age. We are currently conducting studies to determine whether early PCB exposure predisposes animals to noise-induced hearing loss later in life.

Although exposure to PBDEs alone at the doses we employed did not produce cochlear dysfunction, combined exposure to PBDE and PCB doses that alone did not have an effect on cochlear function did produce significant decreases in DPOAE amplitudes and increases in DPOAE thresholds across all frequencies tested. Co-exposure to 3 mg/kg PCB plus 5.7 mg/kg PBDE produced effects roughly equivalent to the effect of a 6 mg/kg dose of PCBs alone, suggesting an additive effect on cochlear
function. In contrast, exposure to 6mg/kg PCBs plus 11.4 mg/kg PBDEs did not produce DPOAE deficits any greater than those observed with exposure to 6 mg/kg of PCBs alone, suggesting that this dose of the PCB mixture may produce a maximal effect on cochlear function. We have never tested higher doses of PCBs, so it remains unknown whether larger deficits in cochlear function could be produced with larger doses of PCBs. The lack of further deficits in the high combined group could be due to the fact that PCB exposure specifically targets the outer hair cells in the cochlea. Previous work has shown that developmental exposure to PCBs (Aroclor 1254) led to a loss of outer hair cells, but inner hair cells were not affected (Crofton et al., 2000a). Even severe damage that is specific to the outer hair cells may not cause more than a moderate hearing loss because the role of the outer hair cells is to act as a cochlear amplifier: increasing the sensitivity to low level sounds and fine tuning frequency discrimination. The inner hair cells and the auditory nerve can still be excited in the absence of the cochlear amplifier as measured by auditory brainstem responses (Adelman et al., 2010).

Although the PCB and PBDE doses used in this study produce body burdens which are significantly higher than the levels found in most environmentally exposed humans, it is widely accepted according to the allometric scaling that higher administered doses are needed in rodents to produce the same internal doses and health effects observed in humans (Sarver et al., 1997; Sharma and McNeill, 2009). We estimate based on preliminary data that the internal PCB body burdens resulting from the doses we administered are similar to the body burdens of humans living near former PCB manufacturing sites in eastern Europe (Trnovec et al., 2008; Trnovec et al., 2010).
Importantly, a recent study in Slovakia has confirmed that early exposure to PCBs was associated with decreased TEOAE and DPOAE amplitudes in 12-yr old children from one such contaminated site (Trnovec et al., 2010).

In summary, our data highlight that it is important not only to assess exposures to individual toxicants during critical periods of development, but also to study mixtures of toxicants as they may exist in the environment. Both the Fox River PCB mixture and DE-71 are environmentally relevant mixtures that individually pose health risks. Our findings contribute to the evidence that these structurally similar chemicals may also have additive effects on various health endpoints, including hearing.

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FIG. 3.1. An example of a DPOAE recording from a rat. The primary tones ($f_1$ and $f_2$) are presented simultaneously at a fixed ratio ($f_2/f_1=1.2$) into the sealed ear canal. The distortion product is at the $2f_1-f_2$ frequency.
FIG. 3.2. Group mean DPOAE amplitudes of control rats compared with treated rats. Exposure to PCBs only (A), PBDEs only (B), or PCBs + PBDEs (C). *Main effect is significantly different from controls (p<0.05).

FIG. 3.3. Group mean DPOAE thresholds of control rats compared with treated rats. Exposure to PCBs only (A), PBDEs only (B), or PCBs + PBDEs (C). *Main effect is significantly different from controls (p<0.05).
FIG. 3.4. Pup serum T4 concentrations taken at weaning. * indicates a significant difference from control (p<0.01). + indicates a significant difference from PBDE only groups (p<0.05).
CHAPTER 4

THE IMPACT OF DEVELOPMENTAL PCB EXPOSURE ON SUSCEPTIBILITY TO NOISE-INDUCED HEARING LOSS IN ADULTHOOD

ABSTRACT
There is evidence that developmental exposure to polychlorinated biphenyls (PCBs) causes cochlear dysfunction via outer hair cell (OHC) damage. One of the functions of the OHCs is to protect the cochlea from noise-induced damage, thus developmental PCB exposure could potentially increase susceptibility to noise-induced hearing loss later in life. In the current study, we exposed rat dams to an environmentally relevant PCB mixture during gestation and lactation, and then later exposed their offspring to loud noise in adulthood. Female Long-Evans rats were exposed to corn oil vehicle, or 1, 3, or 6 mg/kg/day PCBs throughout gestation and lactation. At weaning, two males and two females from each litter were retained until adulthood. One male and female pair from each litter was assigned to the noise exposure group (4 hours a day for 5 consecutive days of 97dB octave-band noise centered at 8 kHz) and the other pair was assigned to the no-noise group. Cochlear function of the rats was tested at baseline, 1 day after the noise exposure, and 28 days after the noise exposure using distortion product otoacoustic emissions (DPOAEs), a method that assesses the functional integrity of the OHCs. Baseline DPOAE amplitudes were decreased in the PCB groups, as previously published, with the clearest effect in the 6 mg/kg PCB group. Unexpectedly, a large percentage of rats in the 6 mg/kg PCB group exhibited behaviors
characteristic of audiogenic seizures at the onset of noise exposure and had to be excluded from the noise study. Rats in the 0, 1 and 3 mg/kg PCB groups all showed a temporary hearing loss 1 day after noise exposure, with partial recovery by 28 days after noise exposure. However, the PCB-exposed groups did not show any evidence of an increased susceptibility to the noise.

Key words: PCBs, noise-induced hearing loss, auditory system, seizures.
INTRODUCTION

Polychlorinated biphenyls (PCBs) are industrial chemicals that have been banned from production since the 1970s, but due to their chemical stability and long half-lives, they continue to persist in our environment. They have bio-accumulated and bio-magnified, particularly in the aquatic food chain, and the main route of exposure to humans is through the consumption of contaminated fish and seafood (Crinnion, 2011). PCBs can cross the mammalian placenta and affect the developing fetus as well as enter breast milk and affect the developing infant (Jacobson et al., 1984).

PCBs have been shown to cause ototoxic effects in both laboratory animals and humans (Crofton et al., 2000a; Powers et al., 2006; Trnovec et al., 2010). Specifically, developmental PCB exposure has been shown to lead to long-lasting hearing loss by damaging or altering the function of the outer hair cells (OHCs) of the cochlea (Crofton et al., 2000a; Lasky et al., 2002; Powers et al., 2006). One of the functions of the OHCs is to protect against noise-induced hearing loss (NIHL) through the stiffening of their stereocilia, which in turn protects against metabolic damage to the cochlea during exposure to loud noise (Lim, 1986).

NIHL is a common occupational disorder with nearly 30 million U.S. workers exposed to potentially harmful levels of noise according to the National Institutes of Occupational Safety and Health (NIOSH) (Franks et al., 1996). Recently younger populations are also suffering from higher rates of NIHL as they increasingly expose themselves to noise trauma such as music concerts and digital music players (Daniel, 2007). Shargorodsky et al. (2010) reported that the prevalence of hearing loss in US adolescents was 19.5% in 2005-2006, which was a 31% increase since 1988-1994.
Previous studies have shown that ototoxic chemicals can interact with loud or moderate noise to exacerbate hearing loss. In a series of studies, co-exposure to chemicals such as toluene, styrene, jet fuel or acrylonitrile and also to noise between 92 and 102 dB either once or chronically over days led to greater elevations of auditory thresholds, greater decreases in distortion product otoacoustic emission (DPOAE) amplitudes and more OHC loss when compared to exposure to either the noise alone or the chemical alone (Fechter et al., 2007; Lataye et al., 2000; Lataye and Campo, 1997; Pouyatos et al., 2005). Because PCBs have been shown to cause OHC dysfunction, they may also render the cochlea more vulnerable to noise, increasing the risk for noise-induced hearing loss (NIHL).

