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Auditory Deficits in Rats Exposed to an Environmental PCB Mixture during Development

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Previous studies have indicated that developmental exposure to polychlorinated biphenyls (PCBs) may result in hearing impairment in rats. The cochlea is the suggested site of action, based upon one study demonstrating a loss of outer hair cells on the basilar membrane, and another demonstrating deficits in distortion product otoacoustic emissions (DPOAEs). The current study was conducted to assess the possible ototoxic effects of a unique PCB mixture formulated to model the congener profile of PCBs found in fish consumed by a human population in northeastern Wisconsin. Female Long-Evans rats were dosed orally with the PCB mixture beginning 28 days prior to breeding and continuing until the pups were weaned. Dams were fed one-half of a cookie onto which was pipetted 0, 1, 3, or 6 mg/kg of the PCB mixture dissolved in a corn oil vehicle. On postnatal day (PND) 21, pups were weaned, and one male and one female from each litter were randomly selected for auditory assessment. DPOAEs were measured to assess cochlear function, and auditory brainstem responses (ABRs) were measured to determine effects on central nervous system auditory pathways. DPOAE amplitudes were decreased, and DPOAE and ABR thresholds were elevated across a range of frequencies in PCB-exposed rats. These results support and extend previous reports of auditory impairment in PCB-exposed rats. Developmental exposure to PCBs may also result in subtle auditory impairments in humans, and if so, this may contribute to some of the cognitive deficits that have been observed in epidemiological studies.

Key Words: **PCBs; auditory system; DPOAE; ABR.**

Polychlorinated biphenyls (PCBs) belong to a class of environmental contaminants known as halogenated aromatic hydrocarbons. Due to their chemical and physical stability, PCBs were effective for various industrial purposes. PCBs were used extensively as lubricants, flame retardants, and as dielectric fluids in transformers and capacitors before their production was banned in the 1970s. Although environmental levels of PCBs have been on the decline, they persist in the food chain and continue to accumulate in the adipose tissue of humans and wildlife (Dietz *et al.*, 2000; Fisk *et al.*, 2005). PCBs are capable of transport across the placenta and are present in breast milk (Jacobson *et al.*, 1984), thus posing a particular threat to developing organisms. PCB exposure has been shown to cause a wide spectrum of toxic responses in humans and in animal models (Safe, 1993). In particular, PCBs affect nervous system and endocrine function, with the thyroid hormone system being especially vulnerable to PCB exposure (Brouwer *et al.*, 1999; Goldey *et al.*, 1995; Morse *et al.*, 1996).

Laboratory studies using reflex modification audiometry have demonstrated that developmental exposure to PCBs can cause hearing impairments in rats. In one study, maternal exposure to a commercial PCB mixture, Aroclor 1254 (A1254), resulted in low-frequency hearing loss in adult offspring (Goldey *et al.*, 1995; Herr *et al.*, 1996). The study hypothesized that the hearing loss was due to the PCB-induced reduction in circulating thyroid hormone, which is necessary for normal development of the cochlea (Uziel, 1986). This idea was supported when it was shown that postnatal thyroxine replacement partially ameliorated the A1254-induced auditory deficit (Goldey and Crofton, 1998). Additional studies have also implicated the cochlea as a likely site of action for the PCB-induced hearing loss. Specifically, developmental exposure to A1254 has been shown to cause a loss of outer hair cells on the basilar membrane of the cochlea, with the affected area of the basilar membrane corresponding to the area responsible for low-frequency hearing (Crofton *et al.*, 2000). Furthermore, Lasky *et al.* (2002) have reported that developmental exposure of rats to A1254 impairs distortion product otoacoustic emissions (DPOAEs), which are acoustic responses generated by the cochlea when it is stimulated by two pure tones. DPOAEs are directly related to outer hair cell function, and thus damage to the outer hair cells results in a loss or decreased amplitude of the DPOAE (see Lonsbury-Martin and Martin, 1990, for review).

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More recently Meerts *et al.* (2004) assessed auditory brainstem response (ABR) peak latencies and thresholds across a range of tone frequencies in rats exposed to either A1254 or a hydroxylated PCB metabolite (4-OH-CB107). The 4-OH-CB107-exposed rats showed prolonged peak II latencies relative to control and A1254-exposed rats, suggesting possible damage to the central auditory pathway. There were no significant increases in ABR threshold relative to controls in either the A1254 or the 4-OH-CB107-exposed rats, although the threshold appeared to be increased somewhat in the A1254 rats at 1 kHz. This is in line with Crofton's early finding of a low-frequency hearing loss following A1254 exposure.

Auditory function has not been assessed in most epidemiological studies of developmental PCB exposure, but PCB exposure has been associated with a hearing deficit in the Faroe Islands study (Grandjean *et al.*, 2001). In this study, hearing thresholds were examined in children aged seven, who were exposed to PCBs during development. Exposure was associated with increased thresholds on an audiometry task, although only in the left ear at two of the eight evaluated frequencies. Similarly, Longnecker *et al.* (2004) have investigated the relationship between PCB levels in maternal serum during pregnancy and audiometrically determined hearing thresholds among 8-year-old offspring. While this study reported a slight increase in hearing threshold at 2 kHz in the left ear and 4 kHz in the right ear, it was concluded that background-level exposure to PCBs in humans was not associated with clinically significant hearing loss. Currently, there are no published studies using DPOAEs to assess hearing in PCB-exposed children, although this approach can easily be applied in humans.

