Published in final edited form as:

Neurotoxicol Teratol. 2014 ; 46: 68–76. doi:10.1016/j.ntt.2014.10.003.

Associations between Prenatal and Recent Postnatal Methylmercury Exposure and Auditory Function at age 19 years in the Seychelles Child Development Study

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Abstract

Objectives—To determine if prenatal or recent postnatal methylmercury (MeHg) exposure from consuming ocean fish and seafood is associated with auditory deficits in young adults. Some investigators have reported adverse associations while others have found no associations. Ocean fish is an important nutrient source for billions of people around the world. Consequently, determining if there is an adverse association with objective auditory measures is important in assessing whether a risk is present or not.

Design—The peripheral and central auditory function of 534 subjects in the Seychelles Child Development Study (SCDS) Main Cohort was examined at age 19 years. The auditory test battery included standard pure-tone audiometry, tympanometry, Auditory Brainstem Response (ABR) latencies, and both click-evoked and distortion-product Otoacoustic Emissions (OAE). Associations with MeHg were evaluated with multiple linear regression models, adjusting for sex, recent postnatal MeHg exposure, and hearing loss.

Results—Bilateral hearing loss (defined as a mean pure-tone threshold of greater than 25 dB) was present in 1.1% of the subjects and was not associated with prenatal or recent postnatal MeHg exposure. As expected, absolute and interwave ABR latencies were shorter for women as compared to men, as the stimulus presentation rate decreased from 69.9 to 19.9 clicks/sec and as the stimulus intensity increased from 60 to 80 dBnHL. Similarly, larger OAE amplitudes were elicited in women as compared to men and in the right ears as compared to the left. There was no association of prenatal MeHg exposure with hearing loss, ABR absolute and interwave latencies or OAE amplitudes. As recent postnatal MeHg increased, some associations were found with a few ABR absolute and interwave latencies and a few OAE amplitudes. However, the direction of these associations was inconsistent. As recent postnatal MeHg levels increased the wave I absolute latencies were shorter at 80 dBnHL for all 3 click rates, but the interwave I-V latency was longer for males for the 80 dBnHL 19 clicks/sec and for the III-V interwave latencies for males and females for the dBnHL 69 clicks/sec. Similarly, smaller OAE amplitudes were found at 1500, 2000, and 4000 Hz for males while larger OAE amplitudes were found for females at 1500 and 2000 Hz as the recent postnatal MeHg levels increased.

Conclusions—No consistent associations were present in this study between prenatal MeHg exposure from consumption of oceanic fish and seafood during pregnancy and auditory functions at 19 years of age. Given the level of prenatal exposure to MeHg, the number of audiologic measures tested, and the presence of the expected sex, click rate, and level dependent findings, it seems unlikely that an association was present and not detected. As recent postnatal MeHg exposure increased, a few associations with ABR latencies and OAE amplitudes were found. The direction of these associations was inconsistent as some showed improved performance as MeHg exposure levels increased while others showed poorer performance. The presence of the inconsistent postnatal MeHg exposure findings are intriguing and deserve further clarification.

Keywords

Methylmercury; Mercury; Auditory; Auditory Brainstem Responses; Otoacoustic Emissions; Fish Consumption; Seychelles Child Development Study

1.0 Introduction

Methylmercury (MeHg) is a neurotoxicant that adversely affects central nervous system function in adequate doses (Clarkson, 1995). Human exposure to MeHg is widespread, almost exclusively from consuming fish, and at dosages substantially below those known to cause signs or symptoms of poisoning. All fish contain small amounts of MeHg. In aquatic environments, bacteria transform inorganic mercury (Hg) into MeHg which is then bio-accumulated up the aquatic food chain. Large predatory fish can bio-accumulate MeHg up to 1.0 ppm or greater (WHO, 1990).

The developing nervous system is especially sensitive to MeHg exposure as demonstrated by observational studies of poisoning and experimental studies. The auditory system is an optimal organ system with which to detect subtle peripheral and central neurologic effects. The measurement of peripheral end-organ function (inner hair-cell function) can be assessed with Otoacoustic Emissions (OAE) and central nervous system (CNS) auditory processing function (auditory nerve conduction speed) with Auditory Brainstem Responses (ABR). These can be directly measured and appear to be independent of each other (Lonsbury-Martin and Martin, 1990; Moore et al., 1995).

Auditory Brainstem Responses represent the summed electrical potentials of sequential activation in the auditory system in response to a repeated stimulus such as a tone burst or click. The ABR waveform has distinct peaks and troughs and represents the summed action potential propagating from the auditory nerve through the cochlear nucleus to the inferior colliculus (Moller et al., 1982).

ABR latencies have been shown to correspond to the degree of myelination in the auditory pathway (Moore et al., 1995; Knipper et al., 2000; Ito et al., 2004), as well as providing information on synaptic maturation and axonal structure (Rance, 2005; Wang and Manis, 2005). For example, broader response peaks and prolonged latencies were found in animal models with myelin deficiencies (Naito et al., 1999; Ito et al., 2004; Roncagliolo et al., 2000; and El-Badry et al., 2007). In addition, neurofilament deficiencies and altered axonal structure also have been shown to prolong latencies (Sheykholeslami et al., 2001; Jones et al., 2008).