The present study used a rat model to investigate whether early PCB exposure increases susceptibility to NIHL in adulthood. Rats were exposed throughout gestation and lactation to an environmentally relevant PCB mixture (0, 1, 3, or 6 mg/kg/day). In adulthood they were exposed to 97dB of octave-band noise 4 hours/day for 5 days. This degree of noise exposure has been shown to cause temporary hearing loss in control rats (Pouyatos et al., 2005). Cochlear function was tested using DPOAEs before noise exposure, 1 day after 5 consecutive days of noise exposure, and 28 days after noise exposure to reveal if temporary and/or permanent cochlear dysfunction occurred.
MATERIALS AND METHODS

Animals. Primiparous female Long-Evans rats, approximately 8-10 weeks of age, were purchased from Harlan (Indianapolis, IN) in three cohorts. The rats were maintained in facilities accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care. They were individually housed in standard polycarbonate plastic shoebox cages with corn-cob bedding, in a temperature- and humidity-controlled room (22°C, 40–55% humidity) on a 12/12-hr light cycle (lights on at 0830 hr). Rats were fed rat chow (Harlan Teklad rodent diet (W) 8604) and water *ad libitum*. All procedures were approved by the Institutional Animal Care and Use Committee at the University of Illinois at Urbana-Champaign and were in accordance with the guidelines of the National Institutes of Health (2002) and National Research Council (2003).

Exposure. After 1 week of adaptation to the vivarium, female rats were assigned to exposure groups (distributed evenly by body weight) and given one of four treatments consisting of corn oil vehicle or PCBs in corn oil. Each exposure group was represented in each cohort. Exposure to the treatments began 28 days prior to breeding and continued until weaning of the pups on postnatal day (PND) 21. The PCB mixture (Fox River PCB mixture) was formulated to mimic the congener profile found in walleye from the Fox River in northeast Wisconsin. The mixture consisted of 35% Aroclor 1242 (Monsanto lot KB 05-415; St. Louis, MO) 35% Aroclor 1248 (AccuStandards lot F-110; New Haven, CT), 15% Aroclor 1254 (Monsanto lot KB 05-612), and 15% Aroclor 1260 (AccuStandards lot 021-020) (Kostyniak *et al.*, 2005). The doses of the PCB mixture (1, 3 and 6 mg/kg/day) were selected based on the results of earlier studies assessing the
in vivo developmental toxicity and auditory toxicity of the mixture in rats (Kostyniak et al., 2005; Powers et al., 2006). The PCBs were diluted in corn oil (Mazola) and pipetted onto one-half of a vanilla wafer cookie (Keebler Golden Vanilla Wafers) at a volume of 0.4 mL/kg. To arrive at a dose of 0.4 mL/kg, the individual dosing solutions were mixed at concentrations of 2.25 mg/mL, 7.5 mg/mL and 15 mg/mL for the PCB doses of 1mg/kg, 3 mg/kg and 6 mg/kg. The PCB treated cookies were fed to the female rats daily at approximately 1100 hr with the amount of dosing solution applied to the cookies adjusted daily to account for weight gain. Corn oil only was pipetted onto cookies for rats in the vehicle control group.

**Breeding, pregnancy, and weaning.** After four weeks of PCB exposure, each female was paired with an unexposed male Long-Evans rat (Harlan, Indianapolis, IN) in a hanging wire cage for 8 consecutive days with food and water ad libitum. The females were returned to their home cages each day for PCB dosing. The females were monitored for the presence of a sperm plug in order to establish gestational day 0. Females who did not give birth were retained and their uteri examined for implantation sites.

On the day of parturition (PND 0), the pups were examined for abnormalities, sexed and weighed, and the number of stillbirths was noted. On PND 2, the litters were culled to 10 pups (five males and five females when possible), and litters with at least 7 pups had extra pups cross-fostered into them from the same treatment group to bring the litters to 8–10 pups. Cross-fostered pups were marked and not used for the experiment. The day of eye opening was recorded and pups were weighed weekly.
There were 42 successful litters. Of the remaining dams, 9 were not pregnant and 9 had litters too small to be included in the study (≤7 pups). Overall the non-pregnant dams and dams with small litters were evenly distributed across the treatment groups.

Dosing continued until the pups were weaned on PND 21. On PND 21, the dams were euthanized and the liver weight and number of uterine implantation sites were recorded. Four pups per litter, two male and two female, were retained for the experiment with only one male and one female per litter being exposed to noise. Organ weights (brain, liver, and thymus) were recorded from one male and female per litter. Pups retained on the day of weaning were pair-housed in same-exposure, same-sex pairs with food and water available ad libitum. Auditory testing and noise exposure began at approximately PND 200.

**Distortion product otoacoustic emissions.** DPOAEs are auditory responses measurable from the cochlea when it is stimulated by two pure tones (called $f_1$ and $f_2$ primaries). DPOAE testing was conducted in a sound-attenuated chamber, lined with sound-absorbing foam in an isolated laboratory. Rats were sedated with 0.5 mL/kg ketamine/xylazine (87:13) intraperitoneally. Once sedated, rats were placed on their sides on a thermo-regulating heating pad (no. 50-7053-R, Harvard Apparatus, Holliston, MA) to maintain body temperature, and the DPOAE probe was positioned in the left ear canal.

DPOAEs were recorded using Tucker Davis Technologies (TDT; Alachua, FL) System 2/System 3 digital signal processing hardware and software. Stimuli were directed into the ear canal through a single ear probe that consisted of two speakers
and a microphone with a soft rubber tip that sealed the ear canal from external noise. All DPOAE stimuli were created using TDT SigGen software and recordings were made using TDT BioSig software. Details regarding the instrumentation can be found in Powers et al. (2006).

As in our previous studies, the DPOAEs were generated by simultaneously presenting two sinusoids, $f_1$ and $f_2$ ($f_2/f_1 = 1.2$), into the sealed ear canal of the rat. The sound levels for the $f_1$ and $f_2$ primaries were calibrated to 60 dB sound pressure level (SPL) and 50 dB SPL (0 dB = 20 μPa), respectively, using a pressure field microphone (no. 4192, Brul and Kjaer, Norcross, GA) in a 2-cc calibration coupler (no. 4946, Brul and Kjaer). The amplitude of the distortion product at the frequency defined by $2f_1 - f_2$ was measured by recording the pressure in the sealed ear canal. In mammals, the $2f_1 - f_2$ distortion product is the most robust and is commonly measured as a reliable indicator of outer hair cell function (Lonsbury-Martin and Martin, 1990). Six stimulus pairs were used for DPOAE testing ($f_2 = 2, 3, 4, 6, 8, \text{ and } 12 \text{ kHz}$).

DPOAE testing consisted of measuring suprathreshold DPOAE amplitudes at each of the six stimulus pairs, beginning with 2 kHz. Each distortion product was the averaged response of 100 stimulus pair presentations (presentation rate = 6/sec). The final distortion product represented an average of two trials (each having 100 separate stimulus presentations). DPOAE amplitudes were calculated by subtracting surrounding noise from the $2f_1 - f_2$ distortion product. The noise was defined as the average of the 10 neighboring frequencies (five above and five below the $2f_1 - f_2$ distortion product).
Noise exposure. Baseline auditory function for all rats was assessed at approximately PND 200 by measuring their DPOAEs as described above. After baseline recordings, animals in the noise exposure groups (one male and one female from each litter) were placed in perforated stainless steel cages (7 in. x 7 in. x 7 in. per rat), and those cages were placed in wooden noise exposure chambers (30.5 in. x 11 in. x 9 ¾ in.), lined with sound insulating material (Soundsoak, Armstrong World Industries, Lancaster, PA). Each chamber had six equally spaced tweeter speakers (3" OEM cone tweeter, MCM Electronics, Dayton, OH) lining the back and top of the chambers. Using an oscilloscope (Tektronix 465), a microphone (Etymotic ER-10B), and a sound pressure level meter (RadioShack SPL-meter 33-4050), the noise levels were calibrated to 97dB when the chambers were closed. The tweeter speakers were connected to an amplifier (Techron 5507 power supply amplifier) which was connected to a music player (Sandisk Sansa Clip+ 4GB MP3 player) loaded with the octave-band noise with a center frequency of 8 kHz and played on a loop. The noise was created using MATLAB software (The MathWorks, Inc.) (Figure 4.1).

On each noise-exposure day, rats in the noise-exposure group were placed in the noise chambers and exposed to 15 min of gradually increasing intensities of noise starting at 75dB and monitored until the noise level reached the 97dB. This was done to habituate the rat to the loud noise. Once the noise level reached 97 dB, rats remained in the chambers for 4 hours. Rats were monitored every 10 min for the first 30 min of the 97 dB noise exposure, and then every 30 min for the remaining 3.5 hours of exposure to the 97dB noise. Noise exposures were administered for 4 hours/day for 5 consecutive days. DPOAE measurements were repeated 1 day after noise exposure.
and again 28 days after noise exposure to determine whether there were temporary or permanent auditory deficits. DPOAEs of littermates not exposed to noise were measured at all of the same time points as the noise-exposed rats.