Although considerable knowledge has been gained from the study of developmental exposure to commercial PCB mixtures and individual congeners, no commercial mixture or individual congener accurately models environmental exposure in humans. An important source of human exposure to PCBs is through the consumption of contaminated fish and seafood. The FRIENDS Children's Environmental Health Center is currently investigating the potential health risks associated with prenatal exposure to PCBs through maternal consumption of contaminated fish from the Fox River and other local bodies of water in northeastern Wisconsin. Laboratory animal studies—including those described in this report—are being used to guide the selection of appropriate neurodevelopmental outcome measures for use in the epidemiological research. An important goal of these animal studies has been to model human exposure to PCBs as closely as possible. To accomplish this, PCB congener data from walleye caught in the Fox River were obtained from the Wisconsin Department of Natural Resources (WDNR), and differing percentages of four commercial PCB mixtures (Aroclors 1242, 1248, 1254, and 1260) were combined to achieve a PCB congener profile that resembled the congener pattern in Fox River walleye as closely as possible (Kostyniak *et al.*, 2005).

The purpose of the current experiment was to assess the effects of the Fox River PCB mixture on auditory function in developmentally exposed rats. Based on the effects of PCBs on the auditory system that have been previously reported, two different methods were used to assess auditory function. Adult rats, exposed to the Fox River PCB mixture during development, were assessed for outer hair cell function by the measurement of DPOAEs. Secondly, auditory brainstem responses were measured to determine if the central processing of auditory information was affected.

MATERIALS AND METHODS

Animals. Primiparous female Long-Evans rats, approximately 60 days of age, were purchased from Harlan (Madison, WI) in two cohorts spaced approximately 6 months apart. The rats were housed in an AAALAC-approved facility, and all procedures were performed in accordance with protocols approved by the Institutional Animal Care and Use Committee at the University of Illinois at Urbana-Champaign. The rats were individually housed in standard plastic cages with corncob bedding, in a temperature- and humidity-controlled room (22°C, 40–55% humidity) that was maintained on a 12-h reverse light-dark cycle (lights off at 0900 h). Food and tap water were available *ad libitum.*

Exposure. After a 2-week adaptation period, the rats were assigned to exposure groups (distributed evenly by weight) and began receiving daily PCB doses of 0, 1, 3, or 6 mg/kg body weight. Exposure began 28 days prior to mating and continued until pups were weaned on postnatal day (PND) 21. The PCB mixture, formulated to mimic the congener profile that is observed in walleye taken from the Fox River in northeast Wisconsin, consisted of: 35% Aroclor 1242, 35% Aroclor 1248, 15% Aroclor 1254, and 15% Aroclor 1260 (Kostyniak *et al.*, 2005). Since the mixture contains a large percentage of A1242 and A1248, it has a greater preponderance of lightly chlorinated PCB congeners than A1254. More information about the congener profile and toxicity of the mixture relative to A1254 is provided in Kostyniak *et al.* (2005). The PCB mixture was 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. The PCB-contaminated cookies were fed to the female rats daily at approximately 11:00 A.M. Doses were adjusted daily to account for weight gain.

Breeding. Four weeks after the initiation of PCB exposure, each female was paired with an unexposed male Long-Evans rat (Harlan, Madison, WI) in a hanging wire cage for eight consecutive days. The females were returned to their home cages for approximately 2 h each day for dosing. Consumption of the cookie was confirmed before they were returned to the breeding cages. The females were monitored twice daily for the presence of a sperm plug (i.e., gestational day, or GD 0). All of the rats were paired for eight consecutive days regardless of whether a sperm plug was observed. Females that did not give birth were retained, and their uteri were examined for implantation sites.

On the day of parturition (PND 0), the pups were examined for gross abnormalities, the number of stillbirths was noted, and all of the pups were sexed and weighed. On PND 2, the litters were culled to 10 pups (5 males and 5 females when possible), cross-fostering with extra pups from the same treatment group as needed to assure all litters had 8–10 pups. Cross-fostered pups were marked by ear clip and not used as subjects. Between PND 7 and PND 70, the pups were weighed weekly to monitor for signs of overt toxicity. There were 41 successful litters (control = 11, 1 mg/kg = 11, 3 mg/kg = 10, 6 mg/kg = 9). The remaining females were either not pregnant or gave birth to a litter that was not large enough for inclusion in the study.

Dosing of the dams continued until the pups were weaned on PND 21. One male and one female from each litter were randomly selected to serve as subjects for this study. The pups were housed in same-exposure, same-sex pairs or triplets. Beginning at approximately 100 days of age, food access was

restricted to reduce body weights to about 85% of free-feeding weight. Food was restricted so that food rewards could be used as motivation to learn a series of behavioral tasks (not described). Food restriction has been used routinely in studying the behavioral effects of PCB exposure, and there is no evidence that it confounds PCB-mediated effects. DPOAE and ABR testing began at approximately 200 days of age.