ABRs have been used to quantify damage to the developing auditory system caused by high dosages of neurotoxicants such as heavy metals and organic compounds (see Dyer, 1985; Otto et al., 1988). A few adults with Minamata disease have shown hearing loss or elevated hearing thresholds as a clinical feature of accidental poisoning (McAlpine and Araki, 1958; Kurland et al., 1960; Nosaka et al., 1970; Fujisaki et al., 1971; Mizukoshi et al., 1975; Ino and Mizukoshi, 1977). Prolonged ABR latencies were also reported in patients diagnosed with Minamata disease, although exposure verification was not possible (Hamada et al., 1982; Inayoshi et al., 1993).

Inconsistent and sporadic associations between certain absolute and interwave latencies and prenatal MeHg exposure from the consumption of oceanic fish and sea mammals have been reported in populations in the Faroe Islands, Greenland, Japan, and Madeira. Some studies have reported prolonged ABR latencies (Grandjean et al., 1997; Murata et al., 1999a, b;

Murata et al., 2004a), while others have found no association (Weihe et al., 2002; Murata et al., 2004b). In these studies various metrics of prenatal MeHg exposure were used including cord blood or maternal hair samples taken at birth (Grandjean et al, 1997; Murata et al, 1999a, Murata et al, 2004a) or during testing when the children were several years of age (Murata et al, 1999b; Murata et al, 2004b). These studies have not been replicated to date in a population in which the source of MeHg exposure was primarily from consumption of oceanic fish, the entire period of prenatal exposure was characterized and there was minimal or no exposure to other contaminants.

To our knowledge no human studies have used the OAEs to examine the peripheral cochlear function in a cohort of individuals exposed to low dose MeHg (Dziorny et al., 2012). Otoacoustic emissions (OAEs) provide a quantitative assessment of the function of the peripheral auditory system (outer hair cells), which cannot be obtained by measuring ABRs alone. Otoacoustic emissions measure sound pressure variations in the ear canal and are thought to reflect induced movement of the basilar membrane by outer hair cells (OHCs) (Kemp et al., 1990; Lonsbury-Martin and Martin, 1990). The amplitude of OAEs is decreased or absent when OHCs are missing or not functioning properly (Liberman et al., 2002; Colombari et al., 2011). For example in experimental animals, polychlorinated biphenyl (PCB) exposure has been shown to decrease the amplitude and increase the threshold of distortion product OAEs (DPOAE) (Lasky et al., 2002). In addition, environmental noise exposure can disrupt auditory function causing both threshold shifts in ABRs and reduced amplitude OAEs (Daniel, 2007; Marshall et al., 2009).

High dose exposure to MeHg can be very toxic to not only the auditory system but the entire neurologic system and even cause death. A few studies of subjects exposed to multiple toxicants have reported an association of low level MeHg exposure and psychometric outcomes after adjusting statistically for other contaminants (Grandjean, 1997; Debes et al., 2006; Boucher et al., 2014). However, studies of subjects consuming oceanic fish and not contaminated with other known toxicants have not confirmed these low level associations (Davidson et al., 2011; Llop et al., 2011; Strain et al., 2012). This study evaluated the association between a subject's prenatal and recent postnatal MeHg exposure and the peripheral and central auditory function in participants enrolled in the Seychelles Child Development Study Main Cohort at 19 years of age. This study tested subjects with common hearing evaluation procedures as well as both ABR and OAE testing procedures to determine if prenatal or recent postnatal exposure to MeHg primarily from ocean fish consumption is associated with changes in auditory function.

2.0 Methods

2.1 Study Population and MeHg Measurements

The SCDS is a longitudinal cohort study in a population which is exposed to MeHg from ocean fish and seafood consumption (Marsh et al., 1995). Sea mammals are not consumed in Seychelles. At enrollment of the Main Cohort, mothers reported consuming on average 12 fish meals per week. The cohort consists of 779 mother-infant pairs that were enrolled in 1989-90 (Marsh et al., 1995). Eighty-two subjects were excluded for specific reasons including lack of maternal hair for exposure assessment, medical conditions affecting

development or study withdrawal (Myers et al., 2003). At 19 years of age there were 697 young adults (89.5%) eligible for this study. A total of 534 subjects (76.6% of the eligible cohort) participated in this evaluation. Their ages ranged from 18.1 to 20.6 years. Six subjects were excluded because of abnormal tympanometric findings and 11 had incomplete audiometric data leaving a total of 517 subjects for analysis. Not all subjects were able to complete all audiometric test procedures and the number of subjects included in each analysis varies.

Our biomarkers of MeHg exposure were measurements of total Hg in hair. Prenatal MeHg exposure was measured as total Hg (THg) in maternal hair growing during pregnancy as described previously (Cernichiari et al., 1995). Recent postnatal exposure was determined by measuring THg in a 1-cm section of the participant's hair closest to the scalp collected at testing. Methylmercury constitutes over 80% of THg in hair (Cernichiari et al., 1995). These are subsequently referred to throughout as prenatal and postnatal exposure. Measured exposure to lead, polychlorinated biphenyls (PCBs), and pesticides in Seychellois subpopulations is low (Cernichiari et al., 1995; Davidson et al., 1998). The demographics of this cohort have been reported previously (Shamlaye et al., 1995). Prenatal maternal diet and nutrition status were not quantified in this cohort. The study protocol was reviewed and approved by the Ethics Committee of the Republic of Seychelles and the Research Subject Review Board at the University of Rochester.