**Statistical analysis.** All statistical analyses were conducted using SPSS for MS Windows (version 15.0; SPSS Inc., Chicago, IL) with statistical significance set at \( p < 0.05 \). In some cases of repeated measures factors, a sphericity violation occurred. In such cases, a Greenhouse-Geisser correction was used to reduce the risk of a Type I error if \( \varepsilon < 0.75 \) and a Huynh-Feldt correction was used when \( \varepsilon > 0.75 \). Data were analyzed via repeated measures analysis of variance (ANOVA). Baseline DPOAE amplitudes of both noise and non-noise groups combined were analyzed via three-way ANOVAs (treatment X sex X frequency). The magnitude of DPOAE amplitude changes between baseline vs. 1 day, 1 day vs. 28 days, and baseline vs. 28 days were analyzed via three-way ANOVAs (treatment X sex X frequency), with noise and non-noise exposed groups analyzed separately. The litter was the unit of analysis, with sex nested within the litter, and frequency was a repeated measure. Post hoc Tukey’s tests were used to compare PCB-exposed groups to the control groups.

Reproductive data including litter size, percent male births, percent live births, percent gestational weight gain, percent lactational weight gain, ratio of liver:body weight, and uterine implantation sites in the dam at weaning were analyzed to verify the overall toxicity of the PCB doses used in the study. For each dependent variable, ANOVA was conducted using treatment as a between-subjects factor. When significant treatment effects were obtained, post hoc Tukey’s tests were done to allow
comparisons between treatment groups and the control group as well as comparisons between low, medium and high dose groups.

Developmental data included average day of eye opening, postnatal weight gain, brain weight, and organ:body weight ratios of the pups. The body weight data was analyzed via mixed ANOVA with treatment as a between-subjects factor, sex (nested within litter) and age as repeated measures factor. Organ:body weight ratios for the liver, and thymus, and whole brain weights were measured at the day of weaning and analyzed via mixed ANOVA with treatment as a between-subjects factor, and litter as a unit of variance with sex nested within litter. Post hoc Tukey’s tests were conducted to examine treatment effects.

RESULTS

Reproductive/developmental endpoints. As previously published for the Fox River PCB mixture (Poon et al., 2011; Powers et al., 2006; Powers et al., 2009; Sable et al., 2009), there were no signs of overt toxicity in the dams. There were no significant effects of treatment on percent live births, litter size, percent male births, number of implantation sites, percent gestational weight gain, percent lactational weight gain, and dam liver weight to body weight ratio.

Analysis of toxicity in the pups revealed no significant main effect of treatment on average day of eye opening or on brain weight. Analysis of liver:body weight ratio did reveal a main effect of treatment \( [F(3,38)=74.283, \ p<0.001] \) and, as we have reported previously, Tukey’s test revealed that all PCB treatment groups had significantly higher liver:body weight ratios compared to the control group. Analysis of thymus:body weight
ratios also revealed a main effect of treatment \[F(3,38)=11.012, p<0.001\], and, similar to our previous studies, Tukey’s test revealed that the 3 and 6 mg/kg PCB groups and significantly lower thymus:body weight ratios compared to the control group.

Analysis of pup postnatal weight gain revealed a main effect of treatment \[F(3,41)=19.738, p<0.001\] and a time x treatment interaction \[F(9,123)=18.229, p<0.001\]. Simple ANOVA of treatment at each time point revealed that the 3 and 6 mg/kg PCB groups had small, but significant decreases in body weight at the 7, 14, and 21 day time points compared to the control group, with the 3 mg/kg group decreased 6-7.5% and the 6 mg/kg group decreased 14-16% compared to controls at those time points.

**DPOAES.** The numbers of litters used in the analysis of baseline DPOAEs were 10, 9, 10, and 6 for the control, 1 mg/kg PCB, 3 mg/kg PCB, and 6 mg/kg PCB groups. Figure 4.2 illustrates baseline DPOAE amplitudes at \(f_2\) frequencies of 2, 3, 4, 6, 8, and 12 kHz before noise exposure separated by sex. There was a significant main effect of treatment \[F(3,31)=15.411, p<0.001\], a significant main effect of frequency \[F(3.322, 102.985)=79.543, p<0.001\], and a sex X frequency x exposure interaction \[F(9.544,98.622)=2.301, p<0.05\]. Further analysis of the interaction revealed that females (Figure 4.2A) in the 6 mg/kg PCB group had significantly lower DP amplitudes compared to controls at the 2, 3, 4, 8, and 12 \(f_2\) frequencies, the 3 mg/kg groups had significantly lower DP amplitudes compared to controls at the 2, 3, 4, and 8 \(f_2\) frequencies, and the 1 mg/kg PCB group had significantly lower DP amplitudes at the 2 and 3 \(f_2\) frequencies. Analysis of the males (Figure 4.2B) revealed that that the 6 mg/kg
PCB group had significantly lower DP amplitudes across all frequencies compared to controls, and the 3 mg/kg PCB had significantly lower DP amplitudes compared to controls at the 2, 3, 4, and 8 f<sub>2</sub> frequencies.

Interestingly and quite unexpectedly, a behavior characteristic of audiogenic seizures (AGS), wild running (Ross and Coleman, 2000) was observed in most of the rats in the 6 mg/kg PCB group and some of the rats in the 3 mg/kg PCB group on the first day of testing during the initial 15 min when the noise was gradually increased from 75dB to 97dB (Table 4.1). Due to the high percentage of rats in the 6 mg/kg PCB group that showed this seizure-like behavior when exposed to noise, this dose group had to be dropped from the noise exposure study.

Figure 4.3 represents the magnitude of change in DPOAE amplitudes. Because there was a treatment effect on baseline DPOAE amplitudes, the magnitude of change between the time points (baseline to 1 day after noise, 1 day to 28 days after noise, baseline to 28 days after noise) was used as the dependent variable to assess the effects of noise exposure. Litter sizes were 10, 9, and 10 for the control, 1 mg/kg PCB, and 3 mg/kg PCB groups in the non-noise exposed groups and 10, 8, and 7 for the control, 1 mg/kg PCB, and 3 mg/kg PCB groups in the noise exposed groups after the rats that showed behaviors characteristic of AGS were removed from the study. As expected, there were no significant changes in DPOAE amplitudes over time in the non-noised exposed groups. Figure 4.4 represents the magnitude of change of DPOAE amplitudes from baseline to 1 day after noise, from 1 day to 28 days, and from baseline to 28 days in the noise exposed groups. There were significant main effects of frequency for baseline vs. 1 day [F(2.769, 60.964)=16.890, p<0.001] showing a
depression of DPOAE amplitudes at the highest frequencies 1 day following noise exposure, for 1 day vs. 28 days [F(3.702, 81.438)=12.278, p<0.001], a recovery of DPOAE amplitudes at the highest frequencies after 28 days, and, similarly for baseline vs. 28 days [F(2.312, 60.102)=3.340, p<0.05], but there were no significant main effects of treatment or sex on the magnitude of change. Tukey’s test on the DPOAE amplitudes from baseline to 28 days at each frequency showed that the magnitude of change was 5 dB greater at 12 kHz than at 2 kHz (p<0.05), revealing that there was slight permanent cochlear dysfunction at the highest frequency.

DISCUSSION

As with previous studies from our lab using the Fox River PCB mixture (Powers et al., 2006; Powers et al., 2009), this study further confirmed that developmental exposure to this environmentally relevant PCB mixture causes long-lasting cochlear dysfunction. DPOAE amplitudes were decreased in both male and female rats exposed to either 3 or 6 mg/kg PCBs, although the 3 mg/kg rats did not differ from controls at the highest frequency tested (12 kHz).

This study originally set out to investigate whether developmental exposure to PCBs would result in more severe cochlear dysfunction after exposure to noise. Unexpectedly, more than 50% of the rats in the 6 mg/kg group displayed wild running, a behavior characteristic of audiogenic seizures (AGS) during the noise habituation period at the onset of noise testing, and this group had to be dropped from further noise exposure. In the 1920s by both Pavlov and the Wistar Institute in Philadelphia (reviewed Ross and Coleman, 2000), discovered that rodents would display AGS in
response to intense noise. Since that time, the first stage of AGS has been described as the wild running phase in which the rat runs uncontrollably at full speed around the testing chamber. Wild running can then progress into more severe stages such as clonus (reviewed Ross and Coleman, 2000).