DPOAEs. DPOAE testing was conducted in a sound-attenuated plywood chamber within an isolated laboratory room. All of the interior surfaces of the sound attenuated chamber were lined with 2-in.-thick sound-absorbing foam. Prior to testing, the rats were sedated with 0.5 ml/kg ketamine/xylazine (87:13) ip. Once sedated, the rats were placed on a thermo-regulating heating pad (#50–7053-R, Harvard, Fishers, IN) in which a rectal thermometer regulated the temperature of the heating pad to maintain the body temperature of the rat throughout testing. All of the rats were placed on their right sides (left ear up), and the ear probe was positioned in the left ear canal.

The DPOAEs were recorded using Tucker Davis Technologies (TDT, Alachua, FL) System 2/System 3 digital signal processing hardware and software. The instrumentation used to present the DPOAE stimuli consisted of a digital/analog converter (TDT # DA-1) that was synchronized using a timing generator (TDT #TG-6), two programmable attenuators (TDT # PA5) that were used to control the stimulus levels, and a single ear probe unit to direct the stimuli into the ear canal. The ear probe contained two Etymotic ER-2 insert earphones and an Etymotic ER-10B ear canal microphone and had a soft rubber tip that served to seal the ear canal from external noises. Recording of the responses in the sealed ear canal was accomplished with an Etymotic ER-10B ear canal microphone, a preamplifier (Etymotic ER-10B amplifier) that provided 40 dB of gain, and a microphone amplifier (TDT # MA-2) that provided an additional 45 dB of gain to the DPOAE responses prior to digital conversion (TDT # AD-1). The sampling rates to generate stimuli and digitize the responses were 50 kHz. All DPOAE stimuli were created using TDT SigGen software, and ear canal recordings were conducted using TDT BioSig software using an Intel microprocessor-based computer.

The DPOAEs were generated by simultaneously presenting two sinusoids differing in frequency (the lower frequency labeled f_1 and the higher frequency f_2) into the sealed ear canal of the rat. 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). Seven stimulus pairs were selected for DPOAE testing, which included $f_2 = 1.0$, 2.0, 3.0, 4.0, 6.0, 8.0, and 12.0 kHz ($f_2/f_1 = 1.2$). The sound levels for the f_1 and f_2 primaries were calibrated to 60 dB Sound Pressure Level (SPL) and 50 dB SPL, respectively, using a pressure field microphone (#4192, Bruel & Kjaer, Norcross, GA) in a 2-cc calibration coupler (#4946, Bruel & Kjaer). Figure 1 provides an example of recorded output showing the f_1 and f_2 primaries along with the $2f_1–f_2$ distortion product.

First, a series of test frequencies were measured to ensure that the probe was inserted correctly and probe placement was consistent across rats. DPOAE testing consisted of measuring suprathreshold DPOAE amplitudes followed by DPOAE thresholds at each of the seven stimulus pairs, beginning with $f_2 = 1.0$. Each distortion product was the average response of 100 stimulus pair presentations (presentation rate = 6 stimulus presentations/s). This cycle of 100 stimulus presentations per distortion product was repeated so that the final distortion product represented an average of the two trials (each having 100 separate stimulus presentations). Suprathreshold amplitudes were defined as the signal:noise ratio of the $2f_1–f_2$ distortion product for the 60 dB and 50 dB SPL f_1 and f_2 primaries, respectively, and were calculated by subtracting the $2f_1–f_2$ distortion product (i.e., ''signal'') from the surrounding noise. The noise was defined as the average of the 10 neighboring frequencies (5 above and 5 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 and were defined as the lowest f_2 dB level at which the $2f_1–f_2$ distortion product was more than 6 dB above the surrounding noise.

ABRs. ABR testing was conducted in the same chamber, and rats were prepared for the procedure as they were for DPOAE testing. ABRs were recorded using the same TDT hardware. The ABR stimuli were presented using a programmable attenuator (TDT# PA-5) and an electrostatic speaker (TDT# EC-1) driven by an electrostatic speaker driver (TDT# ED-1). The EC-1 speaker presented the ABR stimuli through a 10-cm tube, the end of which was fitted with a rubber tip that allowed for the ABR stimuli to be presented into the sealed ear canal. The ABR responses were recorded using needle electrodes (FE-2, Grass, West Warwick, RI) connected to a headstage (TDT# HS-4) and bioamp controller (TDT# DB-4) that amplified the analog voltage recordings 60,000 times and band pass filtered them with 3 dB cutoffs at 100 Hz and 3 kHz before being digitally converted (TDT# AD-1). The sampling rates to generate stimuli and digitize the responses were 125 kHz and 20 kHz, respectively.