2.2 Auditory Measurements

An audiologist (MSO) oversaw the auditory test battery. Three nurses who were specifically trained in the procedures administered the tests. All clinical personnel were blinded to exposures. Subjects were tested in a quiet room at the Child Development Centre (CDC). The subjects were tested in a reclined position for the ABRs and seated upright for the pure-tone screening, tympanometry and OAEs.

Standard pure-tone audiometric testing was administered to quantify any degree of hearing loss. Testing was completed on both ears in octave intervals from 500-8000 Hz (Hertz) (500, 1000, 2000, 4000, and 8000 Hz) using EarTone 3A insert receivers and a calibrated Grasen Stadler (GSI 39) portable audiometer/tympanometer. Normal hearing was defined as having a threshold of less than 25 dB HL (decibels Hearing Level), a mild hearing loss was defined as a mean threshold (at 500, 1000, 2000, 4000 and 8000 Hz) of between 25 - 34 dB HL, and moderate hearing loss was defined as a mean threshold between 35-55 dB HL.

Tympanometric testing was used to assess and exclude any subject that had abnormal middle ear function. Subjects were eliminated from analysis if they were classified as a type B or C tympanogram based on a modification of Jerger's classification (Jerger, 1970): middle ear compliance < 0.2 cc or middle ear pressure < -150 daPa (decaPascals). The tympanometric testing was carried out using the autoTymf function on a GSI 39 portable tympanometer with appropriately fitted TS212 tympanometry tips.

ABRs were obtained using 100 μ sec click stimuli presented to the right and left ear at 60 and 80 dBnHL (decibel above normal hearing level), at 19.9, 39.9 and 69.9 clicks/sec. The nurse prepared the electrode sites with an electrode prep pad and the recording electrodes were

placed on the forehead, and ipsilateral earlobe. The contralateral earlobe served as ground. The nurse checked electrode impedance (which was recorded and saved for later review), placed insert receivers into the ear canals, and monitored the subject's behavior during the recording procedure. The actual recording was automated. Subjects were instructed to close their eyes, lie quietly without moving and encouraged to sleep if possible. Three runs of 1024 clicks were amplified (x150,000) and filtered (300-3000 Hz) using a Biologic NavPro with insert receivers. Waveforms were recorded using an automated procedure that initially tested the right ear at the higher intensity levels (80dBnHL) for the 3 rates (19.9, 39.9 and 69.9 clicks/sec). Then testing was continued at the lower intensity levels (60dBnHL) for all 3 rates. Once testing on the right ear was complete, testing was then repeated on the left ear.

Outer hair cell function was evaluated using distortion products (DPOAE) and click-evoked (CEOAE) emissions. Subjects were seated comfortably and a small ear probe was placed snugly in their outer ear canal. The signal-to-noise ratio (SNR) of the cubic difference ($2F_1 - F_2$) DPOAE was measured for the F_2 frequencies 1000, 2000, 3000, 4000 and 6000 Hz. The two DPOAE tones had levels $L_1 = 70$ dB and $L_2 = 60$ dB, and the F_2 / F_1 ratio was 1.22. CEOAE was evoked using an 80 dB (± 3 dB) broadband click stimulus, with the SNR of the resulting OAE measured in 1/3 octave bands from 1000 – 4000 Hz. OAE testing used the Otodynamics Echoport ILO92 USBII clinical OAE analyzer connected to a laptop computer.

All data were transmitted to the University of Rochester where they were analyzed by an audiologist (MSO) blinded to subject identity and exposure status. For the ABR response, two of the three visually closest waveforms at each presentation level, rate and ear, were averaged and analyzed offline. Absolute latencies of waves I, III and V and I' and V' of the averaged waveform were identified and recorded. The recorded absolute latencies were saved to spreadsheet software and interwave I-III, III-V and I-V latencies calculated.

2.3 Statistical Analysis

Associations with prenatal MeHg exposure and the auditory outcomes were examined using covariate-adjusted linear regression models. All models were adjusted for sex, recent postnatal MeHg exposure and hearing loss. These models were specified *a priori* in analysis plans and all associations were tested with two-sided alternatives at the 0.05 significance level. Adjustments for family stressors, social economic status and maternal IQ were not made since there is no evidence they influence audiometric endpoints. However, since they were covariates in the 19 year neurodevelopmental and behavior analyses we did examine their relationship with the audiometric endpoints. Over 60% of the correlations between these psychometric covariates and ABR or OAE outcomes were <0.05 and 96% were <0.10 .

Each of the ABR endpoints was fit in a separate model. Each model included measurements from both the left and right ears of each subject and included a random subject's effect to account for the correlation between these two measurements. Each of the OAE endpoints was fit with two separate models (one for each ear). Models were first fit with sex by prenatal MeHg and sex by recent postnatal MeHg interactions. When the interaction was significant, separate associations were tested on males and females. These interactions were removed if not significant and the models were rerun. Model assumptions were checked

using standard methods, including checking for constant variance, nonlinearity, and normally distributed residuals. No transformations were necessary for any covariates or outcomes. Outliers were identified with the absolute value of standardized residuals 3.

Subjects missing an audiometric test for hearing loss or a recent postnatal MeHg measurement were not included in the covariate-adjusted regression models. For each wave of the ABR and each frequency of the OAE, an analysis of variance was used to test for differences in the value of each audiology outcome between subjects included and excluded from the regression analyses. This comparison between the two groups was repeated on 36 ABR and 22 OAE endpoints and found no significant differences in audiology outcomes based on exclusion from the regressions after Bonferroni adjustment.

