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ADDENDUM TO THE TOXICOLOGICAL PROFILE FOR MERCURY
(Alkyl and Dialkyl Compounds)

Agency for Toxic Substances and Disease Registry
Division of Toxicology and Human Health Sciences
Atlanta, GA 30333

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ADDENDUM FOR ORGANIC MERCURY COMPOUNDS (Alkyl and Dialkyl Mercury Compounds)
Supplement to the 1999 Toxicological Profile for Mercury

Background Statement

This addendum for Organic Mercury Compounds supplements the Toxicological Profile for Mercury that was released in 1999. Inorganic Mercury and Compounds will be addressed in a separate addendum.

Toxicological profiles are developed in response to the Superfund Amendments and Reauthorization Act (SARA) of 1986 which amended the Comprehensive Environmental Response, Compensation, and Liability Act of 1980 (CERCLA or Superfund). CERCLA mandates that the Administrator of ATSDR prepare toxicological profiles on substances on the CERCLA Priority List of Hazardous Substances and that the profiles be revised "no less often than once every three years". CERCLA further states that the Administrator will "establish and maintain inventory of literature, research, and studies on the health effects of toxic substances" [Title 42, Chapter 103, Subchapter I, § 9604 (i)(1)(B)].

The purpose of this addendum is to provide, to the public and to federal, state, and local agencies a non-peer reviewed supplement of the scientific data that were published in the open peer-reviewed literature since the release of the profile in 1999.

Chapter numbers in this addendum coincide with the Toxicological Profile for Mercury (1999). This document should be used in conjunction with the profile. It does not replace it.

2. HEALTH EFFECTS

2.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

2.2.1 Inhalation Exposure

2.2.2 Oral Exposure

2.2.2.2 Systemic Effects

Cardiovascular Effects.

A number of studies regarding the potential cardiovascular effects of exposure to methylmercury (MeHg) through consumption of contaminated fish have been published since the release of the last update of the Toxicological Profile for Mercury in March of 1999. The results of those studies are inconsistent, with some showing a relationship between mercury in human tissues and cardiovascular effects, and other studies failing to identify such a relationship.

Guallar et al. (2002) examined a population of 684 males, 70 years-of-age or younger from eight European countries and Israel. These individuals were identified from coronary care units of various hospitals as having had their first myocardial infarction (MI), as confirmed by characteristic electrocardiographic changes and elevated enzyme levels. A control population of 724 men without a history of MI was selected as being representative of the same populations. Toenail samples were used to evaluate mercury exposure, and docosohexaenoic acid (DHA) levels were determined from samples of adipose tissue. After adjustment for DHA levels and coronary risk factors, mercury levels in the study population were 15% higher than in controls. It was concluded that total toenail mercury levels were directly associated with the risk of MI, while the adipose tissue DHA level was inversely associated with risk (Guallar et al., 2002).

Yoshizawa et al. (2002) studied the correlation between toenail mercury levels and MIs in male health professionals who had not previously had an MI and who ranged in age

from 40 to 75 years in 1986. In 1987, toenail clippings were collected from 33,737 cohort members. In the ensuing 5-year follow-up period, 470 cases of coronary disease were documented. A matched control subject for whom toenail clippings had been collected in 1987 and who was still alive at the time the MI was confirmed, along with his matched counterpart, were selected for comparison. Among the study participants, dentists and those who consumed more fish had significantly higher levels of mercury in their toenails. However, when compared against the matched controls, the authors concluded that the study data did not support an association between mercury levels and an increased risk of coronary heart disease. When dentists were excluded, there was a positive, but not statistically significant, association between mercury and coronary heart disease.

Yaginuma-Sakurai et al. (2010) studied the effects of MeHg intake through fish consumption at the Japanese provisional tolerable weekly intake (PTWI) of 3.4 ug/kg bw/week on the resting heart rate. Fifty-four healthy volunteers who were either students or graduates of universities in Sendai, Japan were enrolled in the study. The experimental and control groups each consisted of 14 men and 13 women. Subjects in the experimental group were instructed to consume an amount of bigeye tuna and swordfish during a 14-week period. The actual amount of fish consumption was calculated for each subject in the experimental group based on their individual body weights. Consumption of the tuna and swordfish was terminated after 14 weeks, and restricted (presumed to mean forbidden) during the following 15-week period. Intake of both bigeye tuna and swordfish was restricted for the entire 29-week period in the control group, which was otherwise instructed to continue their usual diets. The levels of MeHg were determined from two bigeye tuna and one swordfish purchased from a nearby wholesale fish firm. Total mercury concentrations in these fish were determined from 20 samples to be 1.08 (+/- 0.07) μg/g (ppm) for the tuna and 1.008 (+/- 0.11) ug/g for 10 samples from the swordfish. MeHg concentration was assumed to be 94% of the total mercury content. Hair mercury concentration was considered to be the indicator of MeHg exposure. Hair of all subjects grew at an average of 1.3 (+/- 0.2) cm every four weeks, and hair samples were thus collected from the 1.3 cm nearest the scalp at 4-week

intervals. Heart rate variability (HRV) was measured in all subjects three times: before exposure, after MeHg exposure for 14 weeks, and after the 15-week "wash-out" period following cessation of exposure. Subjects were also asked their health condition, such as the presence of tremor or finger numbness every four weeks at hair sampling, but no such findings were reported in the study. Yaginuma-Sakurai et al. (2009) reported that experimental and control hair mercury levels differed significantly ($p < 0.001$) at 9, 13, 17, 21, 25, and 29 weeks.

A number of parameters were used by Yaginuma-Sakurai et al. (2010) to evaluate HRV, with low frequency component coefficient of variation variability (CCV_{LF}) and power spectral density (PSD_{LF}) used to evaluate sympathetic cardiac activity and high frequency component coefficient of variation variability (CCV_{HF}) and power spectral density (PSD_{HF}) used to evaluate parasympathetic cardiac activity. The measurement protocol consisted of the subject resting in a supine position for 10 minutes, followed by the measurement of 300 R-R intervals of the ECG. Consecutive 100 R-R intervals with a minimal standard deviation (SD) were then automatically extracted by computer to minimize measurement bias. While several parameters indicated an increase in sympathetic activity after 14 weeks of exposure, heart rate remained unchanged; and only CCV_{LF} was statistically significant compared to total hair mercury. All such effects had disappeared by week 29. The authors concluded that MeHg consumption in their study induced a temporary sympathodominant state, and that long-term exposure to MeHg may pose a potential risk for cardiac events involving sympathovagal imbalance in fish-consuming populations. The levels of polyunsaturated fatty acids in plasma were found to be reduced in the experimental population.

As part of the Shiwha-Banwol Environmental Health Study in Korea, Lim et al. (2010) investigated an association between mercury and heart rate variability in 1,589 study participants with an average age of 33 years (range 5 to 83 years of age). Hair mercury levels ranged from 0.01 to 13.36 ppm, with an arithmetic mean of 1.02 ppm (S.D. +/- 0.77) and a geometric mean of 0.83 ppm (S.D. +/- 1.80). HRV was assessed in both time and frequency domains using standardized methods of the Task Force of the European

Society of Cardiology and the North American Society of Pacing and Electrophysiology (1996). High frequency (HF), low frequency (LF), very low frequency (VLF), total power (TP), LF/HF ratio, LF norm (LF/LF+HF) and H3F norm (HF/LF+HF) were used to evaluate the frequency domains. Standard deviation of the NN intervals (SDNN), which is the standard deviation of the average R-R intervals of the EKG calculated over a 24-hour period, was used as the index of time domain measure. SDNN is considered to reflect both sympathetic and parasympathetic influence on heart rate variability.