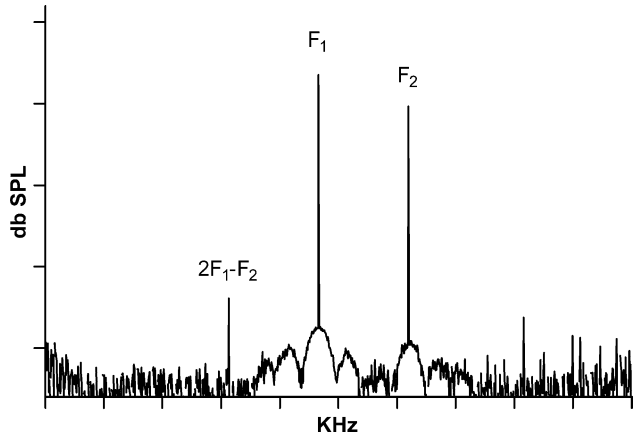

FIG. 1. An example of a DPOAE recording from a rat. The primary tones (f_1 and f_2) are presented simultaneously into the sealed ear canal. The primary tones are always presented at a fixed ratio ($f_2 / f_1 = 1.2$). The distortion product at the $2f_1–f_2$ frequency is commonly measured as a reliable indicator of outer hair cell function.

The ABR stimulus was a 65 dB SPL sinusoid presented at a rate of 21.8/s, with 1/3 rise, 1/3 plateau, and 1/3 fall times. The frequencies selected for testing included 0.75, 1.5, 3.0, 6.0, 12.0, 24.0, and 48.0 kHz and were presented sequentially beginning with the lowest frequency. The stimulus duration was set at 6 ms for the 0.75 kHz, 3 ms for the 1.5 kHz, and 1.5 ms for the frequencies between 3.0 and 48.0 kHz to minimize the differences in energy (i.e., number of cycles per stimulus presentation) between the stimuli. The ABR waveforms were produced via differential voltage recordings from needle electrodes placed under the skin on the scalp at the vertex (noninverting electrode) and ipsilateral mastoid (inverting electrode). The ground electrode was placed on the back of the neck. Each ABR waveform represented the average response to 500 stimulus presentations, and the final analyzable ABR waveform was determined by averaging the waveforms from two consecutive ABR trials (each the product of 500 stimulus presentations). Figure 2 shows a representative ABR waveform, indicating peaks 1–4. Latency and amplitude (peak to following trough) of each of the first four positive peaks of the response to the 65 dB SPL stimuli (suprathreshold) were measured. ABR thresholds were then determined by reducing the stimulus level in 5 dB steps, and were defined as the lowest dB level at which peak 2 could be positively identified.

Statistical analysis. Data were analyzed via repeated measures analysis of variance (ANOVA) using SPSS 9.0 for MS Windows. The litter was the unit of analysis in all analyses, and sex was nested within litter. Cohort was analyzed as a between-litter variable. DPOAE signal-to-noise ratios at the highest stimulus intensity and DPOAE thresholds were analyzed via separate four-way ANOVAs [exposure (4) × cohort (2) × sex (2) × frequency (6)]. DPOAE signals were not consistently observed at $f_2 = 1$ kHz, and therefore, this frequency was omitted from the analysis. ABR peak amplitudes and peak latencies were analyzed via separate five-way ANOVAs [exposure (4) × cohort (2) × sex (2) × frequency (5) × peak (4)], and ABR threshold was also analyzed by four-way ANOVA

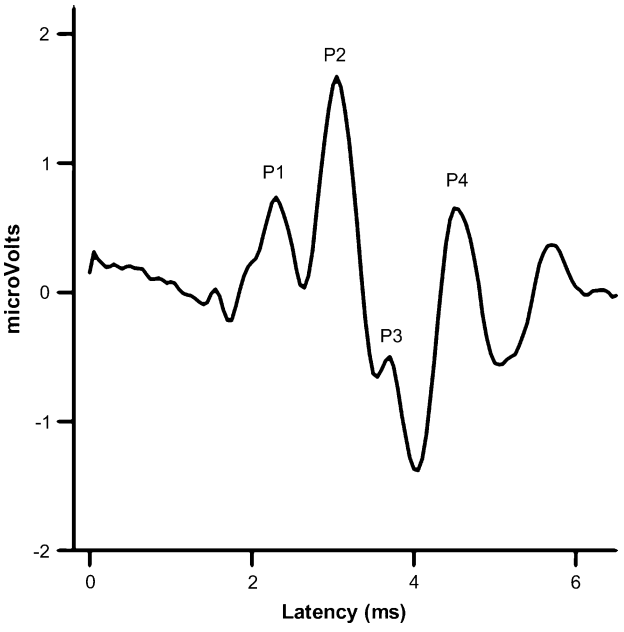

FIG. 2. An example of an auditory brainstem response (ABR) waveform. The first four positive peaks are labeled according to the nomenclature adopted by Herr *et al.* (1996).