The proportion of identifiable ABR waves under each condition are different percentages between 0% and 100%, therefore a beta regression for proportional outcomes was fit to test whether these proportions were significantly related to the response peak, intensity, presentation rate, ear or sex. (Ferrari and Cribari-Neto, 2004). Further an analysis of variance was performed to test for differences in the length of ABR absolute latencies and interwave latencies as a function of sex, ear, stimulus rate, and intensity level. Finally, an analysis of variance was also performed for the distortion product and click-evoked OAEs to test for differences in the amount of emission as a function of sex, ear, and stimulus frequency.

3.0 Results

3.1 Methylmercury Exposure

Prenatal MeHg exposure and audiometric data was available on all 517 subjects. However, recent postnatal MeHg exposure as estimated by the mean child hair concentration at time of testing was available on only 481 (90.1%) subjects because some males in Seychelles shave their heads or cut their hair very short. In addition there were 15 of these subjects who were excluded because of abnormal tympanometric values or lacked an audiogram leaving 466 for analysis. Among subjects with a complete set of covariates, prenatal MeHg exposure was 6.89 ppm. Among the 51 subjects without a complete set of covariates, prenatal MeHg exposure was 6.81 ppm. While prenatal MeHg exposure was similar for both sexes, there was a significant sex difference in recent postnatal MeHg exposure with males being higher (see Table 1). Moreover, male recent postnatal MeHg exposure levels were nearly double their prenatal levels while females' recent postnatal exposure increased only slightly over their prenatal levels.

3.2 Audiometric Testing Results

3.2.1 Pure Tone Audiometry—Of the 517 subjects with audiometric data measured in both ears there were 50 with some degree of hearing impairment. Only 45 of the children had a postnatal MeHg exposure available. No child had a severe hearing loss. Six children had bilateral hearing loss and 44 had monaural hearing loss. Monaural hearing loss was present in the left ear for 19 and the right ear for 25 subjects. While the elevated level of Hg in the children with hearing loss is of interest, the difference in average MeHg exposure

between participants with and without hearing loss did not meet our pre-specified level of significance ($p=0.064 > 0.05$). Further, those subjects with a moderate hearing loss had lower levels of exposure than those with mild hearing loss.

3.2.2 Auditory Brainstem Responses—No differences were seen between the percentage of identifiable response peaks as a function of ear ($p=0.648$) (SDC Table 1s). Female subjects had a greater percentage of identifiable response peaks as compared to males ($p<0.001$). Waves I, III and V were almost always detected for both the male and female subjects at 80 dBnHL regardless of click rate. As the intensity of the stimulus decreased from 80 to 60 dBnHL and the rate increased from 19.9, to 39.9 to 69.9 clicks/sec, the percentage of identifiable waves I and III decreased regardless of sex ($p<0.001$). When collapsed across ear and sex, waves I, III and V are present over 90% of the time at 80 dBnHL. Wave III and V were identified 96-97% of the time regardless of click rate while wave I was detected 96%, 94% and 90% of the time at 19/sec, 39/sec and 69/sec respectively. As the intensity of the stimulus decreased from 80 to 60 dBnHL and the rate increased from 19.9 to 39.9 to 69.9 click/sec, the percentage of identifiable waves I, and III decreased regardless of sex ($p<0.001$). At 60 dBnHL wave V, the most robust response, continued to be detected approximately 95% of the time regardless of click rate (94%, 93% and 92% of the time at 19/sec, 39/sec and 69/sec respectively). Wave I, however, was only detected at click rates of 19/sec, 39/sec and 69/sec 68%, 53% and 37% of the time respectively and wave III at 87%, 80% and 71% of the time respectively. As expected, as the intensity of the stimulus decreased and the rate increased, detecting the earlier less robust response (waves I, and III) became more difficult.

As expected female subjects have significantly shorter absolute ($p<0.001$) and interwave ($p<0.001$) latencies (SDC Figures 1s and 2s and SDC Tables 2s and 3s) even after controlling for response peak, rate and intensity. As the stimulus intensity decreased from 60 to 80 dB the absolute ($p<0.001$) and interwave ($p<0.001$) latencies increased decreased. As the rate increased from 19/sec to 69/s3ec the absolute ($p<0.001$) and interwave ($p<0.001$) latencies increased at both 60 and 80 dBnHL. Sample sizes for ABR waves varied based on wave identification, but in general waves at lower intensities and higher rates were less easily identified and thus have smaller sample sizes.

Prenatal MeHg exposure was not significantly associated with any of the ABR absolute or interwave endpoints studied at 60 dBnHL (SDC Figure 3s) and 80 dBnHL (SDC Figure 4s) endpoints studied

Recent postnatal MeHg was significantly associated with several endpoints. As recent postnatal MeHg levels increased, the wave I absolute latencies decreased for 80 dB for all 3 click rates (Figure 1) regardless of sex. Interwave latencies were prolonged for the I-V interval at 80 dB (19 clicks/sec) in males (Figure 1), and for the III-V interval at 60 dB (69 click/sec) in both sexes as recent postnatal MeHg levels increased (Table 2 and SDC Figure 5s).

Statistical outliers were identified in some of the ABR statistical models where the absolute value of standardized residuals was greater than 3. For each model with statistical outliers,

the model was re-fit without the outliers and in all but one model the coefficient estimates were changed only slightly and the significant covariates did not change. The model for wave III latency at 80 dB and 69/sec stimulus was the exception. In that model both minimal and moderate hearing loss slopes reached significance when outliers were excluded.