Audiogenic seizures differ from other types of seizures in that they begin with wild running (which is not seen with other types of seizures) and mainly require activation of brainstem auditory pathways, especially at the level of the inferior colliculus (Faingold, 2002; Ross and Coleman, 2000). C-fos mRNA expression studies revealed that AGS induced c-fos mRNA in the brainstem, mainly in the inferior colliculus, with weak hippocampal expression (Ishida et al., 1995), while forebrain seizures induced Fos gene expression in forebrain areas such as the cerebral cortex, piriform cortex, and amygdala (Eells et al., 2004). With repeated exposure to loud noise, AGS can eventually recruit forebrain structures as evidence of kindled AGS resulting in increased Fos gene expression in the cortex, hippocampus, medial hypothalamus, and amgydala (Simler et al., 1994).

Although there are no prior studies in the literature indicating that PCB-exposed rodents have increased susceptibility to AGS, there is one study that suggests PCB-exposed rats have a lower threshold for other types of generalized seizures. In that study, rats were exposed to a specific PCB congener (PCB 95) at doses of 1 or 6 mg/kg/day in the maternal diet from gestational day 5 until weaning at PND 21 and their seizure susceptibility was tested using the convulsive drugs, flurothyl and pentylenetetrazole (PTZ) (Lein et al., 2010). Rats in the 1 mg/kg PCB group had significantly shorter latencies to the first stage of myoclonus and a tendency to have
shorter latencies to the onset of tonic-clonic seizures when exposed to flurothyl. Rats in the 1mg/kg PCB group also kindled significantly faster than the other groups when exposed to PTZ. The investigators suggested that the lower seizure threshold may be related to the discovery that PCBs bind to the ryanodine receptor (RyR), which regulates calcium release from intracellular stores and alters neuronal excitability (Wong et al., 1997). Exposure to PCB 95, a potent RyR activator, in hippocampal slices ex vivo caused an imbalance between inhibitory and excitatory circuits, and a similar effect was observed in the auditory cortex in vivo using whole-cell voltage clamp recordings (Kenet et al., 2007; Kim and Pessah, 2011). As Faingold (2002) has reviewed, a deficit in GABA-mediated inhibition in the inferior colliculus is a critical mechanism for the induction of AGS. Although no studies have shown that there is an imbalance between inhibitory and excitatory circuits in the inferior colliculus of PCB-exposed rats, the hippocampal studies, suggest that this could be a fruitful area for further research.

Another mechanism that could be related to the AGS-like behaviors we observed is reduced thyroid hormone levels. Previous studies indicate that hypothyroid rats are more susceptible to AGS. Rat pups treated with propylthiouracil (PTU) during PND 0-19 had higher incidences of AGS at 4 months of age (Kato et al., 1996). Another study fed rat dams low iodine diets throughout gestation and lactation and the hypothyroid offspring had a high incidence (89%) of AGS when exposed to loud noise (Van Middlesworth, 1977). Deficiency of T4 during neocorticogenesis in rats (embryonic day 13-15) also caused about 20% of the glutamatergic neurons to migrate to abnormal locations in the primary somatosensory cortex and these rats were also more susceptible to AGS, which suggests a potential role for excitatory/inhibitory imbalance in
the cortex for AGS (Auso et al., 2004). Previous studies with PCBs, including the Fox River PCB Mixture have established that developmental exposure lowers thyroid hormone (T4) in pups (Crofton et al., 2000b; Goldey et al., 1995; Poon et al., 2011). The T4 levels taken at weaning of littermates tested in this study were dramatically reduced compared to controls (see chapter 5). Therefore the lowering of T4 levels during development is another mechanism through which PCBs could have increased in the susceptibility to AGS.

Based on the groups that could be tested (1 and 3 mg/kg PCBs), there was no evidence of more severe or more lasting cochlear dysfunction after noise exposure in the PCB-exposed groups relative to the control group. The exposure to noise was centered at 8 kHz and, as expected because DPOAE damage is frequency specific in the cochlea (Emmerich et al., 2005), for all groups, the largest reduction in DPOAE amplitude was seen at f2 frequencies of 8-12 kHz.

This study used an approach similar to published studies in which rats exposed to either acrylonitrile (ACN) or JP-8 jet fuel were co-exposed to loud noise to induce NIHL (Fechter et al., 2007; Pouyatos et al., 2005). In those studies, the toxicant-exposed rats exhibited either a permanent cochlear dysfunction (ACN) that did not recover with time, or an additive effect (JP-8 jet fuel) whereby groups exposed to both the chemical and the loud noise exhibited greater reductions in DPOAE amplitudes than those exposed to either the chemical alone or the noise alone. We did not see either of these patterns in the 1 and 3 mg/kg PCB groups; e.g. a permanent reduction in DPOAEs or an additive effect of PCBs and noise on cochlear function. Rather, we saw a similar pattern in the PCB-exposed and control groups: temporary OHC dysfunction 1
day after noise at most of the frequencies tested and a slight (about 5dB) reduction in DPOAE amplitude that still persisted 28 days after noise exposure at the 12 kHz frequency. We also did not expose our rats concurrently to the toxicant and noise, like in the studies described above. Our study was a developmental exposure to the toxicant and an adult exposure to noise. Also, we were not able to test the 6 mg/kg group, which showed the clearest reduction in DPOAE amplitudes prior to noise exposure, and this may have limited our ability to detect an additive effect of PCBs and noise.

In summary, developmental PCB exposure has been shown to cause cochlear dysfunction that persists into adulthood. In this study our aim was to investigate whether early PCB exposure would interact with exposure to loud noise in adulthood to worsen its effect on the cochlea. Unlike previous studies that co-exposed rats to solvents or jet fuel and loud noise, we did not observe any interactive effects of PCBs and loud noise. However, very unexpectedly, a large percentage of the rats in our highest PCB dose group displayed what appeared to be AGS when exposed to noise. Future studies designed to specifically assess seizure activity will be needed to confirm that developmentally PCB-exposed rats are more prone to audiogenic seizures and to determine whether this phenomenon is limited to noise exposure or represents a more generalized reduction in threshold for seizures, which could have important human health implications.
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FIG. 4.1. Frequency response of the octave-band noise centered at 8 kHz.
FIG. 4.2. Group mean DPOAE amplitudes of control and PCB-treated rats. a indicates the 6 mg/kg PCB group significantly different from control (p<0.05), b indicates the 3 mg/kg PCB group significantly different from control (p<0.05), and c indicates the 1 mg/kg PCB group significantly different from control (p<0.05).

FIG. 4.3. Group mean DPOAE amplitudes of control and PCB-treated rats in the no noise groups. Magnitude of change of amplitudes between the baseline and 1 day after noise time points (A). Magnitude of change of amplitudes between the 1 day after noise and 28 days after noise time points (B). Magnitude of change of amplitudes between the baseline and 28 days after noise time points (C).
FIG. 4.4. Group mean DPOAE amplitudes of control and PCB-treated rats in the noise-exposed groups. Magnitude of change of amplitudes between the baseline and 1 day after noise time points (A). Magnitude of change of amplitudes between the 1 day after noise and 28 days after noise time points (B). Magnitude of change of amplitudes between the baseline and 28 days after noise time points (C).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% Showing Seizure Activity</th>
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<tr>
<td>6 mg/kg PCB</td>
<td>53%</td>
</tr>
<tr>
<td>3 mg/kg PCB</td>
<td>24%</td>
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<tr>
<td>1 mg/kg PCB</td>
<td>5%</td>
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<tr>
<td>Control</td>
<td>0%</td>
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TABLE 4.1. Percentage of total animals per treatment group that exhibited wild running or clonus during the first 15 mins of noise habituation (75-97dB).
CHAPTER 5

THE IMPACT OF DEVELOPMENTAL PCB EXPOSURE ON SUSCEPTIBILITY TO AUDIOGENIC SEIZURES IN ADULTHOOD

ABSTRACT
Developmental exposure to polychlorinated biphenyls (PCBs) causes auditory deficits via outer hair cell damage. A function of the outer hair cells is to protect the cochlea from loud noise. Thus we recently conducted a study to investigate if developmental PCB exposure would exacerbate noise-induced hearing loss (NIHL). Unexpectedly some PCB-exposed rats exhibited seizure-like behaviors when exposed to loud noise. Thus, we conducted an experiment to determine if adult rats exposed to PCBs perinatally are more susceptible to audiogenic seizures (AGS) using a standard audiogenic seizure paradigm. Adult male and female rats exposed to PCBs during gestation and lactation (0, 1, 3 or 6 mg/kg/day) and previously tested in the NIHL study were exposed to 2 minutes of noise at 100dB, and re-exposed after 24-48 to hours at 105dB and then at 110dB if they did not exhibit clonus at the lower noise intensities. Female rats exposed to either 3 or 6 mg/kg PCBs had significantly higher incidences of AGS compared to controls. Male rats exposed to 6 mg/kg PCBs had higher incidences of AGS compared to noise-naïve (NN) controls, but not compared to controls used in the earlier NIHL study. Thyroxine measured at weaning was significantly lower in all PCB groups compared to controls, suggesting a potential mechanism for the increases
in AGS. This is the first study to show that developmental PCB exposure increases the susceptibility to AGS in adulthood.