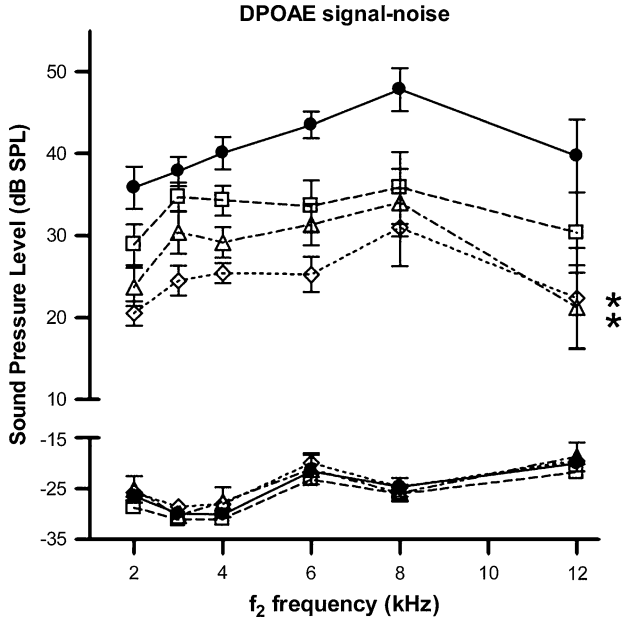

FIG. 3. Group mean DPOAE amplitudes and noise levels (in dB SPL) presented as a function of f_2 frequency for rats in the control group (filled circles), 1-mg/kg group (open squares), 3-mg/kg group (open triangles), and 6-mg/kg group (open diamonds). The data are plotted as mean ± SEM. *The main effect across all frequencies is significantly different from controls ($p < 0.05$).

[exposure (4) × cohort (2) × sex (2) × frequency (6)]. ABRs were sparsely observed at 0.75 kHz, and therefore, this frequency was omitted from the analysis. The ABR waveforms generated by the 1.5-kHz stimuli were irregular in appearance, in that peak 2 was identifiable while peaks 1, 3, and 4 were not. Therefore, the 1.5-kHz data was used in the analysis for ABR thresholds, but not ABR amplitudes and latencies. Significant effects were analyzed further by post hoc multiple comparison tests using the Dunnett's two-sided test (the controls were the comparison group). Significance was ascribed at $p < 0.05$.

RESULTS

Reproductive and Developmental Outcomes

There were no overt signs of clinical toxicity in the dams from any of the treatment groups. Gestational weight gains, liver weights, litter sizes, percent live births and percent male pups were similar to the controls (Kostyniak *et al.*, 2005). Pups in all three exposure groups were smaller than controls at birth, with pups in the highest exposure group (6 mg/kg/day) weighing about 10% less than controls. Pups in all three exposure groups remained smaller than controls at weaning. Again, the largest body weight deficit was in the 6 mg/kg/day group—approximately 19% less than controls. A regression analysis showed that the body weight decrease was linearly related to dose. Liver-to-body-weight ratios were significantly increased in pups from all three exposure groups, likely reflecting liver enzyme induction, but brain-to-body-weight and thymus-to-body-weight ratios were not altered.

DPOAEs

Some rats were lost under sedation, and others had incomplete data sets due to waking during the procedure or external noise during the procedure. As a result, the numbers of litters used in the analysis of DPOAEs were 9, 11, 8, and 7 for the control, 1 mg/kg, 3 mg/kg, and 6 mg/kg groups, respectively. Figure 3 illustrates DPOAE amplitudes as a function of f_2 frequency for control and PCB-exposed rats. The DPOAE amplitudes of control rats were 10–20 dB larger than those of PCB-exposed rats across all of the frequencies that produced reliable distortion products ($f_2 = 2, 3, 4, 6, 8$, and 12 kHz). This is confirmed by a significant main effect of exposure [$F(3, 27) = 7.053$; $p = 0.001$]. There was also a significant effect of frequency [$F(5, 139) = 12.387$; $p < 0.001$]. There was no observed effect of sex, nor were there significant sex × exposure, sex × frequency, or frequency × exposure interactions. There was no cohort effect. Post hoc analysis revealed significant differences between the control group and the 3-mg/kg group ($p = 0.006$) and 6-mg/kg group ($p = 0.001$).

Figure 4 depicts the DPOAE thresholds at each frequency for the control and PCB-exposed rats. DPOAE thresholds for PCB-exposed rats were elevated approximately 10–15 dB in comparison to control rats. Statistical analysis revealed a significant main effect of exposure [$F(3, 27) = 11.255$; $p < 0.001$]. There was also a significant effect of frequency [$F(5, 139) = 89.653$; $p < 0.001$]. There was no observed effect of sex, nor were there significant sex × exposure, sex × frequency, or frequency × exposure interactions. There was no cohort effect. Post hoc analysis indicated no difference between the control group and the 1-mg/kg group, but did confirm significant differences