Male subjects generally had significantly longer ABR wave latencies as compared to female subjects. Hearing loss was also significantly associated with increased wave I latency under four of the six click stimulation conditions. Moderate hearing loss was significantly associated only with increased interwave I-V latency with the 80dB, 39/sec stimulus.

3.2.3 Oto Acoustic Emissions—There were 524 subjects with both Distortion Product and Click-Evoked OAEs in at least one ear and of those 466 subjects had tests adequate for the regression analyses. Subjects were excluded from the CEOAE regression analysis and were classified as invalid when the recorded stimulus level was $> \pm 3$ dB from the specified recording level of 80dB SPL. In the right ear 80 subjects (15 %) were excluded from analysis while in the left ear 111 subjects (21%) were excluded. On average, for both sexes the subjects had relatively large click and distortion product OAEs in both ears. The signal-to-noise ratio in both genders increased as frequency increased until the 2000-3000 Hz region in the click evoked OAE and to the 3000-4000 Hz region with distortion product OAEs in both ears and then tended to decrease at higher frequencies for both measures. As expected, female subjects had larger DPOAEs from 4000-6000 Hz ($p=0.013$) and CEOAEs from 1000 to 4000 Hz than males ($p<0.001$). In addition, the right ears of male and female subjects had larger signal to noise ratios (SNR) for both DPOAE ($p=0.003$) and CEOAEs ($p=0.004$) across frequencies (SDC Figure 6s).

Prenatal MeHg exposure was not significantly associated with any DPOAE or CEOAE endpoint in any model (Table 3).

Recent postnatal MeHg exposure was not significantly associated with any DPOAE endpoint. However, recent postnatal MeHg exposure was significantly associated with mid-frequency CEOAEs (1500 and 2000 kHz) in only the left ears of both male and female subjects and at 4000 kHz in the right ear of male subjects (Table 3). Otoacoustic emission models had very few statistical outliers and none were influential. Sex was significantly associated with several endpoints and females generally had larger SNR than males. One CEOAE endpoint (2000 kHz left ear) demonstrated a significant association with severe hearing loss, resulting in a lower SNR.

4.0 Discussion

We studied the association of prenatal exposure from ocean fish consumption with physiologic measures of hearing at 19 years in the Main Cohort of the SCDS. The mean prenatal MeHg exposure of participants during gestation was 6.89 ppm, approximately ten times that of the US population and substantially high than that of other studies. We expected to find changes in the ABR and the OAE if prenatal MeHg exposure at these levels causes auditory system damage. ABR latencies are highly dependent on the function of the myelin sheath covering the auditory nerve (Reuhl and Chang, 1979) and children diagnosed

with Fetal Minamata disease are reported to have abnormal myelin profiles (see Dzorny et al 2012 for a review). However, there were no consistent associations present between prenatal MeHg exposure and the 36 ABR and 22 OAE endpoints measured.

Recent postnatal MeHg levels of males were significantly higher than female subjects. It is presently unclear why a difference in MeHg levels exists between the sexes. One could speculate that the sex differences are likely due to dietary differences or dietary preferences of these young adults. Male subjects may be more likely to consume greater quantities of fish or eat a variety of fish with higher concentrations of MeHg than females. To date no complete dietary study has been completed to determine possible causes of these sex differences found in this investigation.

Our test battery was comprehensive and included ABR testing methodology (for example, presentation levels and click rates) that were the same as prior studies along with additional parameters and OAEs. The ABR testing was sensitive enough to find the expected associations such as shorter absolute and interwave latencies in females as compared to males, and as the presentation rates decreased and the stimulus intensity levels increased (Stockard et al, 1979; Beagley and Sheldrake, 1978; Paludtti et al., 1983; Lasky, 1984; Suzuki et al., 1986; Chiarenza et al., 1988, Trune et al., 1988; Lopez-Escamez et al., 1999; Stuart and Yang, 2001). Regarding OAEs, we found the expected greater amplitude OAEs in female subjects as compared to the males and in the right ears of participants as compared to their left (Bilger et al, 1990; Talmadge et al., 1993; McFadden, 1993; Morlet et al., 1995; McFadden et al, 1996; McFadden and Pasanen 1998; Lamprecht-Dinnesen et al, 1998; Ismail et al., 2003; McFadden et al., 2008). Given the level of the subjects' exposure to prenatal MeHg, the number of audiometric measures tested, and the presence of the expected associations on both ABR and OAE tests, it seems unlikely that an association with prenatal MeHg exposure was present and not detected.

Our study of prenatal exposure is consistent with other studies in Greenland and Japan that reported finding no associations between MeHg exposure and ABR latencies (Weihe et al., 2002 Murata et al., 2004b). Some epidemiologic studies have reported an adverse association between prenatal MeHg exposure and the ABR. A study in the Faroe Islands reported an association between cord blood MeHg and prolongation of wave III ($p = 0.06$) and V ($p = 0.01$) latencies at 20 and 40 clicks/sec in children examined at age 7 years (Grandjean et al., 1997). A subsequent reanalysis of a subcohort of those children who had the most reliable testing was reported as showing an association of cord blood MeHg with prolongation of wave III and interwave I-III latencies at 20 and 40 clicks/sec (Murata et al, 1999a). When the same cohort was re-examined at age 14 years the authors reported an association of cord blood MeHg with prolongation of wave III and V latencies and of maternal hair MeHg with prolongation of wave III and interwave I-III latencies (Murata et al, 2004a). Murata and colleagues (1999b) carried out a similar study in 7 years old children in Madeira. They reported an association of MeHg exposure measured in maternal hair taken when the children were evaluated at age 7 years with prolongation of the wave III and V and interwave I-V and III-V latencies.