Lim et al. (2010) found that the hair mercury concentration negatively affected both the time and frequency measures in general, and particularly the HF parameter. Using multiple linear regressions and adjusting for selected variables, HRV was found to be decreased by 8.4% for each 1 ppm increase in hair mercury concentration. Hair mercury concentration was significantly higher ($p < 0.0001$) in those eating fish one or more times a week than in those consuming less than one fish meal per week. The authors pointed out that their results suggested that low levels of mercury intake may cause parasympathetic dysfunction, and that their findings were consistent with the findings of Sorensen et al. (1999), Grandjean et al. (2004), and Murata et al. (2006).

Blood pressure (BP) and HRV were studied in 205 Nunavik Inuit adults (arithmetic mean age of 52.1 years; range 40-73 years) living in northern Quebec, Canada (Valera et al. 2008). The gender composition of the study population was 85 men (mean age: 53.0 years) and 120 women (mean age 51.2 years). The specific measures of HRV were divided into time and frequency domains. Time domain measures were the average of all EKG R-R intervals (NN), the standard deviation of R-R intervals (SDNN), the standard deviation of the average R-R intervals calculated over 5-minute periods (SDANN), the square root of the mean squared differences of successive R-R intervals (rMSSD), and the proportion of interval differences of successive R-R intervals > 50 ms (pNN50). The rMSSD and pNN50 measures are indices of cardiac parasympathetic modulation. CVRR was calculated as (SDNN/NN) x 100. Frequency domain measures used were very low frequency (VLF, 0.0033-0.04 Hz), low frequency (LF, 0.04-0.15Hz), and high frequency (HF, 0.15-0.40 Hz). VLF was considered an index of parasympathetic activity and the

stimulus of neuroendocrine activity and thermogenesis. LF is an index of both sympathetic and parasympathetic activity, and HF is solely an indicator of parasympathetic activity. The LF/HF ratio represents sympathovagal balance. After adjustment for confounders, a statistically significant, positive correlation between MeHg and SDANN, SBP, and PP ($p = 0.026$, $p = 0.01$, and $p = 0.0036$, respectively) was found. CVRR tended to decrease with blood mercury concentrations, but this trend was not statistically significant ($p = 0.056$) (Valera et al., 2008).

In a study of 7-year-old Japanese children exposed to MeHg *in utero* through maternal fish consumption, Murata et al. (2006) assessed cardiac autonomic function in 136 subjects for whom umbilical cords had been preserved. MeHg in cord tissue was used as the biomarker of prenatal exposure. Of the maternal population, 15 women (11%) reported some drinking during pregnancy, and 10 (7.4%) reported smoking during pregnancy. No mothers ate shark or whale during pregnancy.

Immediately prior to testing, the children remained quietly in a supine position for five minutes (Murata et al. 2006). Then, 300 R-R intervals were measured, and consecutive 100 R-R intervals with the minimal standard deviation were automatically extracted by computer to avoid measurement error. No abnormal ECG findings were reported in the 7-year-olds, but cord tissue MeHg was correlated in a negative fashion with CCVHF and PSD_{HF} ($p = 0.027$), and positively with the LF/HF ratio and %LF ($p = 0.016$), indicating a negative parasympathetic effect and positive sympathetic effect on cardiac activity. Test results and cord MeHg for individual children were not reported, so it is not possible to determine the precise prenatal exposure level at which an adverse effect might have been seen. Likewise, it was not indicated whether there was a higher prevalence of autonomic effects in children of alcohol consuming mothers than in the children of mothers who did not report alcohol consumption during pregnancy. It has been previously reported that CCVLF and PSDLF seem to be reduced by chronic high-level exposures to alcohol, but be unaffected by low-level alcohol exposures during pregnancy (Murata and Araki 1995). The authors also calculated a maternal hair mercury equivalent concentration using the equation of Akagi et al. (1998): maternal hair Hg (in μg/g) at parturition =

25.24 x cord-tissue concentration (in ug/g dry weight). Murata et al. (2006) thereby estimated a median maternal hair Hg concentration of 2.4 μg/g (or 2.4 ppm; range: 0.43 to 9.26 μg/g), and suggested that a maternal hair concentration at parturition may be associated with reduced parasympathetic activity and/or sympathovagal shift.

The possible affect of prenatal MeHg exposure on blood pressure was also examined for the Seychelles Child Development Study (SCDS) cohort (Thurston et al. 2007). The measurements were obtained at participant target ages of 12 and 15. The 12-year-old subjects consisted of 313 boys whose mothers had a mean hair concentration of 6.6 ppm (range: 0.5-23.1 ppm) at parturition and 331 girls whose mothers had an average parturition hair concentration of 7.0 ppm (range: 0.7-26.7 ppm). The 15-year-old subjects consisted of 267 boys with a mean maternal hair mercury concentration of 6.5 (range: 0.5-20.0 ppm) at parturition and 292 girls with a corresponding mean maternal hair mercury concentration of 7.0 ppm (range: 0.8-26.7 ppm) at parturition. For girls, there was no significant correlation between prenatal MeHg exposure and blood pressure at either 12 or 15 years of age. Similarly, there was no correlation between prenatal exposure and blood pressure in boys at 12 years of age. However, diastolic blood pressure was found to be significantly increased in the15-year-old boys with increasing prenatal MeHg exposure ($p = 0.003$) using a linear regression model (compared with a p value of 0.59 for girls). Systolic blood pressure was unaffected. There was also a significant ($p < 0.001$) association between BMI (kg/cm^2) and diastolic blood pressure for both boys and girls.

As part of the Kuopio Ischemic Heart Disease Risk Factor Study, Rissanen et al. (2000) randomly selected 1,871 men aged 42-60 years who had no clinical coronary heart disease at baseline examination. After an average follow-up time of 10 years, 194 coronary events were reported. Of these, 100 were definite (fatal and non-fatal) and 60 were probable MIs; 34 were typical episodes of acute chest pain. At follow-up in the 194 subjects with coronary events, serum fatty acids were measured, and the percent proportion of the sum of docosohexaenoic acid (DHA) and docosapentaenoic acid (DPA) was calculated. The mean percent of serum DHA + DPA of all fatty acids was 3.01 +/-

0.79% (range: 1.39% to 7.51%). The test subjects were divided into five groups based on DHA + DPA measurements: <2.38%; 2.38% to 2.73%; 2.74% to 3.07%; 3.08% to 3.58%; and > 3.58%. The lowest fifth was then used as a comparison against the other groups. Men in the highest fifth of DHA + DPA in all fatty acids had a 44% reduced risk of acute coronary events compared to men in the lowest fifth. Men in the highest fifth of DHA + DPA who had low hair mercury (2 ppm or lower) had a 67% reduced risk of acute coronary events, compared with men in the lowest fifth DHA + DPA with a hair mercury level above 2 ppm (comparison group). The authors of that study concluded that their data provided support for the concept that fish oil-derived fatty acids reduce the risk of acute coronary events (Rissanen et al. 2000).