Key words: PBCs, audiogenic seizures
INTRODUCTION

Polychlorinated biphenyls (PCBs) are industrial contaminants that were manufactured for use in dielectric fluids, transformers, and capacitors (Crinnion, 2011). Their production has been banned since the 1970s, but due to their chemical stability and lipophilicity, they continue to persist in the environment. PCBs have bio-accumulated and bio-magnified up the food chain and human exposure is primarily via consumption of contaminated fish and seafood (Crinnion, 2011). PCBs readily cross the mammalian placenta and are mobilized from body fat into breast milk during lactation, putting the developing offspring at risk. One cause for concern is that developmental exposure to PCBs has been associated with long-lasting hearing deficits in both animal models and humans (Crofton et al., 2000; Goldey et al., 1995; Poon et al., 2011; Powers et al., 2006; Trnovec et al., 2010).

Exposure to PCBs during gestation and lactation has been shown to cause long lasting hearing deficits in rodent offspring, and functional studies suggest that the site of action is at the level of the outer hair cells (OHCs) of the cochlea. An early study found that maternal exposure to Aroclor 1254 (a commercial PCB mixture) led to increased auditory thresholds at low frequencies, and this was accompanied by a sharp reduction in circulating thyroid hormone T4 concentrations (Goldey et al., 1995). Thyroxine replacement during cochlear development partially ameliorated the low-frequency hearing loss (Goldey and Crofton, 1998). Crofton (2000) also reported the loss of OHCs in the region of the cochlea responsible for low-frequency hearing in adult rats after perinatal exposure to Aroclor 1254. These findings led to other experiments to further elucidate the cochlear site of action using distortion product otoacoustic emissions.
(DPOAEs), which test the integrity of the OHCs, and auditory brainstem responses (ABRs), which measures the integrity of the auditory neuronal pathway. Exposure to Aroclor 1254 or to an environmentally relevant PCB mixture (Fox River PCB Mix) during development resulted in decreased DPOAE amplitudes that lasted into adulthood (Lasky et al., 2002) in the absence of any changes in ABR amplitudes or latencies, further suggesting that PCBs act at a cochlear site of action to produce auditory deficits (Powers et al., 2006; Powers et al., 2009).

Given this evidence that PCBs act at the site of the cochlea, namely at the OHCs, we investigated the interaction between PCB exposure during cochlear development and exposure to loud noise in adulthood (see chapter 4). OHCs can protect the inner ear from noise-inducing hearing loss (NIHL) through the stiffening of the stereocilia which protects against metabolic damage to the cochlea (Lim, 1986), thus we hypothesized that early PCB exposure might increase the likelihood of NIHL later in life. Developmentally PCB-exposed rats were subjected to 97dB of noise for 4 hours a day, for 5 consecutive days. Before each testing day, rats were habituated to the noise by gradually increasing the noise intensity from 75 to 97dB over a period of 15 min. During this initial 15 min of noise exposure on the first day of testing, many of the rats in the highest dose group (6 mg/kg PCB) exhibited bouts of wild running followed by a period when they were unresponsive to stimuli. This behavior appeared to be similar to behaviors that occur in the first stage of audiogenic seizures (AGS) (reviewed in Ross and Coleman, 2000).

Audiogenic seizures are seizures that are elicited by exposure to loud noise. They are generalized seizures which require the activation of the auditory brainstem
and are initiated mainly in the inferior colliculus (Coleman et al., 1999; Eells et al., 2004; Ishida et al., 1995; Faingold, 2002; Ross and Coleman, 2000). They usually consist of a set of defined stages (see Ross and Coleman, 2000). The first stage consists of a running fit, in which the animal runs wildly around its test space. The second stage can include a second running fit after a brief 3-4 second period of rest. Those that experience two running fits are usually considered to have experienced a less severe seizure than one running fit. The third stage consists of a generalized clonus state or sometimes a tonic-clonic state. Finally after the seizure ends, the animal goes into postictal recovery during which it is immobile and unresponsive for approximately 15 min. These seizures present differently from forebrain induced seizures which start with facial and forelimb tremors and can progress to a clonic or clonic-tonic state (Racine, 1972).

Some rodent species and strains are more susceptible to AGS than others. Specifically in rats, there exists the genetically prone epilepsy rat (GEPR), the Wistar Audiogenic rat (WAR), and Sprague-Dawley rats that are more susceptible to seizures (Ross and Coleman, 2000). Long-Evans rats, which were used in our studies, are reported to be less susceptible to seizures (Ross and Coleman, 1999). Rat strains that are less susceptible to AGS can be made more susceptible by priming them with damaging noise during the critical period of cochlear development. One hypothesis for this induced sensitivity to AGS is that priming to loud noise during cochlear development results in short-term hearing loss so that the rat will be hypersensitive to intense stimulation later in life (Ross and Coleman, 2000). Our Long-Evans rats were not primed with loud noise during cochlear development, but they were exposed to
PCBs during cochlear development which our findings (see chapter 4) suggest could have primed the rats to be more susceptible to audiogenic seizures in adulthood through damage to the OHCs.

In this study, our goal was to formally investigate whether developmental exposure to the Fox River PCB mixture would make rats more susceptible to AGS in adulthood. We used a classic AGS paradigm in which the rats were exposed to a brief (2 min) high intensity noise (Ross and Coleman, 1999) and the incidence of seizure behaviors was observed.

MATERIALS AND METHODS

Animals. Primiparous female Long-Evans rats, approximately 8-10 weeks of age, were purchased from Harlan (Indianapolis, IN) in three cohorts. They were individually housed in standard polycarbonate plastic shoebox cages with corn-cob bedding, and fed rat chow (Harlan Teklad rodent diet (W) 8604) and water *ad libitum*. All rats were housed in a temperature- and humidity-controlled room (22°C, 40–55% humidity), on a 12/12-hr light cycle (lights on at 0830 hr). The rats were maintained in facilities accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care. All procedures have been approved by the Institutional Animal Care and Use Committee at the University of Illinois at Urbana-Champaign and are in accordance with the guidelines of the National Institutes of Health (2002) and National Research Council (2003).
Exposure. The female rats were randomly assigned to exposure groups and given one of four treatments consisting of corn oil vehicle or PCBs in corn oil. Each exposure group was represented in each cohort. Exposure began 28 days prior to breeding and continued until weaning of the pups on postnatal day (PND) 21. The PCB mixture (Fox River PCB mixture) was formulated to mimic the congener profile found in walleye from the Fox River in northeast Wisconsin. The mixture consisted of 35% Aroclor 1242 (Monsanto lot KB 05-415; St. Louis, MO), 35% Aroclor 1248 (AccuStandards lot F-110; New Haven, CT), 15% Aroclor 1254 (Monsanto lot KB 05-612), and 15% Aroclor 1260 (AccuStandards lot 021-020) (Kostyniak et al., 2005). The doses of the PCB mixture (1, 3 and 6 mg/kg/day) were selected based on the results of earlier studies assessing the in vivo developmental toxicity and auditory toxicity of the mixture in rats (see Kostyniak et al. 2005; Powers et al. 2006). The PCBs diluted in corn oil (Mazola) or the corn oil vehicle alone were pipetted onto one-half of a vanilla wafer cookie (Keebler Golden Vanilla Wafers) at a volume of 0.4 mL/kg. To arrive at a dose of 0.4 mL/kg, the individual dosing solutions were mixed at concentrations of 2.25 mg/mL, 7.5 mg/mL and 15 mg/mL for the PCB doses of 1mg/kg, 3 mg/kg and 6 mg/kg. The PCB and vehicle treated cookies were fed to the female rats daily with the amount of dosing solution applied to the cookies adjusted daily to account for weight gain.