The different audiometric associations that have been reported may be due to differences in the populations studied, diets, biomarkers of prenatal MeHg exposure, toxicant co-exposures or other factors. In the Seychelles, exposure to MeHg is gradual and sustained from daily fish consumption. Seychellois citizens do not consume sea mammals and consequently are not exposed to high levels of PCBs or other toxicants present in such food sources. The Seychelles has no industrial pollution so exposure to environmental toxicants is primarily due to background levels that are present throughout the oceans. PCBs are known to be associated with ABR latencies and OAE amplitudes. PCBs are reported to result in prolonged ABR latencies, decreased ABR amplitudes (Herr et al., 1996), elevated ABR thresholds (Powers et al., 2009), and reduced OAE amplitudes (Herr et al., 1996; Powers et al., 2009; Chen and Hsu, 1994; Grandjean et al., 2001; Lasky et al., 2002). A secondary analysis of the Faroe Islands 7 year evaluations reported an association between PCB levels and prolongation of the wave III latency at 40 click/sec and of wave V latency at 20 click/sec (Grandjean et al., 2001). When that analysis was adjusted for MeHg, only the wave V latency was adversely associated with the PCB exposure. Other studies examining prenatal PCB exposure have also reported abnormal ABRs (Chen and Hsu, 1994). Coexposure to MeHg and PCBs exposure is reported to have synergistic adverse effects (Bemis and Seegal, 1999; Risher et al., 2003). Some have also measured umbilical cord blood or at the time of testing measured mercury in maternal hair or the child's blood or hair. The correlation between these different biomarkers is not clear.

The absence of significant associations between prenatal MeHg exposure and auditory endpoints in the Seychelles could have several explanations. Prenatal MeHg exposure may have no adverse influence at the level we studied, or it may require co-exposure with other neurotoxicants to influence auditory measures. Alternatively, the beneficial influence of long chain polyunsaturated fatty acids (LCPUFA) or other nutrients which are abundant in fish may protect the auditory system in individuals that consume fish on a regular basis (Myers et al., 2007; Davidson et al., 2011).

The Seychelles Main Cohort continues to find no associations or improved performance between prenatal MeHg exposure and developmental outcomes over the 19 year longitudinal study. Findings from the present investigation are consistent with previous data reported from this cohort at 6, 19, 26, 66, 107 months and 10.5, 17 and 19 years. Over 19 years of life, no consistent adverse pattern for prenatal MeHg exposure has emerged. Measures of global and specific neurocognitive outcomes, achievement and problematic behaviors have not shown any consistent pattern of adverse association with prenatal MeHg exposure from a population that primarily consumed oceanic fish (Davidson et al., 1998, Davidson et al., 2011, Myers et al., 2003, Van Wijngaarden et al., 2013).

Although this study was not designed to test postnatal MeHg exposure, we did measure recent exposure in the subjects' hair and included it in the analyses. Hearing loss was reported in children poisoned by MeHg in Japan and Iraq and animal models of acute, high-dose postnatal MeHg exposure have been shown to disrupt myelination and damage central auditory pathways (Dziorny et al., 2012). We did not find a clear or consistent pattern of associations, but there was an association with wave I absolute at all 3 click rates and interwave latencies at III-V and I-V at a specific rate and level in males only. The wave I

absolute latencies at 80 dB for all 3 click rates was shorter as MeHg exposure levels increased. We know of no biological reason to believe this could be associated with postnatal MeHg exposure. However, there is evidence that nutrition might play a role in ABR maturation in premature infants (Amin et al., 2000) and it is possible that maturation or preservation of the auditory system may be related to nutrients present in fish such as LCPUFA. The limitations of our metric for postnatal MeHg exposure make us cautious in interpreting this finding, but it is intriguing and further exploration of such associations seem warranted. We did also find prolongations of interwave latencies I-V (80 dB, 19 clicks/sec) and III-V (60 dB, 69 clicks/sec) in male subjects as recent postnatal exposure increased.

This study has a number of strengths. The cohort was large and their MeHg exposure is from the regular consumption of fish that has MeHg levels similar to that of commercial fish in developed countries. Testing was automated, carried out by specially trained nursing staff in a clinical center specifically dedicated to the study. Testing procedures replicated previously reported ABR testing with additional audiometric parameters such as a faster presentation rate and 2 OAE measures. Oversight and scoring of test results was by the first author, an experienced audiologist. The audiologist, other clinicians, and families were not aware of subjects MeHg exposure. Analyses were carried out using an *a priori* analysis plan based on a biological hypothesis with control for confounding factors such as sex and middle ear function that could result in a misinterpretation. The study also has limitations. Epidemiology studies are not able to confirm that no associations exist. Prenatal LCPUFA were not measured in this cohort or in similar studies by other investigators. However, recent publications have suggested that they may influence audiometric measures

4.1 Summary

No associations between prenatal low-level MeHg exposure from fish consumption during pregnancy and subjects' audiometric parameters at 19 years of age were present in a population exposed through gradual and sustained oceanic fish consumption containing concentrations similar to those found in ocean fish and seafood sold in developed countries. Recent postnatal exposure was also examined and there were a few associations with ABR latencies and OAE amplitudes, but there was no consistent pattern. These postnatal findings are intriguing and deserve further study.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

This research was supported by grants RO1-ES010219, P30-ES01247, ES 08442, and T32-ES007271 from the US National Institute of Environmental Health Sciences, National Institutes of Health and by the Government of the Republic of Seychelles.