A prospective case-control study design was used by Hallgren et al. (2001) to investigate the relationship between high fish intake and congestive heart disease. This study used the cohort from The Vasterbotten Intervention Programme, an on-going community intervention program designed to prevent cardiovascular disease and diabetes. At the ages of 30, 40, 50, and 60, the study subjects were asked to complete a health survey including questions regarding social background, medical history, intake of drugs, education (years), and various lifestyle factors, including diet, daily current smoking, drinking habits, and stress. In the overall cohort, there were 243 cases of diagnosed myocardial infarction (MI), of which 78 qualified participants had been diagnosed with a first-ever MI after entering the intervention study. Control subjects (156 individuals with no history of MI or stroke) were randomly selected from the overall population completing the same health survey and were matched for age and geographic location of residence. There were 119 individuals from the first-MI and control populations who reported consuming one or more fish meals per week (high-intake group) and 110 individuals reporting the intake of less than one fish meal per week (low-intake group). Levels of erythrocyte-Hg and the *n*-3 fatty acids DHA and eicosapentaenoic acid (EPA) were significantly higher in subjects in the high intake group ($p < 0.001$) for total fish consumption. A negative correlation was found between the PUFAs and systolic blood pressure ($p = 0.029$), but not between the PUFAs and diastolic pressure or cholesterol. Univariate analysis revealed a decreasing risk of MI with increasing PUFA levels ($p =$

0.02), but increased with increasing serum cholesterol ($p = 0.05$). Hallgren et al. (2001) also reported a significant decreasing risk of MI with increasing erythrocyte-Hg levels, but the *p*-value for that relationship was only 0.07 (not generally considered statistically significant). Further, multivariate analysis of the data revealed that subjects with high erythrocyte-Hg and high PUFA levels had a markedly lower risk. The authors concluded that their data show as strong inverse association between the risk of first-ever MI and the biomarkers of fish intake, and that the erythrocyte-Hg and PUFA association is independent of traditional risk factors for MI.

Sorensen et al. (1999) evaluated the cardiovascular effects of approximately 1,000 7-year-old children in the Faroes Islands. These study subjects were part of the 1,022 cohort of children born to mothers who had ingested fish, pilot whale meat, and pilot whale blubber during pregnancy. Heart rate in the children was found to decrease with increasing body weight, height, and BMI. Although mean heart rate itself was found not to be associated with mercury exposure, the variability in heart rate did decrease slightly with mercury exposure, particularly in boys. Increased heart rate was found to be associated with increased diastolic and systolic blood pressures. After adjustment for body weight, blood pressure underwent an increase that reached a plateau as a function of prenatal MeHg exposure. However, the magnitude of this effect was not dose-dependent, with the greatest mercury-associated changes in blood pressure occurring in the low-level exposure range. Adjusted diastolic and systolic blood pressures decreased by 13.9 mmHg and 14.6 mmHg, respectively, when the cord blood mercury concentration increased from 1 µg/L to 10 µg/L. Above the 10 µg/L cutoff, blood pressures were not associated with mercury exposure. The authors concluded that their findings suggest that prenatal exposure to methylmercury may affect the development of cardiovascular homeostasis (Sorensen et al., 1999).

Fillion et al. (2006) studied 251 persons from six communities along the Tapajos River, a major tributary of the Amazon. The mean age of the study population was 35.2 years (range: 15-89 years), with 19.9% being over 50 years-of-age. Although 17.5% of the study population was regarded as overweight (BMI > 25 kg/m^2), only 4% were

considered obese (BMI > 30 kg/m^2). Fish consumption, blood pressure (systolic and diastolic), and hair mercury levels were examined. The weekly fish consumption level was 6.8 +/- 4.7 meals in the week preceding the measurement (3.3 +/- 3.6 piscivorous fish meals and 3.4 +/- 3.2 non-piscivorous fish meals per week). The mean hair mercury level was 17.8 µg/g (range: 0.21 – 77.2 µg/g), with 69.9% of the study participants having hair mercury levels of 10 µg/g or greater.

The mean systolic pressure in the Fillion et al. (2006) study was 113.9 mmHg (range 90-170) and the mean diastolic pressure was 73.7 mmHg (range: 60-110 mmHg). Univariate analysis revealed that systolic blood pressure was positively associated with age ($p < 0.001$), BMI ($p < 0.001$), and hair mercury level ($p = 0.046$). Systolic pressure was also higher in males ($p = 0.001$) and in smokers ($p < 0.01$). Elevated diastolic pressure was significantly associated only with BMI ($p = 0.01$). No association was observed between fish consumption and blood pressure.

There are differences of opinion regarding just how to interpret the collective volume of all published studies regarding the possible causal relationship of methylmercury to the effects observed. Mozaffarian (2009) and Stern (2005) both addressed this in their respective reviews of the current evidence on this subject. Mozaffarian (2009) pointed out that the conflicting results of prior studies of mercury and cardiovascular disease are not dose-related, with some studies with relatively low exposures reporting a positive association and other studies finding no significant associations at similar or higher exposures. Mozaffarian also notes that growing evidence suggests that fish consumption has benefits for health outcomes beyond CHD mortality, including non-fatal cardiovascular events. The positive effects of PUFA, including omega-3 fatty acids, appeared to provide an overall positive effect of fish consumption. Stern (2005), however, after reviewing the available literature on the same subject, arrived at the conclusion that a statement indicating that the benefits of fish intake exceed the potential risks is unsupported by the available data. He strongly felt that the data support the existence of a net negative effect of fish consumption on cardiovascular health.

The above studies considerably strengthen the data base on cardiovascular effects of MeHg exposure; however, there is no clear preponderance of data to unequivocally determine that MeHg can present significant health effects as a result of consuming contaminated fish. The data previously reported in the 1999 Toxicological Profile for Mercury do indicate that MeHg-induced effects might occur at exceptionally high dosages, but not at levels of MeHg seen from consuming fish.

Ocular Effects. The effects of MeHg on eye movements were examined in Cree Indian subjects from northern Quebec by Beuter and Edwards (2003). Measurements from 36 exposed Cree subjects, a group of 21 patients with Parkinson's disease, and a control group of 30 subjects were examined with an infrared eye-movement recording system. The tests consisted of recording pursuit, fixation, and prompted and remembered saccades for both eyes along both horizontal and vertical axes. The authors reported that eye movements were qualitatively different in MeHg-exposed Cree subjects than in both age-matched control subjects and subjects with Parkinson's disease. When Cree subjects were broken down into more-exposed and less-exposed groups, the average scores of the more-exposed group were reported to be clearly separated from those of the less-exposed and control groups for characteristics of fixation, pursuit, and accuracy and sharpness of prompted saccades.

2.2.2.3 Immunological and Lymphoreticular Effects

Methylmercury

Ortega et al. (1997) examined the effects of four different forms of MeHg in male Sprague-Dawley rats. Groups of six animals were given either tap water or water containing 5 or 500 µg of MeHg/L in the form of $MeHgS^-$, $(MeHg)_2S$, $(MeHg)_3S^+$, or MeHgCl for a period of eight weeks. The amount of water ingested by each animal was recorded daily. At the end of the study, it was determined that a 550-600 g rat drank approximately 30 ml H_20/day, equivalent to 0.35-0.46 µg Hg/kg/day.

Ingestion of the lower dosage of any of the four MeHg forms for eight weeks had no obvious adverse effect on general health, as assessed by total body weight and serum levels of BUN creatinine, and GPT (Ortega et al. 1997). No statistically significant differences were noted in these parameters for any experimental group or the control group. However, results of the lymphocyte proliferative response tests showed significant increases in immune response after mitogen stimulation in the 5 µg/L exposed groups. Compared to controls, the $MeHgS^-$ and $(MeHg)_2S$ exposed groups showed a two- to three-fold increase in the proliferative response, whereas the $(MeHg)_3S^+$ group had a significant decrease in the proliferative response compared to the control group. The MeHgCl exposed group showed nearly a four-fold increase in lymphocyte proliferative response. In comparison, in the 500 µg/L exposed animals, there was a significant decrease in the proliferative response in the $(MeHg)_3S^+$ and MeHgCl groups, with the other two exposed groups showing an enhanced response above the control group. Ortega et al. (1997) concluded that low-dose levels of MeHg can alter the lymphocyte proliferative response to mitogen stimulation, although the specific response may vary with the specific form of MeHg consumed. Further, that response can be elicited at doses near levels considered to be safe for human consumption.