Breeding, pregnancy, and weaning. After the four weeks of PCB exposure, each female was paired with an unexposed male Long-Evans rat (Harlan, Indianapolis, IN) in a hanging wire cage for 8 consecutive days with food and water ad libitum. The females
were returned to their home cages each day for PCB dosing. The females were monitored for the presence of a sperm plug in order to establish gestational day 0. On the day of parturition (PND 0), the pups were examined for abnormalities, sexed and weighed. On PND 2, the litters were culled to 10 pups (five males and five females when possible), and litters with at least 7 pups had extra pups cross-fostered into them from the same treatment group to bring the litters to 8–10 pups. Cross-fostered pups were marked and not used for the experiment. There were 42 successful litters. Of the remaining dams, 9 were not pregnant and 9 had litters too small to be included in the study (≤7 pups). Overall the non-pregnant dams and dams with small litters were evenly distributed across the treatment groups.

Dosing continued until the pups were weaned on PND 21. One male and one female per litter were exposed to noise in a noise-induced hearing loss (NIHL) study beginning at approximately PND 200 (see chapter 4) and the AGS study began at approximately PND 400.

Some of the rats (mainly the control and 1 mg/kg PCB animals) used in this study had previously been subjected to noise (97db) 4 hours per day for 5 consecutive days, whereas others (some of the 3 mg/kg rats and most of the 6 mg/kg rats) were not exposed to the noise due to the seizure-like behaviors they displayed at the onset of noise exposure. Therefore, we also included a noise-naïve (NN) control group to control for that fact that previous noise exposure could potentially sensitize the rats to be more prone to audiogenic seizures. Eight Long-Evans females and eight Long-Evans males (Harlan, IN) aged 10-12 months were used as the NN control group.
**Total Thyroxine Radioimmunoassay.** Serum taken from littermates of the rats tested in this study at weaning (PND 21) was assayed for total t4 concentrations using a Total T4 Coat-A-Count Kit (Siemens) and counted in a gamma counter (Packard Cobra Autogamma II).

**Audiogenic Seizures.** Approximately 4-5 months after the rats completed testing for the NIHL study (see chapter 4) those from the second and third cohorts (one male and one female from each litter) were tested in the AGS study. For testing, each rat was placed individually in a cylindrical plexiglass tube (12 ft X 12 ft), lined on the bottom with sound insulating material (Soundsoak, Armstrong World Industries, Lancaster, PA). The chamber had a removable top lined with four equally spaced tweeter speakers (3” OEM cone tweeter, MCM Electronics, Dayton, OH). Using a sound pressure level meter (RadioShack SPL-meter 33-4050), the noise level was calibrated to 100dB, 105dB or 110dB when the chamber was closed. The tweeter speakers were connected to an amplifier (Techron 5507 power supply amplifier) which was connected to a music player (Sandisk Sansa Clip+ 4GB MP3 player) loaded with the octave band noise with a center frequency of 8 kHz and played on a loop. The noise was created using MATLAB software (The MathWorks, Inc.).

On the first AGS testing day, each rat was placed in the plexiglass chamber and exposed to 2 min of noise at 100dB and monitored for any audiogenic seizure behaviors. The stages of audiogenic seizures were observed as follows: 0-no abnormal behavior, 1-1st running fit, 2-2nd running fit, 3-clonus with full-body loss of posture, and 4- immobile postictal recovery (Ross and Coleman, 2000). A rat was removed from the
experiment and not tested further if it experienced clonus at any of the noise intensities tested. If a rat did not experience clonus at 100dB, then it was re-tested 24-48 hours later at 105dB. If the rat still did not experience clonus, it was re-tested 24-48 hours later at 110dB.

**Statistical analysis.** All statistical analyses were conducted using SPSS for MS Windows (version 20.0; IBM SPSS Statistics with Exact Tests Module) with statistical significance set at \(p< 0.05\). The incidence of audiogenic seizures was analyzed using a 5x2 Pearson Chi-Square (\(\chi^2\)) test, and comparisons of the incidence of seizures between the control group and each treated group were conducted using a Fischer’s Exact Test with two-sided exact p-values. The data for males and females were analyzed separately.

Circulating T4 concentrations measured at the day of weaning were analyzed via mixed ANOVA with treatment as a between-subjects factor, and litter as a unit of variance with sex nested within litter. Post hoc Tukey’s tests were conducted to examine treatment effects.

**RESULTS**

Data on reproductive/developmental endpoints have been reported elsewhere and thus are only briefly summarized here (see chapter 4). There were no signs of clinical toxicity in the dams from any of the treatment groups. Liver weights were increased and thymus weights were decreased in the PCB-exposed pups at weaning. The 3 and 6 mg/kg PCB-exposed groups also had slightly lower body weights with a 6-7.4% decrease in
the 3 mg/kg group and a 14-16% decrease in the 6 mg/kg group, on postnatal days 7, 14 and 21.

**Audiogenic Seizures.** The numbers of females used in the study were 9, 8, 7, 6, and 6 respectively for the control, NN control, 1, 3, and 6 mg/kg PCB groups. Analysis of the incidence of any seizure behaviors (running fits or running fits progressing into clonus) across all three noise intensities (100dB, 105dB, and 110dB) revealed that there was a significant main effect of treatment ($\chi^2=12.588$, df=4, p=0.01). The comparisons revealed that the 6 mg/kg PCB females had a significantly higher incidence of seizures than the control females ($\chi^2=5.402$, df=1, p<0.05) (Figure 5.1). Analysis of the incidence of seizures that progressed to clonus (severest form) across all three noise intensities also revealed a main effect of treatment ($\chi^2=24.236$, df=4, p<0.001). In this case comparisons revealed that both the 6 mg/kg PCB ($\chi^2=11.250$, df=1, p=0.002) and the 3 mg/kg PCB ($\chi^2=6.873$, df=1, p<0.05) females had a higher incidence of clonic seizures than controls (Figure 5.2). Analysis of the incidence of any seizure behaviors at the lowest noise intensity tested (100dB) was also conducted to determine the incidence of seizures at a noise intensity not typically associated with seizure activity in LE rats (Ross and Coleman, 2000). This analysis also revealed a main effect of treatment ($\chi^2=16.280$, df=4, p=0.002) with the 6 mg/kg PCB ($\chi^2=8.182$, df=1, p<0.05) and 3 mg/kg PCB ($\chi^2=6.873$, df=1, p<0.05) females showing significantly higher incidence of seizure behaviors than the controls (Figure 5.3).

The numbers of males used in this study were 9, 8, 8, 5, and 6 for the control, NN control, 1, 3 and 6 mg/kg PCB groups respectively. Unlike the females, statistical
analysis of the incidence of any seizure behavior across all three noise intensities, the incidence of clonus across all three noise intensities, and the incidence of any seizure behavior at the lowest noise intensity (100dB) did not reveal any significant difference between the treated and control males (Figures 5.4-5.6).

To test whether previous noise exposure may have sensitized control rats to be more prone to audiogenic seizures, a control group not exposed to any previous noise was included. Analysis of the incidence of any seizure behavior across all three noise intensities, the incidence of running fits progressing into clonus across all three noise intensities, and the incidence of any seizure behavior at the lowest noise intensity (100dB) also did not reveal any significant difference between the original controls and the NN controls (see Figures 5.1-5.6). However, visual inspection of the data did suggest that noise-exposed males were somewhat more likely to exhibit seizure activity than NN males. Since about half of the 6 mg/kg males were removed from the NIHL study after only a very brief (less than 15 min) relatively low intensity (less than 97dB) noise exposure, that group was also compared the NN control group (Figures 5.4-5.6), Fisher’s Exact Tests revealed that the 6 mg/kg PCB males had a higher incidence of seizures across all intensities (p<0.01), a higher incidence of clonus seizures (p<0.05), and a higher incidence of seizures at 100dB (p<0.01), relative to the NN controls.