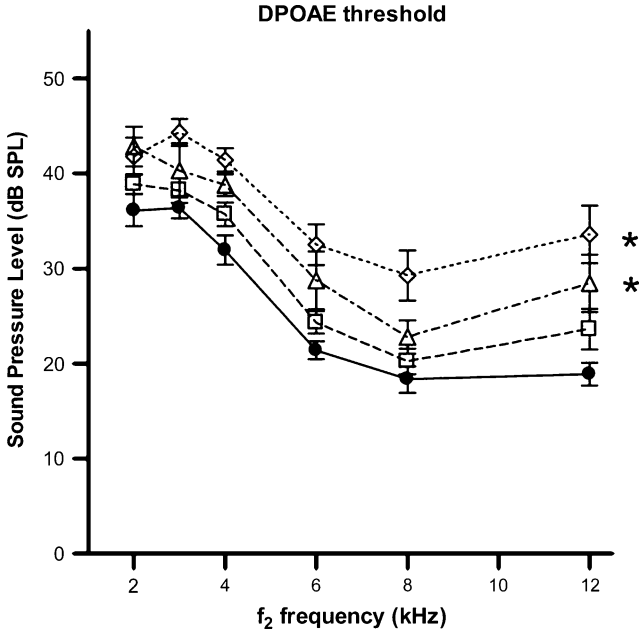

FIG. 4. Group mean DPOAE thresholds (in dB SPL) presented as a function of f_2 frequency for rats in the control group (filled circles), 1-mg/kg group (open squares), 3-mg/kg group (open triangles), and 6-mg/kg group (open diamonds). The data are plotted as mean ± SEM. *The main effect across all frequencies is significantly different from controls ($p < 0.05$).

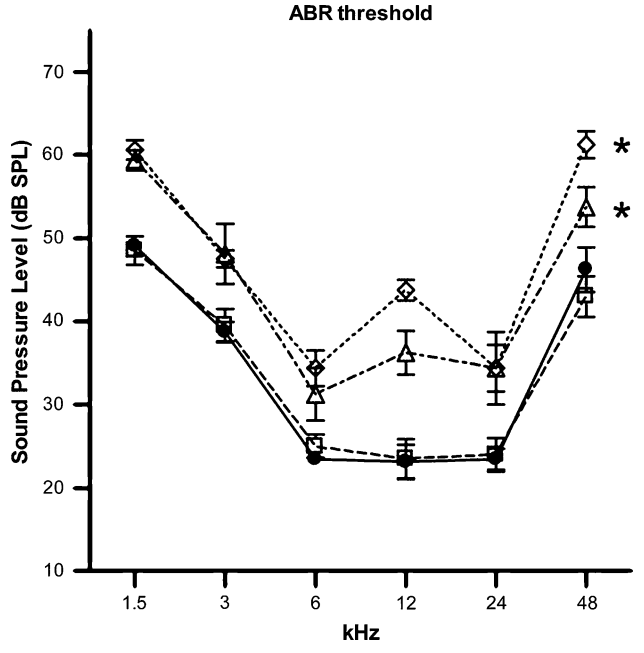

FIG. 5. Group mean ABR thresholds (in dB SPL) for rats in the control group (filled circles), 1-mg/kg group (open squares), 3-mg/kg group (open triangles), and 6-mg/kg group (open diamonds). The data are plotted as mean ± SEM. *The main effect across all frequencies is significantly different from controls ($p < 0.05$).

between the control group and the 3-mg/kg group ($p = 0.003$) and the 6-mg/kg group ($p < 0.001$).

ABRs

Again, some rats were lost under sedation, and others had incomplete data sets due to waking during the procedure or external noise during the procedure. As a result, the numbers of litters used in the analysis of ABRs were 8, 4, 4, and 4 for the control, 1 mg/kg, 3 mg/kg, and 6 mg/kg groups, respectively. Figure 5 shows the ABR thresholds at each frequency that produced a reliable ABR waveform (1.5, 3, 6, 12, 24, and 48 kHz) for control and PCB-exposed rats. Compared to control rats, PCB-exposed rats from the 3- and 6-mg/kg groups demonstrated elevated ABR thresholds (approximately 10–20 dB). This effect was confirmed by a significant main effect of exposure [$F(3, 12) = 16.572$; $p < 0.001$]. There was also a significant effect of frequency [$F(5, 60) = 141.555$; $p < 0.001$] and a significant effect of sex [$F(1, 12) = 6.167$; $p = 0.029$], which resulted from the female rats having lower ABR thresholds than the male rats. There were no significant sex × exposure, sex × frequency, or frequency × exposure interactions. There was no cohort effect. Post hoc analysis indicated no difference between the control group and the 1-mg/kg group, but significant differences from the control group were observed for both the 3-mg/kg group ($p = 0.002$) and the 6-mg/kg group ($p < 0.001$).

Statistical analysis of ABR peak amplitudes revealed no significant main effect of exposure [$F(3, 12) = 2.292$; $p = 0.126$] (data not shown). There was a significant effect of frequency [$F(4, 51) = 121.680$; $p < 0.001$], and a significant effect was observed for sex [$F(1, 12) = 6.693$; $p = 0.023$], due to higher peak amplitudes in female rats. There were no significant exposure × peak, sex × exposure, sex × frequency, or frequency × exposure interactions. There was no cohort effect.

Statistical analysis of ABR peak latencies revealed no significant main effect of exposure [$F(3, 12) = 1.781$; $p = 0.200$] (data not shown). There was a significant effect of frequency [$F(4, 51) = 297.035$; $p < 0.001$]. There was no observed effect of sex, nor were there any significant exposure × peak, sex × exposure, sex × frequency, or frequency × exposure interactions. There was no effect of cohort.