In order to explore whether MeHg can cause the same mercury-induced autoimmunity seen with Hg^{++} exposure, Havarinasab et al. (2007) treated 33 male mice with drinking water containing MeHg at a concentration of 4.2 mg/L (equivalent to 420 ug Hg/kg body weight/day for 30 days. Controls (n = 5) received plain tap water. After 30 days, treatment with MeHg was stopped in all animals, and seven mice were euthanized. Six or seven mice were euthanized 2, 4, 6, and 8 weeks thereafter. Mesenteric lymph nodes, blood, (right) kidney, liver, and spleen were harvested for analysis. Serum anti-nuclear antibodies (ANA) were measured by indirect immunofluorescence. Serum anti-chromatin antibodies, anti-DNP antibodies (for polyclonal B-cell activation), IgG1, IgG2a, and IgE were assessed using the enzyme linked immunosorbent assay (ELISA) method. Speciation was performed for mercury found in mesenteric lymph nodes and the kidney.

Havarinasab found that a substantial titer of the IgG isotype of anti-nucleolar (ANoA) had developed after 30 days of MeHg treatment, compared with no ANoA in controls. Treated mice also showed a 20-fold increase of the mean ACA titer compared with controls ($p < 0.005$). The mean anti-DNP antibody titer was doubled following treatment for 30 days compared with controls ($p < 0.05$). Mean IgG2 concentration was doubled ($p < 0.005$), mean IgG1 was increased by 50% ($p < 0.05$), and mean IgE concentration showed a 6-fold increase, compared to control values, after 30 days of MeHg treatment. At the end of the 30-day treatment period, the kidney contained a total of 42 μg Hg/g tissue, 80% of which was MeHg. Lymphoid tissue contained only about 17% as much Hg as the kidney, but the fraction of MeHg was similar. In animals sacrificed at bi-weekly intervals following the cessation of MeHg treatment, the levels of anti-DNP antibody titer declined rapidly to the control level after just two weeks post-treatment and remained there for the following six weeks. Serum IgG2a declined after stopping MeHg treatment, but was still statistically significant at the two-week measurement, then declined to a non-significant plateau at four weeks post-treatment. IgG1 concentration also declined rapidly and was reduced to a non-significant level two weeks post-treatment. With respect to tissue Hg levels, total Hg had declined by ~ 70% in both kidney and lymph nodes during the first two weeks post-treatment. MeHg in these tissues showed a parallel decline similar to first order kinetics during the first 4-6 weeks post-treatment. A similar decline in the Hg^{++} concentration was seen in these organs during the first 2-4 weeks after cessation of MeHg treatment; it then declined further to a plateau at four weeks post-treatment. The elimination half-life for MeHg in kidney and lymph nodes was estimated to be 8 and 7 days, respectively, compared with an inorganic mercury half-life of 22 and 19 days for the kidney and lymph nodes, respectively. As the MeHg concentration declined, the concentration of Hg^{++} exceeded that of MeHg in the kidney at eight weeks post-treatment and in the lymph nodes at only three weeks post-treatment. The authors attributed the selective increase in Hg^{++} in the lymph nodes to be the likely result of the demethylation of MeHg in the macrophage-rich lymphoid tissue. Other changes were not statistically significant, when compared to control values. Havarinasab et al. (2007) concluded from their data that MeHg is able to induce an

autoimmune effect showing the same features as that caused by treatment with inorganic mercury.

Shenker et al. (2000) investigated the effects on human lymphoid cells in culture. The cells were obtained from the blood of healthy males and females aged 22-40 years. T-cells were isolated and treated with MeHg concentrations ranging from 0 to 5 µmoles. MeHg was found to induce a decline in mitochondrial membrane potential and intracellular pH, as well as the generation of reactive oxygen species. The MeHg-induced mitochondrial dysfunction also caused the release of cytochrome *c*, a factor that promotes apoptosis, from the mitochondria into the cytosol. [Szalai et al. (1999) had previously reported that apoptotic stimuli induce a switch in mitochondrial calcium signaling at the beginning of the apoptotic process by facilitation of a Ca^{++}-induced opening of the mitochondrial permeability transition pore. Thus, signals evoked by large Ca^{++} pulses or IP_3-mediated spikes in cytosolic Ca^{++} trigger mitochondrial permeability transition and subsequently the release of cytochrome c.]

Shenker et al. (2000) reported that whereas inorganic Hg (as $HgCl_2$) caused a significant elevation in T-cell Bcl-2, an anti-apoptotic protein, treatment with MeHg did not alter Bcl-2 levels. Those authors concluded that MeHg is a potent immunotoxicant by virtue of its ability to induce lymphocyte death in a manner consistent with apoptosis. Further, their data show that mitochondrial activity is severely perturbed in MeHg-treated cells.

In a study actually designed to investigate the effects of dietary fats on MeHg-induced acute toxicity, Jin et al. (2007) fed groups of 18 young male Sprague Dawley rats semi-purified casein-based diets containing either soy oil, seal oil, docosahexaenoic acid (DHA), fish oil, or lard for 28 days. Beginning on day 29, the rats were administered 0, 1, or 3 mg MeHg/kg/day by gavage for 14 days. On day 43 of the study, the rats were sacrificed and blood and selected organs were harvested for examination.

Jin et al. (2007) found that MeHg imposed a suppressive effect on the adaptive immune system, and a stimulation effect on the innate immune system. MeHg did not have a significant effect on lymphocyte counts. Granulocytes, however, were impacted by

MeHg exposure. In the 3 mg MeHg /kg/day dose group, monocyte counts were significantly increased in the fish oil group ($p < 0.001$), lard group ($p = 0.034$), DHA group ($p = 0.002$), and soy oil group ($p = 0.003$), compared to those receiving no MeHg. Eosinophil and neutrophil counts were also positively affected by MeHg ($p = 0.004$ and $p < 0.001$, respectively). While serum IgM levels were not significantly altered by MeHg, IgG levels were ($p < 0.001$). The correlation was negative, but varied by dietary fat group.

Hultman and Hansson-Georgiadis (1999) examined the extent to which organic mercury, in the form of MeHg, activates the immune system and causes systemic autoimmunity in a number of strains of mice genetically susceptible or resistant to inorganic Hg, a form of Hg well known to have adverse effects on the immune system. Female SJL/N, A.SW (H-2'), BALB/C, DBA/2 (H-2'), B10S (H-2'), A.TL (H-2'), and B10.TL mice were used in this study. All mice were 9-13 weeks old at the onset of the experiments. Mice were treated with subcutaneous (*s.c.*) injections of 1.0 mg MeHgCl/kg bw diluted with a sterile 0.84% NaCl solution on the dorsum every third day for 27 days. Controls were similarly injected with only sterile 0.84% NaCl. Serum immunoglobulins (Ig), anti-ssDNA antibodies, anti-DNP antibodies were assessed by ELISA. Anti-nuclear antibodies were assessed by indirect immunofluorescence and immunoblotting.

Increases in serum immunoglobulins were seen in some species, but not others (Hultman and Hansson-Georgiadis 1999). Table 1 shows the effects of MeHg treatment on IgE, IgG1, IgG2, and IgM concentrations at various times after the onset of MeHg treatment. Although treatment was for only four weeks, antibody levels continued to be analyzed at periods of up to 10 weeks after beginning MeHg injections (six weeks after the cessation of treatment).

In anti-nuclear antibody (ANA) and anti-nuclear autoantibody (ANoA) tests, differences were again seen among mouse species (Hultman and Hansson-Georgiadis 1999). In A.SW mice, ANoA developed after just two weeks of MeHg treatment, and the ANoA titer continued to increase during the ensuing two weeks. For SJL mice, six of nine had

developed ANoA, which increased another ~33% at 10 weeks, despite having stopped MeHg treatment six weeks prior.