Abbreviations Used

ABR	Auditory Brainstem Response
CEOAE	Click Evoked Otoacoustic Emission
DPOAE	Distortion Product Otoacoustic Emission
Hg	mercury
LCPUFA	long chain polyunsaturated fatty acids
MeHg	Methylmercury
OAE	Otoacoustic Emission
OHC	Outer Hair Cells
PCB	polychlorinated biphenyl
THg	total mercury

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Figure 1.

Exposure-response relationship between recent postnatal MeHg exposure and ABR absolute wave latencies (upper graphs) and interwave latencies (lower graphs) for males (left column) and females (right column) at 80 dBnHL. Each * symbol indicates a linear fit with a slope significantly different from zero. As the recent postnatal MeHg exposure level increases, the absolute wave I latency for both males and females decreases (slopes for 69, 39, and 19/sec are -0.0033, -0.0031, -0.0029, and $p=0.002$, 0.001 , 0.002 , respectively *** shows that all wave I slopes are significant at each repetition rate). As the recent postnatal MeHg level increases, the interwave wave I-V latencies increase for males subjects only (slope of 0.0045, $p=0.025$). The association is estimated in a regression analysis that is adjusted for prenatal Hg exposure and hearing loss. The rug plot above the x axis represents the number of subjects at each exposure level.

Table 1

Descriptive data by prenatal and recent postnatal MeHg exposure levels on the cohort studied as it relates to sex, hearing status, and degree of hearing loss.

Prenatal MeHg Exposure	Mean MeHg Level	N	SD	p value
	6.89 ppm	517	4.53	
Sex				
Males	6.68 ppm	234	4.37	.329
Females	7.07 ppm	283	4.66	
Hearing Status				
Normal Hearing	6.75 ppm	467	4.40	0.064
Hearing Loss	8.26 ppm	50	4.46	
Degree of Hearing Loss				
Mild Hearing Loss Thresholds between 25-35 dB	8.43 ppm	38	5.46	0.705
Moderate Hearing Loss Thresholds between 35-55 dB	7.71 pm	12	5.68	

Recent Postnatal MeHg Exposure	Mean MeHg Level	N	SD	p value
	10.32 ppm	466	6.12	
Sex				
Males	12.60 ppm	196	7.06	<0.001
Females	8.67 ppm	270	4.7	
Hearing Status				
Normal Hearing	10.30 ppm	421	6.19	0.830
Hearing Loss	10.49ppm	45	5.48	
Degree of Hearing Loss				
Mild Hearing Loss Thresholds between 25-35 dB	10.84 ppm	35	5.62	0.402
Moderate Hearing Loss Thresholds between 35-55 dB	9.24 ppm	10	5.05	

A child's degree of hearing loss is based on the ear with the greatest loss

Table 2

Auditory brainstem response regression models. Models with a significant interaction between sex and recent postnatal MeHg exposure show separate recent postnatal MeHg coefficients for males and females. In interaction models, the sex coefficient is not comparable to models without interactions. Coefficients significant at the 0.05 level are displayed in bold.

	Wave	Model p-value (n)	Prenatal MeHg	Recent Postnatal MeHg	Sex male	Mild Hearing Loss (25-35 dB)	Moderate Hearing Loss (>35 dB)
80 dB, 19/sec	I	<0.001 (797)	-0.0009	-0.0029	0.0631	0.0479	-0.0123
	III	<0.001 (804)	-0.0007	-0.0023	0.1686	-0.0054	0.0374
	V	<0.001 (810)	-0.0015	-0.0018	0.2340	-0.0079	0.0010
	I-III	<0.001 (795)	-0.0001	0.0005	0.0994	-0.0528	0.0507
	I-V	<0.001 (797)	-0.0003		0.0712	-0.0499	0.0559
	F			-0.0042			
	M			0.0045			
	III-V	<0.001 (804)	-0.0002		0.0106	-0.0110	-0.0140
	F			-0.0032			
	M			0.0023			
80 dB, 39/sec	I	<0.001 (788)	-0.0011	-0.0031	0.0743	0.0352	-0.0241
	III	<0.001 (799)	-0.0008	-0.0024	0.1680	-0.0083	0.0443
	V	<0.001 (808)	-0.0014	-0.0015	0.2422	-0.0150	0.0618
	I-III	<0.001 (785)	-0.0003	0.0008	0.0867	-0.0335	0.0743
	I-V	<0.001 (787)	0.0000	0.0015	0.1519	-0.0484	0.1378
	III-V	<0.001 (798)	-0.0002	0.0008	0.0672	0.0002	0.0599
80 dB, 69/sec	I	<0.001 (752)	-0.0011	-0.0033	0.0864	0.0725	-0.0256
	III	<0.001 (795)	-0.0018	-0.0019	0.1768	0.0401	0.0797
	V	<0.001 (805)	-0.0024	-0.0029	0.2497	0.0303	0.0250
	I-III	<0.001 (747)	-0.0013	0.0019	0.0811	-0.0169	0.1200
	I-V	<0.001 (749)	-0.0010	0.0009	0.1421	-0.0382	0.0520
	III-V	<0.001 (792)	0.0000	-0.0008	0.0642	-0.0005	0.0636
60 dB, 19/sec	I	<0.001 (570)	0.0000	-0.0012	0.0739	0.1171	-0.0294
	III	<0.001 (728)	-0.0040	-0.0022	0.1936	0.0356	0.1300
	V	<0.001 (781)	-0.0044	-0.0020	0.3047	0.0411	0.0811
	I-III	<0.001 (565)	-0.0009		0.0162	-0.0252	0.1272
	F			-0.0044			
	M			0.0019			
	I-V	<0.001 (570)	-0.0007		0.1068	-0.0544	0.1588
	F			-0.0047			
	M			0.0029			
	III-V	<0.001 (726)	0.0005	0.0009	0.0920	-0.0206	0.0286
60 dB, 39/sec	I	<0.001 (453)		-0.0028	0.1785	0.1499	0.0216