DISCUSSION

This study indicates that developmental exposure to PCBs can result in long-lasting deficits on various measures of auditory function. DPOAE amplitudes were reduced, and DPOAE thresholds were elevated in adult rats exposed to PCBs during development. ABR thresholds were also elevated as a result of PCB exposure. While many of the results are consistent with previous reports, there are some notable differences.

Robust effects were observed on DPOAE amplitude and threshold in PCB-exposed rats, and the effects appear to be

more pronounced with increasing doses. These results are in agreement with previous studies showing hearing deficits that result from developmental exposure to PCBs. However, the current study indicates that deficits extend into the mid- and high-frequency range. DPOAE deficits were observed at all frequencies examined (2–12 kHz). Developmental exposure to A1254 in rats has been shown to result in low-frequency hearing loss (Crofton *et al.*, 2000; Goldey *et al.*, 1995; Herr *et al.*, 1996). Crofton *et al.* (2000) complemented these findings by reporting a loss of outer hair cells in the upper (i.e., apical) turns of the cochlea, where the cochlea is responsive to low-frequency sounds. Later work demonstrates that A1254 exposure reduces DPOAE amplitude and increases DPOAE threshold, with the most pronounced effects at the lowest frequencies examined (Lasky *et al.*, 2002). These studies, along with the current data, implicate the outer hair cells of the cochlea as a site of action for developmental exposure to PCBs.

A strong effect of PCB exposure on ABR threshold was also observed. Across all measurable frequencies (1.5–48 kHz), the ABR thresholds were elevated in rats exposed to the 3- and 6-mg/kg/day doses of the Fox River PCB mixture. Lasky *et al.* (2002) did not find any PCB-exposure related effects on ABRs. Those ABRs, however, were generated using a broadband-click stimulus as opposed to the pure-tone stimuli used in the current study. Herr *et al.* (1996) did report ABR deficits following developmental exposure to A1254, including a decreased peak 1 amplitude at 1 kHz. The results of the current study suggest that ABR thresholds are sensitive measures of PCB-induced deficits, and that ABR deficits extend to higher frequencies than previously reported. These findings are consistent with a dysfunction of the cochlea and/or the auditory nerve.

The major discrepancy with previous reports of PCB-induced hearing deficits is the observation that deficits extend beyond the low-frequency range. It is possible that this can be attributed to a difference in the ototoxicity of the PCB mixtures. The Fox River PCB mixture used in this study includes many PCB congeners that are not present in A1254, and thus the mixtures may have different biologic activity. The discrepant results may also be explained by differences in PCB body burdens in the rat pups. Although previous studies have used the same maternal dose (6 mg/kg) that was used in the current study, the doses differ on a molar basis. The molecular weight of A1254 is ~326, while the molecular weight of the Fox River PCB mixture is ~296 (Hansen, 1999). However, more importantly, the dosing regimen was much longer in the current study in comparison to the previous reports of PCB-induced hearing deficits, with dosing beginning 28 days prior to breeding as opposed to beginning on GD 6. This greater length of exposure may have resulted in much higher PCB body burdens in the rat pups, and this may have contributed to the greater extent of hearing deficits. Lastly, the discrepancy may be due to the method of measuring hearing. It is possible that DPOAEs are more sensitive to the detection of high-frequency deficits than are other testing methods. This hypothesis gains support from a comparison between Goldey *et al.* (1995) and Lasky *et al.* (2002). Both studies used the same dosing regimen of 6 mg/kg A1254 from GD 6 to PND 21, but the method of hearing assessment and the results differed. The former study measured auditory threshold via reflex modification audiometry and observed PCB-induced deficits only at 1 kHz. The latter study measured DPOAEs, and although auditory deficits were most pronounced at low frequencies (2.1–3.2 kHz), deficits were also observed at higher frequencies (3.7–8.6 kHz). Widholm *et al.* (2003) investigated the auditory function of rats exposed to A1254, but in this study maternal exposure began 28 days prior to breeding and continued until PND 16 (similar to the current study). PCB-induced deficits in DPOAE amplitude were observed at 2, 3, 4, and 6 kHz. Furthermore, deficits in DPOAE threshold were observed at as high as 12 kHz. While these results lend support to the idea that longer exposure times resulted in the deficits at higher frequencies, when they are taken together with the data from Lasky *et al.* (2002) it seems likely that the sensitivity of the DPOAE test is also a factor.

The auditory deficits observed in this study may be due in part to PCB-induced disruption of thyroid hormone levels during development. Previous reports of hearing deficits induced by maternal exposure to A1254 have been hypothesized to result from postnatal hypothyroxenemia due to postnatal lactational transfer of PCBs (Goldey *et al.*, 1995), and this has been supported by the finding that thyroxine replacement in the offspring during the lactational period partially ameliorated the hypothyroxenemia and the hearing loss (Goldey and Crofton, 1998). In the rat, there is a critical time period between the onset of fetal thyroid gland function (GD 17–18) and the onset of hearing (PND 12), during which any delay in the rise of thyroid hormone plasma levels leads to permanent hearing defects (Knipper *et al.*, 2000). Interestingly, these authors report that a delay in the normal increase of thyroid hormone during this critical period, induced by treating lactating mothers with methimazole, leads to impaired DPOAE measurements and prolonged ABR peak latencies.