In comparison, SJL controls showed no ANoA. Three of 10 MeHg-treated B10.S mice had a low ANoA titer at four weeks, but these titers had dropped to 0 in one animal, remained unchanged in another, and decreased to one-fourth of the 4-week titer in the third animal. In MeHg-treated A.SW mice, the anti-DNP antibody level was substantially increased after 3-10 weeks ($p < 0.001$). In treated A.TL mice, anti-DNP antibodies were elevated at week two ($p < 0.01$) in one set of experiments and at week three ($p < 0.05$) in a second set of experiments, and the titer of these antibodies was significantly ($p < 0.01$) elevated in BALB/C mice at three and four weeks. There was no significant change in anti-DNP antibodies in SJL or DBA mouse strains (Hultman and Hansson-Georgiadis 1999).

Anti-ss-DNP antibodies were significantly elevated ($p < 0.01$) in A.SW mice at the two-week measurement, in A.TL mice at the two week measurement ($p < 0.001$), in SJL mice at the week three measurement ($p < 0.05$), and in DBA mice at the week four measurement ($p < 0.001$), but not in BALB/C mice (Hultman and Hansson-Georgiadis 1999).

Table 1. Status of Ig levels following MeHg treatment

Strain:	**A.SW**	**A.TL**	**SJL**	**BALB/C**	**DBA**
IgE antibodies	increased at 2 weeks ($p < 0.05$); at 10 weeks, 5-fold increase ($p < 0.001$)	no significant change	increased at week 2, only ($p < 0.05$); fluctuations in both treated and controls	significant increase ($p < 0.05$) only after 10 weeks	no significant change
IgG1	tendency to increase 2-10 weeks; significant ($p < 0.05$) only after 10 weeks	increased 3-6 weeks after onset of MeHg; ($p < 0.05 – 0.001$); depending on week	no significant change	no significant change	no significant change
IgG2a	increased ($p < 0.01$) at 2-4 weeks; ($p < 0.001$); at 8 weeks	significantly increased at weeks 2 ($p < 0.01$) and 3 ($p < 0.001$); only	no significant change	below controls on weeks 4-10; p ranged from $< 0.05 – 0.001$)	no significant change
IgM	Significantly increased ($p < 0.01$); at 3 weeks, only	no significant change	no significant change	significantly increased ($p < 0.001$); at 2 weeks, only	significantly increased ($p < 0.001$); at 2 weeks, only

Source: Hultman and Hansson-Georgiadis (1999)

Hultman and Hansson-Georgiadis (1999) concluded from their experiments that while both MeHg and inorganic Hg induce the production of ANoA/AFA, the specific effect of the two different forms of Hg on the immune system is different both quantitatively and qualitatively. They further noted that the delayed onset of immune effects in MeHg-treated mice indicated the demethylation of MeHg may cause a response pattern characteristic of inorganic Hg; that is to say, the inorganic divalent Hg cation (Hg^{++}) may be the actual cause of the adverse immune sequellae.

Ethylmercury

Delayed-type hypersensitivity reactions following exposure to thimerosal are well-recognized (Ball et al. 2001). Allergic contact dermatitis induced by the mercuric and thiosalicylic acid groups of thimerosal was studied by Lebrec et al. (1999). T-cell responses to such substances involve CD4+ and CD8+alphabeta+ T-lymphocytes, as well as CD4/CD8 gammadelta+ T-cells. While T helper-2 cytokine production by drug-specific human T cells from patients with allergic contact dermatitis has been previously shown, these authors also reported that T helper 1-like and T cytotoxic 1-like responses clearly also play key roles in this cutaneous reaction.

2.2.1.4 Neurological Effects

A number of studies reporting a variety of neurologic effects have been reported since the publication of the Toxicological Profile for Mercury in 1999. Results of both human and laboratory animal studies have confirmed effects suggested prior to that date and expanded our knowledge of the adverse health outcomes that may be associated with exposure to MeHg and other alkyl mercurials. These studies are presented below under sub-headings that specifically refer to the specific type of study recapitulated under each.

Neurobehavioral effects (human studies)

Myers et al. (2003) reported the results of the Seychelles Child Development Study (SCDS) neurobehavioral testing of the 107-month-old cohort. The Seychellois diet contains about 10 times more ocean fish than is typically consumed by U.S. citizens (Davidson et al. 2006). Pre-natal maternal hair mercury levels reported by Myers et al. (2003) ranged from <3 ppm to > 12 ppm, with an arithmetic average maternal mercury concentration of 6.9 ppm for the overall test cohort. Pre-natal hair mercury concentration was determined from maternal hair growing during pregnancy. The mean age of children at testing was 107 months. A broad range of global and domain-specific tests were conducted to assess neurocognitive, language, memory, motor, perceptual-motor, and behavioral functions. Nearly all of the tests that were reported to show significant association with pre-natal exposure in the Faroes were included in this test battery. Statistically significant associations between prenatal MeHg exposure and performance were found for only two of 21 endpoints measured in the Seychelles cohort at 107 months-of-age. There was a significant decrease in performance on the grooved pegboard time for the non-dominant hand in males, and a significant improvement of the hyperactivity index of the Connor's teacher-rating scale as prenatal exposure increased. The authors pointed out that for these two endpoints, there were three outliers in the grooved pegboard non-dominant hand test and two outliers in the Connor's teacher-rating scale test, all of which had prenatal MeHg concentrations of 7.5 ppm or less and low performance on those tests. Interestingly, the Boston Naming Test, which was used as the most critical test in the derivation of EPA's RfD for MeHg, showed no statistically significant association with pre-natal MeHg exposure on this round of testing in the 107-month Seychelles cohort. It was pointed out that the Wexler's intelligence scale for children III full scale Intelligence Quotient (IQ) (WISC III) and Boston Naming Tests were both affected by cultural variation, with lower means for the Seychellois children than for US controls. The Faroes study did not obtain a HOME score or report SES, variables previously suggested to affect child development.

The data from the testing of the 107-month SCDS study (Myers et al. 2003) was used in a benchmark dose analysis reported by van Wijngaarden et al. (2006). The average 95% lower confidence limit (BMDL) of the BMD, across all 26 neurobehavioral endpoints

measured, varied slightly among the three models used (*k*-power, Weibull, and logistic). However, the choice of statistical model was reported to not greatly affect the BMDL estimates. The lowest BMDL of 20.1 ppm (range: 17.2 – 22.5) was calculated using the logistic model, while the highest BMDL calculated, 20.4 ppm (range: 17.9 - 23.0) was determined using the *k*-power model. The lowest individual BMDLs determined in this study were 17.2 ppm for the logistic model with the BMR set at 10% and 15.5 for the *k*-power model with the BMR set at 5%. The study authors recommend presenting an average BMDL and its corresponding range based on all available evidence to provide an indication of the exposure limits within which the true BMDL is likely to fall.

Further testing of the 107-month cohort in the SCDS was reported by Myers et al. (2004). The Child Behavior Checklist (CBCL) was administered to 643 members of the original cohort (enrolled in 1989-1990) in this prospective, longitudinal, double-blind study. The CBCL measures behavior in eight domains, and provides an overall behavioral index and 10 sub-scale indices. The prenatal and postnatal measures of exposure were total mercury in maternal and child hair samples, respectively. Prenatal exposure was determined in the longest available segment of maternal hair representing growth during pregnancy, assuming a growth rate of 1.1 cm per month. Postnatal exposure was measured in the centimeter segment of the child's hair closest to the scalp that was taken at the time of the 107-month evaluation (Myers et al., 2004).

For prenatal exposure, there were two sub-scales that showed significant or marginal *p* values and negative coefficients: Social Problems and Somatic Complaints. The pre-natal association with the Social Problems sub-scale was not significant with outliers ($p = 0.121$), but was without outliers ($p = 0.032$). The prenatal association for the Somatic Complaints sub-scale was not significant with outliers ($p = 0.31$), but was called "marginally significant" ($p = 0.067$) without outliers (Myers et al., 2004). The authors concluded that the (pre-natal exposure) effect on the Social Problems sub-scales was beneficial, while the (post-natal exposure) effect on the Thought Problems sub-scale that appeared to begin around 8 ppm was considered an adverse association. No other

associations with mercury exposure were apparent among the 22 behavioral endpoints studied (Myers et al., 2004).