**Thyroxine Concentrations.** Figure 5.7 shows serum total T4 levels measured at weaning (PNDS 21) in littermates of the rats used in this study. The number of litters was 11, 13, 11, and 7 for control, 1, 3, and 6 mg/kg PCB groups. There was a
significant effect of treatment \([F(3,38)=79.460, p<0.001]\). Tukey’s tests revealed that all treatment groups had significantly lower T4 levels than the control group \((p<0.001)\).

**DISCUSSION**

The typical rat models in audiogenic seizure studies have been genetically susceptible strains including the GEPR rat, and the WAR rat or Sprague-Dawley rats primed with loud noise during the critical period of cochlear development (Ross and Coleman, 2000). In this study we used LE rats, a strain that is considered to be relatively resistant to AGS (Ross and Coleman, 1999). However, our rats were exposed to PCBs during cochlear development. In a previous study designed to assess whether these developmentally PCB-exposed rats would be more susceptible to NIHL in adulthood, we found that a high percentage (53%) of rats in our highest PCB exposure group (6 mg/kg) compared to controls (0%) exhibited AGS-like behaviors shortly after exposure to moderate intensity noise (75-97 dB).

The current study used a systematic AGS testing paradigm to confirm that developmentally PCB-exposed rats are, in fact, more susceptible to AGS when exposed to loud noise in adulthood. There was an increased incidence of AGS, which was most pronounced in female rats. Males showed a similar trend that did not reach statistical significance, most likely due to a higher than expected incidence of AGS in the control males. Because the male controls had higher than expected incidence of seizures, perhaps as a result of their previous exposure to 97dB of noise for 4 hours a day for 5 consecutive days, we also compared the 6 mg/kg PCB males, half of which (3 out of 6)
were not tested in the NIHL study, to the noise-naive (NN) male controls, revealing a significant increase in seizure incidence.

There is a sex effect in susceptibility to AGS in the literature, with females showing a greater susceptibility to AGS, and statistically, our effects were clearer in the females than the males. Specifically for AGS, female GEPR-9 rats had a higher frequency of more severe seizures than males (Mishra et al., 1988). Another study also showed that female Sprague-Dawley rats that were ovariectomized and given testosterone exhibited decreased AGS responses, while castrated males given estrogen exhibited increased AGS responses (Werboff and Corcoran, 1961).

Thyroid hormone levels taken just after the end of the critical period of cochlear development were dramatically reduced in the PCB animals compared to controls. Previous studies have shown that developmental hypothyroidism can increase susceptibility to audiogenic seizures. In one study, it was concluded that rat pups treated with propylthiouracil (PTU) from PND 0-19 had higher incidences of AGS at 4 months of age (Kato et al., 1996). Rat dams fed low iodine diets throughout gestation and lactation had hypothyroid offspring that also had higher incidences of AGS (89%) (Van Middlesworth, 1977). Our PCB rats had lower levels of T4 during cochlear development and other literature has also shown that developmental exposure to PCBs causes hypothyroxinemia (lowered T4, without alterations in TSH) (Goldey et al., 1995; Morse et al., 1996; Poon et al., 2011). Therefore, this developmental hypothyroxinemia could explain why our PCB animals are more susceptible to seizures.

Another possible explanation for the increased susceptibility of the PCB-exposed rats to AGS comes from previous studies that have shown that PCB rats have an
imbalance between the excitatory and inhibitory systems in the brain and potentially a lower threshold for other types of generalized seizures (Kim and Pessah, 2011; Lein et al., 2010). Perinatal exposure to PCB 95 or Aroclor 1254 produced after-discharges (epileptic waveforms) in the evoked potentials of hippocampal slices when challenged with picrotoxin and (Kim and Pessah, 2011). Rats developmentally exposed to PCB 95 at 1 mg/kg/day had significantly shorter latencies to the first stage of myoclonus and the onset of tonic-clonic seizures when exposed to flurothyl, a convulsive drug, and also kindled seizures significantly faster when exposed to pentylenetetrazole (PTZ) (Lein et al., 2010). The investigators speculated that the lower seizure threshold in PCB rats was due to PCBs binding to the ryanodine receptor (RyR) which, in turn, released calcium from intracellular stores, altering neuronal excitability (Wong et al., 1997). Many believe that AGS is induced by deficits in the inhibitory/excitatory GABA and glutamate circuits in the inferior colliculus (Faingold, 2002; Ross and Coleman, 2000). Although PCBs have been shown in vitro to inhibit uptake of glutamate and GABA into rat brain synaptosomes (Mariussen and Fonnum, 2001), there is no direct evidence of imbalance between inhibitory and excitatory circuits in the inferior colliculus of PCB-exposed rats, but this would be a potentially fruitful area for further research.

AGS is usually seen in mammals in which the auditory system matures postnatally (Ross and Coleman, 1999). Genetically susceptible rodents with hearing loss, or rodents primed by sound exposure, sound deprivation, or kanamycin exposure (Pierson and Swann, 1988) during postnatal development show altered excitability levels in the auditory system such that the animals were more susceptible to AGS (Ross and Coleman, 1999). Although AGS does not present itself clinically in humans (Fisher,
1989), many of the same substrates that are involved in AGS are also involved in
generalized human seizures (Ross and Coleman 2000). Therefore, if PCBs are causing
a susceptibility to AGS in rodents, they may also lead to a greater sensitivity to other
types of generalized seizures, such as forebrain seizures that are relevant to humans.

In conclusion, we have shown that developmental exposure to PCBs during the
critical period for cochlear development caused adult rats to be more susceptible to
AGS. The effect was statistically more robust in females than males. Increased AGS
susceptibility was accompanied by significant decreases in T4 during cochlear
development. This is important because hypothyroidism during cochlear development
has been linked to an increase in AGS, suggesting a potential mechanism for the effect.
One published study suggests that developmental exposure to PCBs can also result in
a lower threshold for forebrain seizures (Lein et al., 2010). Together with our findings,
this suggests a generalized reduction in seizure threshold after early PCB exposure.
Future studies will be needed to fully assess whether this is the case, and, if so, how the
biological substrates of these seizures have been altered in the brains of PCB-rats. If
developmental PCB exposure is found to cause a generalized reduction in seizure
threshold this would have very important human health implications.

REFERENCES

alterations of the auditory brainstem response in audiogenic seizure-prone Long-Evans


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FIG. 5.1. Incidence of any seizure behavior (running fits or running fits progressing into clonus) across all three noise intensities in female rats. * indicates the 6 mg/kg group significantly different from control (p<0.05).

FIG. 5.2. Incidence of any clonus seizures across all three noise intensities in female rats. * indicates the 3 and 6 mg/kg groups significantly different from control (p<0.05).
FIG. 5.3. Incidence of any seizure behavior (running fits or running fits progressing into clonus) at 100dB in female rats. * indicates the 3 and 6 mg/kg groups significantly different from control (p<0.05).

FIG. 5.4. Incidence of any seizure behavior (running fits or running fits progressing into clonus) across all three noise intensities in male rats. * indicates the 6 mg/kg group significantly different from NN control (p<0.05).
FIG. 5.5. Incidence of only clonus seizures across all three noise intensities in male rats. * indicates the 6 mg/kg group significantly different from NN control (p<0.05).

FIG. 5.6. Incidence of any seizure behavior (running fits or running fits progressing into clonus) only at 100dB in male rats. * indicates the 6 mg/kg group significantly different from NN control (p<0.05).
FIG. 5.7. Pup serum T4 concentrations taken at weaning. * indicates a significant difference from control (p<0.05).
CHAPTER 6

GENERAL DISCUSSION

CONCLUSIONS

This series of studies has reconfirmed that developmental exposure to an environmentally relevant PCB mixture, the Fox River PCB mix, results in long lasting cochlear dysfunction. These studies have also gone a step further to show that developmental exposure to PCBs interacts with other environmental risks to worsen known effects.