Another possible mechanism for PCB-induced hearing deficits is an alteration of intracellular Ca^{2+} signaling via ryanodine receptor–activated Ca^{2+} stores. A series of experiments demonstrated that PCBs can act on ryanodine receptors by stabilizing a high-affinity binding conformation, resulting in an increased intracellular Ca^{2+} concentration (Wong *et al.*, 1997). Further experiments have demonstrated that the immunophilin FKBP12, an accessory protein associated with the ryanodine receptor, is essential for the PCB-mediated effect on ryanodine receptor activity (Wong and Pessah, 1997), and Zajic *et al.* (2001) have reported that the FKBP12 protein is abundant throughout the rat cochlea, including the outer hair cells. An increase in intracellular Ca^{2+} concentration, through the activation of the ryanodine receptor, may regulate the motion of the outer hair cells (Dallos *et al.*, 1997). Experiments performed by Bobbin (2002) investigated how the release of Ca^{2+} from ryanodine receptor stores in the organ of Corti can affect

cochlear function and found that the administration of caffeine or ryanodine (drugs that act on the ryanodine receptor and induce intracellular Ca^{2+} release) into the perilymph compartment of the guinea pig cochlea has effects on cochlear function. Caffeine and ryanodine reversibly suppressed DPOAEs evoked by low-intensity primaries, suppressed the compound action potential from the auditory nerve, and increased N1 ABR latency at low stimulus intensities. These effects are similar to those observed in the current study and suggest that the PCB-induced auditory deficits may be at least partially explained by PCB-mediated activation of ryanodine receptor channels. Kostyniak *et al.* (2005) report the ryanodine receptor activity of the Fox River PCB mixture and each of the four Aroclor mixtures used to formulate it. All were found to be active, but the EC50 of the Fox River PCB mixture (1.8 μM) was lower than that of A1254 (4.2 μM).

Although experiments utilizing animal models indicate that the auditory system is a target of PCB exposure, relatively few epidemiological studies have investigated the association between PCB exposure and hearing impairment. Grandjean *et al.* (2001) report modest effects of PCB exposure on audiometrically determined hearing thresholds in children. However, Longnecker *et al.* (2004) report no association between background-level maternal PCB exposure and hearing thresholds in children. While PCB exposure levels in this study were higher than current background levels, they were about threefold lower than those reported by Grandjean *et al.* (2001). Other studies of human subjects have utilized auditory event-related potentials (P300) as a means to investigate PCB-induced deficits. A study of 7- to 12-year-old Yu-Cheng children, whose mothers were exposed to PCBs through chronic ingestion of contaminated rice-bran cooking oil, found that P300 amplitudes were reduced and P300 latencies were prolonged in comparison to control subjects (Chen and Hsu, 1994). Another study, evaluating 9-year-old Dutch children, found that children with high prenatal exposure to PCBs exhibited prolonged P300 latencies in comparison to children with low prenatal PCB exposure (Vreugdenhil *et al.*, 2004). These recent results, along with results from animal models of PCB exposure, highlight the need for further study of the effects of PCB exposure on auditory function in humans.

It is important to note that the methods used in the current study have not been used to assess the effects of PCB exposure on hearing in humans. DPOAEs and ABRs can be measured in newborn humans, and in contrast to traditional audiometry, these methods may be able to more reliably detect subtle hearing deficits. There is, however, an obvious difference between humans and rats in that the cochlea develops prenatally in humans. Although prenatal exposure via placental transfer is relatively low in comparison to lactational transfer, prenatal exposure has been more reliably associated with the subtle cognitive deficits in humans. Thus, prenatal PCB exposure may also be capable of inducing subtle hearing deficits in humans. It is possible that hearing deficits, particularly if they are present in the speech sound range, may be related to some of the cognitive impairments that have been associated with early PCB exposure.

The results of this study have determined that DPOAEs and ABRs are sensitive measures of PCB-induced auditory deficits, and thus these methods will be utilized in the epidemiological study conducted by the FRIENDS Children's Environmental Health Center. Newborn infants in the study will not only be tested for DPOAEs and ABRs, but will also be observed for early speech perception and language development. Future work using the rat model will focus on delineating the mechanisms for these effects. It is hypothesized that the auditory deficits described here are due to PCB-induced impairment of the cochlea. The impairment may result from thyroid hormone depletion during development of the cochlea, or a disregulation of ryanodine receptor activated Ca^{2+} stores in outer hair cells, or perhaps both mechanisms contribute to the effect. The suspected damage to the cochlea will be quantified by counting hair cells on surface preparations of the organ of Corti. Experiments will be conducted to test whether the ototoxic mechanism is thyroid hormone mediated or ryanodine receptor mediated. These planned experiments will utilize specific PCB congeners known to have opposing effects on thyroid hormone levels and ryanodine receptor activation. Additionally, investigations of the effects of methylmercury exposure on auditory function, as well as whether coexposure to PCBs and methylmercury exacerbates the effects observed in the current study, are forthcoming.

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