For post-natal exposure, the Poisson model for the Thought Problems sub-scale showed an association with postnatal exposure both with ($p = 0.011$) and without outliers ($p = 0.013$). Using a semi-parametric additive model, some evidence for a non-linear association between post-natal exposure and the Thought Problems subscale was seen ($p = 0.01$). No effects were seen below approximately 8 ppm hair mercury, but an increasing trend toward adversity was observed above that level. The non-linear relationships between prenatal exposure and the Social Problems and Somatic Complaints sub-scales were not statistically significant.

While Myers et al. (2004) concluded that their data showed no "clear pattern of adverse associations between behaviors measured by the CBCL and either pre- or post-natal exposure, they also stated that their data suggest that fish consumption may pose both risks and benefits.

In a subsequent review of the previously reported SCDS 66-month and 107-month data and examination of new data, Myers et al. (2009) focused on post-natal MeHg exposure and any possible adverse health effects. In this study, three types of alternative post-natal exposure metrics were used to examine their association with the children's IQ at 107 months of age. Myers et al. (2009) found four endpoints adversely associated with post-natal exposure in one or both sexes. Post-natal mercury exposure was adversely associated with the Connor's Teacher rating Scale ADHD Index in both sexes. The WISC III FS IQ test, grooved Pegboard with the non-dominant hand test, and the Connor's Continuous Performance Task Risk Taking test revealed adverse post-natal mercury associations in girls only.

These authors pointed out that some associations in their primary analysis were in the direction of declining performance as post-natal exposure increased, while others were in the opposite direction. They did note, however, a reversal of associations between post-

natal exposure and endpoints from improved performance to deteriorating performance between the 66- and 107-month exams, which they felt were unexpected and of unclear significance. Although a number of adverse associations were found, Myers et al. (2009) noted that the results of their testing varied across ages and psychological domains. For example, in evaluating associations with mercury exposure and test performance, Myers et al. (2009) looked at all similar tests and pointed out that the grooved pegboard is a test of fine motor coordination, and that two other tests that also measure fine motor coordination (Finger Tapping and Trail Making A and B) showed no association with recent post-natal exposure. Thus, they state that no consistent or clear pattern of associations has emerged, and that their findings do not provide clear evidence for an adverse association between the levels of post-natal mercury exposure in this cohort. In their summary, Myers et al. (2009) do note that their findings raise intriguing possibilities and suggest that post-natal exposure should be studied prospectively.

The average post-natal hair mercury concentrations in the Seychelles cohort ranged from 6.6 ppm at 6 months of age to 4.8 ppm at 66 months and 6.9 ppm at 107 months. Thus, it is clear that post natal exposure has occurred on a relatively consistent basis since infancy in this fish-eating cohort. When the hair sample was taken for the post-natal measurements, it was taken from the 1cm segment nearest the scalp. As such, those measurements provided information on the most recent month only, and do not necessarily reflect levels of continuous mercury intake. It may be that relatively continuous exposure to small amounts of MeHg from eating fish has resulted in the test scores seen in the 107-month cohort. Future testing should provide more information.

As discussed in the 1999 version of the Toxicological Profile for Mercury, Grandjean et al. (1997) reported another epidemiologic study of MeHg exposure for a population in the Faroe Islands. Although the Faroese are a fishing culture, the major source of MeHg exposure for this population is pilot whale meat, which is intermittently consumed (1-2 meals/week) as part of the cultural tradition. The initial study cohort consisted of 1,022 singleton births occurring in a 21-month window during 1986-1987. At approximately 7 years of age, neurobehavioral testing was conducted on 917 of the

remaining cohort members. No abnormalities attributable to mercury were found during clinical examinations or neurophysiologic testing. A neuropsychologic test battery was also conducted to evaluate possible effects on specific domains of brain function. The neuropsychologic testing indicated mercury-related dysfunction in the domains of language, attention, memory, and visuospatial and (to a lesser extent) motor function, which the authors considered to remain after the children of women with maternal hair mercury concentrations above 10 μg/g (10 ppm) were excluded. A follow-up paper on this population suggested that the time-dependent susceptibility may vary for different brain functions (Grandjean et al. 1999). These researchers reported that the greatest susceptibility to MeHg neurotoxicity occurs during late gestation.

In a follow-up study of pre-natal exposure to polychlorinated biphenyls (PCBs) and deficits in cognitive development in infancy and through the preschool years, Stewart et al. (2003) conducted a reassessment of children in the Oswego Newborn and Infant Development Project at age 54 months. In a previous assessment of members of this study cohort at 38 months, Stewart and his colleagues had found a number of statistically significant predictors of small but measurable deficits on the McCarthy Scales tests. In addition, a statistically significant interaction between cord blood PCBs and maternal hair mercury was seen through compromised performance on McCarthy scales at 38 months in individuals with higher prenatal PCB exposure. The 38-month testing also found that higher PCB levels enhanced the negative association between MeHg and McCarthy performance, but not the other way round (i.e. MeHg did not enhance the negative PCB effect). In the 54-month testing, MeHg associations were not evident until PCB levels were elevated. In referencing the Faroe Islands study, Stewart et al. (2003) speculated that the highest levels of PCBs might have potentiated the MeHg effects, and further noted that the highest quartile of PCB exposure in the Oswego study is lower than the lowest quartile of PCB exposure in the Faroes study (Grandjean et al. 2001). The testing at 54 months showed no relationship between PCBs and/or MeHg. Stewart et al. (2003) suggested that the most highly exposed children had caught up to the least exposed children on McCarthy performance by 54 months, but that follow-up testing at later ages will be necessary to determine whether the PCB x MeHg interaction observed represents

a substantive and replicable interaction between these compounds, or just an isolated finding in a single report at a single stage of development.

Crump et al. (1998) conducted BMD analysis and further regression analyses of data collected in a study in which a series of scholastic and psychological tests were administered to children whose mothers had been exposed to MeHg during pregnancy. Hair samples were collected from 10,970 new mothers in New Zealand in 1977 and 1978. High hair mercury levels were considered to be those over 6 ppm, the hair level predicted to result at steady state from consumption of mercury at the WHO/FAO Provisional Tolerable Weekly Intake of 0.3 mg total mercury/week and 0.2 mg methylmercury/ week). By this criterion, 73 of approximately 1,000 mothers who had consumed fish more than 3 times/week during pregnancy were determined to have high hair mercury levels. In 1985, when the children were 6 to 7 years of age, 61 children (including one set of twins) of the 73 mothers in the high hair mercury group were located; these children constituted the high exposure group, which was matched with three control groups [one with 3-6 ppm (μg/g) maternal hair mercury levels, one with 0-3 ppm maternal hair mercury levels whose mothers had been identified as "high fish consumers," and one with 0-3 ppm maternal hair mercury levels whose mothers had not been high fish consumers]. The entire study cohort consisted of 237 children. A battery of 26 psychological and scholastic tests was administered to the children at school during the year 1985. Mothers were interviewed at the time of test administration to obtain additional data on social and environmental factors. In the high exposure group, one boy's mother had a hair mercury level of 86 ppm, which was more than four times higher than the next highest hair mercury level of 20 ppm. BMDs (10% response rate) calculated from five tests ranged from 32 to 73 ppm, when the child of the mother with the hair mercury level of 86 ppm was included. This corresponded to a BMDL range of 17 to 24 ppm. The BMDL is a modeled number considered to correspond to an experimental no-observed-adverse-effect-level (NOAEL) (Farland and Dourson 1992) . Although none of the test scores of the child whose mother had the hair mercury level of 86 ppm were outliers according to the definition used in the analyses, his scores were significantly influential in the analyses. When this child was omitted from the analyses,

BMDs ranged from 13 to 21, with corresponding BMDLs of 7.4 to 10 ppm. According to this most conservative interpretation of the New Zealand data, no neuro-psychological effects would be seen (and were not seen) in the offspring of women with hair mercury levels at or below the bottom of this BMDL range (i.e., 7.4 ppm maternal hair mercury level).