	Wave	Model p-value (n)	Prenatal MeHg	Recent Postnatal MeHg	Sex male	Mild Hearing Loss (25-35 dB)	Moderate Hearing Loss (>35 dB)
	F		0.0039				
	M		-0.0085				
	III	<0.001 (670)	-0.0010	-0.0029	0.2215	0.0232	0.1506
	V	<0.001 (774)	-0.0030	-0.0030	0.3111	0.0473	-0.0099
	I-III	<0.001 (446)	-0.0009	-0.0001	0.1258	-0.0516	-0.0418
	I-V	<0.001 (453)	-0.0022		0.0915	-0.0655	-0.0187
	F			-0.0054			
	M			0.0046			
	III-V	<0.001 (670)	-0.0023	0.0010	0.0747	0.0308	-0.0275
60 dB, 69/sec	I	<0.001 (311)	0.0008	-0.0030	0.0998	0.0176	-0.0801
	III	<0.001 (590)	-0.0016	-0.0029	0.2571	0.0588	0.1707
	V	<0.001 (758)	-0.0021	-0.0023	0.3127	0.0343	0.0204
	I-III	0.002 (302)	-0.0017	0.0019	0.1342	-0.0125	0.0688
	I-V	<0.001 (311)	-0.0024	0.0045	0.1765	-0.1096	0.1204
	III-V	0.010 (588)	-0.0033	0.0038	0.0415	-0.0025	-0.0485

Table 3

Otoacoustic emission regression model results. Models with a significant interaction between sex and prenatal or recent postnatal MeHg exposure show separate MeHg coefficients for males and females. In interaction models, the sex coefficient is not comparable to models without interaction. Regression analysis of only those values that were significant was included in this table.

	Freq (kHz)	Ear	Model p-value (n)	Prenatal MeHg	Recent Postnatal MeHg	Sex male	Mild Hearing Loss (25-35 dB)	Moderate Hearing Loss (>35 dB)
DPOAE	1.0	R	0.869(382)	0.0227	-0.0770	-0.0980	-1.3441	-0.7076
	1.0	L	0.603(361)	-0.0235	0.0961	0.4388	3.1109	-3.2796
	1.5	R	0.999(382)	-0.0232	0.0108	0.0029	0.2948	1.3218
	1.5	L	0.577(361)	0.0409	0.1047	0.2452	-2.1759	-5.5986
	2.0	R	0.923(382)	-0.0397	-0.0458	-0.5449	0.3471	3.4209
	2.0	L	0.871(361)	0.0281	0.0855	-0.0888	-1.8798	-3.4916
	3.0	R	0.701(382)	0.0379	-0.0926	0.5049	-2.3318	-0.7512
	3.0	L	0.556(361)	0.0505	0.0864	0.0166	0.8822	-6.5930
	4.0	R	0.163(382)	0.0444	-0.0672	-1.9307	-0.2959	-5.9327
	4.0	L	0.437(361)	-0.0739	0.0792	-1.2330	-1.7301	-7.0646
	6.0	R	0.053(382)	0.0522	-0.0850	-2.5499	-0.6892	-4.4729
	6.0	L	0.129(361)	0.0442	-0.0263	-1.8202	-5.0777	-7.7542
CEOAE	1.0	R	0.948(382)	-0.0629	0.0409	-0.6672	0.1026	0.2215
	1.0	L	0.497(361)	-0.0079	0.0543	-1.1538	1.2476	-5.6155
	1.5	R	0.175(382)	-0.0819	0.0245	-2.2278	1.7621	2.8541
	1.5	L	0.002(361)	-0.0093		4.9597	2.4297	-5.4654
		F				0.3482		
		M				-0.3023		
	2.0	R	0.586(382)	-0.0322	-0.0010	-1.3709	0.4387	-1.2601
	2.0	L	0.002(361)	0.0704		4.9454	-2.5683	-8.9630
		F				0.3754		
		M				-0.3134		
	3.0	R	0.054(382)	0.0318	-0.0118	-2.0066	0.0184	-4.1085
	3.0	L	0.048(361)	0.0909	0.0717	-1.9168	0.4570	-5.9516
	4.0	R	0.001(382)	0.0214		-0.1689	0.8401	-3.0746
		F				0.0903		
		M				-0.1760		
	4.0	L	0.017(361)	0.0242	0.0194	-2.5805	-0.2475	-3.2643