In Chapter 3, we examined the effects of co-exposure to PCBs and PBDEs during development on auditory function later in life. Because PBDEs are structurally similar to PCBs and have been shown to have similar toxic effects, we hypothesized that co-exposure to the two chemicals would have additive effects on cochlear function. As we have reported previously, developmental exposure to 6 mg/kg PCBs resulted in decreased DPOAE amplitudes and elevated DPOAE thresholds across all frequencies tested. Developmental exposure to the molar equivalent dose of PBDEs did not produce any DPOAE deficits, suggesting that PBDES are not as potent as PCBs in their impact on auditory function. There was an interactive effect from combined exposure to PCBs and PBDEs such that the individual low doses of PCBs or PBDEs (3 mg/kg PCB or 5.7 mg/kg PBDE) did not result in DPOAE deficits, but the combination of the two produced a deficit that was similar in magnitude to that seen in the 6 mg/kg PCB group. This is consistent with studies that have shown that co-exposure to PCBs and PBDEs had
additive effects on synaptosomal DA concentrations (Dreiem et al., 2010), T4 levels (Miller et al., 2012), and the failure to habituate resulting in hyperactivity (Eriksson et al., 2006) when compared to either toxicant alone. Humans are exposed to a large number of environmental chemicals simultaneously, but the potential for additive effects due to chemicals with similar toxic effects has not been studied in detail. Therefore, our findings together with those from others highlight the need to address the potential for additive effects of PCBs and PBDEs in human populations.

Thyroid hormones are very important in the development of the cochlea and hypothyroidism during the developmental period can lead to permanent hearing deficits (Uziel, 1986). Previous rodent studies found that a 60% decrease in T4 concentrations during the critical period for cochlear development is needed to result in significant hearing deficits (Crofton, 2004; Crofton and Zoeller, 2005). While PCBs did reduce T4 concentrations more than 60% at weaning, the PBDEs were not as potent. This may account for the lack of DPOAE deficits in rats exposed to PBDEs alone.

In Chapter 4, we examined the interaction between PCB exposure during development and noise exposure in adulthood on NIHL. Because PCBs are thought to act at the level of the cochlea, mainly through a loss or loss of function of the OHCs (Crofton et al., 2000), and the OHCs are important in protecting the rest of the inner ear from damage due to loud noise (Lim, 1986), we hypothesized that PCB-exposed animals would be more susceptible to intense noise and exhibit more severe NIHL than control rats. Our study used an approach similar to other studies in which rats were co-exposed to industrial chemicals or JP-8 jet fuel, and intense noise and were found to exhibit either permanent cochlear dysfunction or larger effects on cochlear dysfunction.
than were seen with exposure to either the chemical or the noise alone (Fechter et al., 2007; Pouyatos et al., 2005).

As in our previous studies, baseline DPOAE amplitudes were decreased in the PCB-groups. The clearest reductions were in the 6 mg/kg PCB group, which could not be tested in the noise experiment. Rats in the control, 1 and 3 mg/kg PCB groups all showed the same amount of temporary hearing loss after exposure to noise for 4 hours a day for 5 consecutive days at 97dB, with a partial recovery 28 days after noise exposure. The PCB-exposed groups did not show any evidence of increased susceptibility to the noise. Unexpectedly, many of the rats in the 6 mg/kg PCB group showed characteristics of AGS (Ross and Coleman, 2000), when first exposed to noise and had to be excluded from the study.

In response to the discovery that developmental PCB exposure may increase the susceptibility to AGS, we designed a classical AGS experiment using the rats from the NIHL study (Chapter 5). Adult female rats developmentally exposed to 6 mg/kg PCBs had higher incidences of any AGS behavior (wild running or wild running progressing into clonus), while both the 3 and 6 mg/kg PCB groups had higher incidences of clonus seizures and higher incidences of seizures at the lowest noise intensity (100dB) compared to controls. The 6 mg/kg PCB males also had higher incidences of seizures, but only in comparison to noise-naïve (NN) controls, and not compared to their controls from the NIHL study.

Hypothyroidism during the critical period for cochlear development has been associated with an increase in the incidence of AGS (Kato et al., 1996; Van Middlesworth, 1977), and we speculate that the increase in susceptibility to AGS in
PCB-exposed animals could be related to the sharply reduced T4 concentrations that were present in these animals during early development. The exact mechanism through which lowered TH induces AGS is not fully understood. AGS is a brainstem seizure mediated mainly by the inferior colliculus (Eells et al., 2004; Faingold, 2002; Ishida et al., 1995; Kato et al., 1996), but can recruit forebrain structures as well (Simler et al., 1994). Interestingly, one study revealed that a deficiency in T4 during neurodevelopment caused about 20% of the glutamatergic neurons to migrate to abnormal locations within the primary somatosensory cortex (Auso et al., 2004). Other investigators have found evidence of excitatory/inhibitory imbalance in hippocampus or cortex after developmental PCB exposure (Kenet et al., 2007; Kim and Pessah, 2011). Together, these studies suggest a potential role for excitatory/inhibitory imbalance in both hypothyroid-induced and PCB-induced AGS. If PCB exposure can alter the underlying substrates involved in both AGS and other types of generalized seizures, then this could have important human health implications for seizure susceptibility.

**FUTURE RESEARCH DIRECTIONS**

We did not find any auditory deficits with PBDE doses that were equimolar to the PCB doses we have previously demonstrated to be ototoxic. However, given the prevalence of PBDEs in the environment, it will be important for future studies to test higher doses of PBDEs to see if they cause auditory deficits. Other environmental chemicals are also known to disrupt the thyroid system, such as bisphenol A (BPA), for example (Zoeller et al., 2005), and they too should be tested for their potential to cause auditory deficits and/or to cause additive effects when animals are co-exposed. This is
important for human health because very little is known about the potential for mixtures of chemicals that exist in the environment to have additive effects on auditory function.

Currently, we are examining if there are histological abnormalities in the cochlea of the rats developmentally exposed to PCBs that could play a role in both the auditory deficits and AGS we observed. Croften et al. (2000) has found that rats developmentally exposed to Aroclor 1254 showed a loss of OHCs in regions associated with the low frequency hearing loss they observed, with no other histological abnormalities to the rest of the organ of Corti. We would like to see if there are any structural abnormalities, such as a possible loss of OHCs, loss of ganglion cells, or an abnormal tectorial membrane in developmentally PCB-exposed animals.

Future directions to elucidate if PCBs can interact with noise to increase the susceptibility to NIHL could include lowering the noise intensity to around 85dB, as no animals had any AGS at this level, and increasing the noise exposure time to 8 hours. According to the NIOSH recommendations (1998), noise is potentially harmful starting at 85 dB over an 8-hour work day. Therefore that noise exposure procedure might be more relevant to humans.

One of the most surprising discoveries in this series of studies was that developmental PCB exposure markedly increases the susceptibility to AGS in adulthood. This was a striking effect and a new discovery that has not been published in the previous PCB literature. Mechanistically, it would be interesting to look at GABA and glutamate levels in the inferior colliculus of PCB exposed animals, as excitatory/inhibitory imbalances in that area of the brain have been shown to be important in the activation AGS (Eells et al., 2004; Faingold, 2002; Ishida et al., 1995;
Kato et al., 1996). Although GABA and glutamate have not been studied in the inferior colliculus after PCB exposure, a previous study suggests that synaptosomes from PCB-exposed rats have reduced reuptake of glutamate and GABA (Mariussen and Fonnum, 2001). It would also be interesting to examine if postnatal thyroid hormone replacement in PCB rats could attenuate AGS susceptibility, similar to a study where postnatal thyroxine replacement was found to attenuate the hearing deficits seen PCB rats (Goldey and Crofton, 1998).

Only one other lab has published on the ability of perinatal exposure to PCBs to affect seizure susceptibility. They found a single PCB congener, PCB 95, to lower the threshold for forebrain seizures (Lein et al., 2010). It would be interesting to replicate those experiments using our environmentally relevant PCB mix (FRM). This would address whether early PCB exposure causes a generalized reduction in the seizure threshold, vs. a specific effect on AGS threshold. Possible experiments would include measuring field excitatory post-synaptic potentials (fESPS) from FRM-exposed hippocampal slices to examine if they present epileptic waveforms, and conducting \textit{in vivo} experiments using PTZ to examine whether FRM-exposed rats kindle after fewer repeated doses, or to examine if FRM-exposed rats have lower thresholds for forebrain seizures. If PCB mixtures are also shown to increase the susceptibility to forebrain seizures, then this would have very important human health implications.

In summary, although it is important to study the effects of single toxicants or risk factors, it is equally important to examine the interactions of relevant risks that humans are exposed to in everyday life. Through this series of studies, developmental exposure to an environmentally relevant toxicant, PCBs, has been shown to interact with other
environmental chemicals and with loud noise to cause more detrimental outcomes than does exposure to PCBs alone. It is important to continue the study of interactions and co-exposures, as they may lead to important discoveries that will help us to gain a better understanding of human diseases.

REFERENCES