Following the publication of the results of the Faroes (Grandjean et al. 1997) and New Zealand (Crump et al. 1998) studies, Palumbo et al. (2000) conducted a reanalysis of the possible association of MeHg with performance on the McCarthy Scales of Children's Abilities (MSCA) in the 66-month Seychellois cohort. Since no association between MeHg exposure and performance on the MCSA General Cognitive Index had been found in this cohort (Davidson et al. 1998), Palumbo and co-workers conducted further analyses to determine whether associations on specific subscales of the MSCA could be identified. After analysis of standard MSCA subscales, more specific subscales of the MSCA were defined and analyzed using a neuropsychological approach. In this process, subscales were recombined to approximate the domains of cognitive functioning evaluated in the Faroes and New Zealand studies. Palumbo et al. (2000) found that analyses of both the standard and recombined MSCA subscales showed no adverse associations with methylmercury exposure and the neuropsychological endpoints examined.

Debes et al. (2006) reported the results of neuropsychological testing of the Faroes child cohort at 14 years of age. In this paper, 878 members of the initial 1,022 mother-child pair cohort underwent detailed neurobehavioral examination. Eighteen of the participating children were excluded due to existing neurological disorders. The neuropsychological test battery used was based on the same criteria as applied for previous testing at 7 years of age. Debes et al. (2006) reported that higher prenatal MeHg exposure, as indicated by cord blood mercury concentration, was associated with lower finger tapping scores, increased reaction time, and lower cued naming scores. Maternal air mercury concentration showed significant or near-significant associations with deficits only on three conditions of finger tapping and the two measures of reaction time. Yet another measure of exposure, cord tissue mercury, showed associations with deficits

on the naming and verbal learning results only. As with the Seychelles testing, not all associations with pre-natal mercury exposure were negative. Higher MeHg exposure was associated with better scores on the WMS-II Spatial Span test in this Faroes cohort.

Debes et al. (2006) also reported that prenatal MeHg exposure seemed a less important predictor of neuropsychological performance at age 14 than at age 7 years; however, they noted that this risk factor (prenatal exposure) appeared to represent about the same proportion of the total variance explained by the regression model at the two testing occasions.

Post-natal exposures were, in general, reported by Debes et al. (2006) to be only weakly related to cognitive test scores at 14 years, with the only statistically significant association being the NES2 finger tapping score for the preferred hand. Likewise, PCB exposure, although only available for half of the subjects, was reported to show only weak associations with the outcomes, none of them reaching statistical significance.

Jedrychowski et al. (2006) evaluated the effects of prenatal MeHg exposure through maternal fish consumption on cognitive and motor function in 1-year-old infants in Poland. The study cohort consisted of 233 infants born between January 2001 and March 2003. The *in utero* period of the infants ranged from 33 to 42 weeks, with 91% being born after 38 weeks of gestation. The maternal population consisted of Krakow residents, who attended ambulatory prenatal clinics in the first and second trimesters of pregnancy and who had singleton births. Those women ranged in age from 18 to 35 years, and all were non-smokers. Maternal blood (30 to 35 ml) was collected at delivery, as was a comparable amount from venal umbilical cord blood. The geometric mean blood mercury level was 0.55 µg/L (range: 0.10 to 3.40 µg/L) for mothers at delivery. Of these subjects, 75% had whole blood mercury levels <1 µg/L; and 90% had levels not greater than 2 µg/L. The geometric mean of cord blood mercury was 0.88 µg/L (range: 0.10 to 5.00 µg/l). Of the infants, 60% had cord blood mercury levels <1 µg/L and 90% had cord blood mercury levels below 2 µg/L. The Bayley Scales of Infant Development

II (BSID-II) tests were conducted to assess infant mental and motor function at approximately one year of age (Jedrychowski et al. 2006).

On testing, Jedrychowski et al. (2006) found that 197 infants had normal performance on the Motor and Mental scales of the BSID-II, while 36 infants showed delayed motor and/or psychomotor performance on these tests. Of the infants with normal test performance, the geometric mean maternal mercury blood level was 0.52 μg/L, whereas the mothers of infants showing delayed performance had blood mercury levels with a geometric mean value of 0.75 μg/L. The difference in these two values was statistically significant at the $p < 0.01$ level. In contrast, the difference between geometric mean cord blood levels in the normal-result and delayed performance infant groups was not statistically significant ($p = 0.07$). The geometric mean cord blood values in Jedrychowski et al. were 0.85 μg/L for the normal group and 1.05 μg/L for the delayed performance group. However, blood mercury levels and test performance data were not reported for individual mother-infant pairs (Jedrychowski et al. 2006).

Carta et al. (2003) investigated the possible sub-clinical neurotoxic effects associated with relatively low levels of mercury ingested through fish consumption in adults. The subjects were divided into two groups. The test group consisted of 22 males with a median age of 51.5 years (range: 35-61) who were habitual consumers of fresh tuna and had no recent or previous occupational exposure to mercury or other CNS toxicants. This group consumed an average of 2.5 fish meals per week (range: 1-7). The control group consisted of 22 administrative clerks with a median age of 51.1 years (range: 32-57) and with no occupational exposure to mercury or other neurotoxicants. The control group consumed an average of 1 fish meal per week (range: 0-3). All subjects were administered a cross-sectional field study battery, including neurobehavioral tests of vigilance, psychomotor function, and hand tremor, as well as a serum prolactin (PRL) assessment and urine mercury analysis. Blood organic mercury measurements were available for only 10 exposed and six control subjects. Median values for blood organic mercury were 41.5 μg/L (range: 13-85) for the exposed group and 2.6 μg/L (range: 0.8-4.0) for the control group. Total blood mercury median values were 44.0 μg/L (range:

15-93) for the exposed group and 3.9 ug/L (range: 1.2-5.4) for controls. Both of those differences were reported to be highly significant ($p < 0.001$). Median urine mercury values (in µg Hg/g creatinine) were significantly different between the two groups, with a median value of 6.5 (range: 1.8-21.5) for the exposed group and 1.5 (range: 0.5-5.3) for the control group ($p = 0.001$). Serum PRL values were 12.6 ng/ml and 9.1 ng/ml for the tuna consumers and controls, respectively, and the difference was statistically significant ($p = 0.0006$). Statistically significant decrements in performance were found between the two groups for the color and word reaction time ($p = 0.003$), digit symbol reaction time ($p < 0.001$), and finger tapping (both hands) ($p = 0.037$) speed tests.

In a similar study, Yokoo et al. (2003) examined 52 men and 77 women with a mean age of 35 (range: 17-81 years) for neurocognitive deficits that may be associated with MeHg intake through fish consumption. The subjects were from six villages located along the Culaba River in Brazil. The mean hair mercury concentration of the 129 subjects was 4.2 µg/g (range: 0.56-13.6 µg/g). The neurocognitive screening battery included tests from the Wechsler Memory Scale and Wechsler Adult Intelligence Scale, Concentrated Attention Test of the Toulouse-Pierron Factorial Battery, Manual Ability Subtests of the Tests of Mechanical Ability, and Profile of Mood States. Statistically significant decrements in performance were reported for fine motor speed ($p = 0.005$), logical memory ($p = 0.014$), digit span ($p = 0.05$), digit span backward ($p = 0.03$), easy learning ($p = 0.03$), and errors-commissions tests ($p = 0.015$), and the decreased performance was correlated with increasing hair mercury levels.

Axelrad et al. (2007) used a Bayesian hierarchical model to integrate data from the Faroes, Seychelles, and New Zealand studies to estimate a dose-response relationship between maternal mercury body burden and subsequent childhood decrements in IQ. All cognitive end-points reported in these three studies were considered for inclusion in the model (*i.e.*, endpoints from the Faroes testing at 7 years, the Seychelles testing at 9 years, and the New Zealand testing at 6 years). This integrated analysis produced a central estimate of -0.18 (95% CI, range -0.378 to –0.009) IQ points for each ppm maternal hair mercury. The authors concluded that while IQ does not represent all neurodevelopmental

deficits associated with mercury, it nevertheless provides a broad-based measure of effects on cognitive development and can be readily applied to estimate benefits of reducing mercury exposures in the population.

Gao et al. (2007) investigated the effects of prenatal Hg exposure on neurobehavioral development in neonates born to exposed mothers in Zhoushan City (Zhejiang Province), China. Zhoushan was selected as the site for this study in large part because it is an important coastal fisheries area and is well-known for its fish output and the heavy consumption of seafood among the residents. In the period August-October of 2004, term infants (36-42 weeks of gestation) born in the Zhoushan Women's and Children's Health Hospital were recruited for the study. After screening, 408 mother-infant pairs (203 male and 205 female infants; mean maternal age 26.6 +/- 3.5 years) were included in the study. Hair samples were collected from the mothers 1-3 days after delivery and submitted for total Hg analysis using cold vapor atomic absorption spectrometry. To estimate maternal fish intake and general nutritional status, the authors employed a standardized questionnaire in which each woman was asked to estimate the frequency of fish intake and provide a weekly average intake of shellfish, seafood, and freshwater fish consumed as a main meal. When the infants were three days old, a neonatal behavioral neurological assessment (NBNA) was administered to assess functional abilities, most reflexes and responses, and the stability of behavioral status during the examination. The NBNA consists of five clusters: behavioral (six items), passive tone (four items), active tone (four items), primary reflexes (three items), and general assessment (three items).

Gao et al. (2007) found that 91% of the study women ate fish at least once per week during pregnancy, and 91.9% of those were more likely to consume marine than freshwater fish. None of the women drank alcohol, smoked, had treated hair, or used skin-lightening products during pregnancy, and no family members worked as dentists or thermometer or fluorometry makers. The geometric mean hair Hg level was 1.247 ppm (interquartile range 0.927 – 1.685 ppm), and the geometric mean cord blood level was 5.58 µg/L (interquartile range 3.96 – 7.82 µg/L). The frequency of fish intake during pregnancy was positively associated with both maternal hair Hg and cord blood Hg ($p <$

0.001). Mothers who consumed fish more than five times weekly had much higher (~ 2 times) hair and cord blood Hg levels than those who rarely consumed fish. Of 384 neonates taking the NBNA, 94% were considered well developed in neurobehavioral function, and only 1% were considered abnormal. Over 99% scored full marks in the primary reflexes and general assessment categories. The total NBNA test scores, behavioral scores, and passive tone scores of male newborns were all significantly higher than those of female newborns ($p < 0.05$), but there was no difference between the active tone scores of males and females.

Gao et al. (2007) concluded that both maternal hair and cord blood Hg levels were correlated with the frequency of fish consumption. While it appeared that the behavioral ability of male neonates was significantly affected by prenatal exposure to mercury, no such effect was observed in females. Gao et al. (2007) noted that long-term impact of exposure to Hg on childhood development needed to be investigated to exclude any chance findings.

Fish consumption habits and their effect on fetal and infant neurodevelopment were studied in a population of 100 mother-infant pairs in Amazonian women (Marques et al. 2007). These volunteers, between the ages of 15 and 45 years, were recruited by nurses during routine visits to prenatal clinics in three hospitals in Porto Velho, Brazil. Upon enrollment, a complete clinical evaluation was obtained from medical records, and an interview questionnaire was applied to assess the socioeconomic and educational status of the study participants, as well as their food habits, frequency of fish consumption, and their intention of breastfeeding. The median income of this cohort was $125 U.S. dollars (range: $16.67 - $1,250.00), and 64% did not have indoor plumbing, indicating that the majority of the women in this study were socioeconomically underprivileged. Fish consumption, which was irrespective of income and education, was relatively high, with 57% reporting consumption of fish up to once a week and 43% consuming 2-7 fish meals per week. Only 5% reported no fish consumption, and 4% reported more than 7 fish servings a week.

During the birth visit to the hospital, samples of cord blood, placenta, and hair were obtained from both mothers and newborns (Marques et al. 2007). Median measured Hg values at parturition were 7.44 ppb (range: 0.12-43.74) for umbilical cord, 8.10 ppb (range: 0.37-56.28) for placenta, 0.55 ppb (range: 0.01-9.97) for maternal blood, 5.40 ppm (range: 0.39-62.43) for maternal hair, and 1.59 ppb (range: 0.05-19.65) for fetal/neonatal hair, with 92% of the neonatal hair samples being less than 6 ppb. Placental Hg was also positively correlated with maternal hair-Hg ($p < 0.01$), newborn hair-Hg ($p < 0.05$), maternal blood-Hg ($p < 0.05$), and umbilical cord-Hg ($p < 0.01$). The positive correlation between maternal hair-Hg and infant hair-Hg remained when measured again at 6 months ($p < 0.01$).

At six months of age, only 86 of the initial 100 mother-infant pairs reported for clinical and neurobehavioral examination; additional hair samples were collected at that time (Marques et al. 2007). The infants' developmental assessment was conducted by trained professionals using the Gesell Developmental Schedules, which included all reactions (voluntary, spontaneous, or learned) and reflexes. Postural reactions, hand pressure, locomotion, coordination, constructive ability (influenced by motor development), visible and audible communication, and individual reactions regarding people and stimulations were also examined at that time. The results of these tests were expressed as developmental scores for motor skills, language development, adaptive behavior, and personal social behaviors.

The results of the Gesell Schedules testing revealed that 74% of the infants showed normal schedules, while 26% (21 children) exhibited developmental delay in one or more features of the Gesell (Marques et al. 2007). Of these, 1% showed signs of motor impairment, 9% had language deficits, and 16% had multiple impairments (including motor and language delays). However, no children showed any delay in personal social behavior or social ability. The 26% with neuromotor developmental delays had higher median hair-Hg values, and all were from mothers who had higher hair-Hg levels than mothers of normally-developed infants.

Neurobehavioral effects (laboratory animal studies)

A number of studies of non-human mammals have revealed additional evidence of developmental effects following oral exposure to organic mercury during gestation, lactation, and/or post-weaning since the publication of the Toxicological Profile for Mercury in 1999.

Pregnant transgenic mice (cross between ARE-hPAP and C57BL/6) were administered 0.5 mg Hg/kg body weight/day (as CH3HgOH; equivalent to 0.47 mg/kg/day of Hg) in drinking water from day 7 of gestation through 7 days post-partum (Onishchenko et al. 2007). Control dams received tap water for the same period. Brain tissue Hg was measured in one or two pups from three different litters at post-natal day (PND) 8 (day after termination of maternal exposure) and in 4-week-old mice (before first behavioral experiments). Brain mercury concentrations at PND 8 were 2.60 μg/g (+/- 0.19 μg/g) in dams and 0.93 μg/g (+/- 0.02 μg /g) in pups, compared with control brain Hg levels below 0.006 μg/g.

Pups (6-8 per group) underwent behavioral testing during post-natal weeks 5-15 and 26-36. Onishchenko et al. (2007) monitored the behavior of the offspring using an automated system (Intelli-Cage) designed for continuous long-term recording of home cage behavior in social groups, as well as analysis of basic activities and learning. Spontaneous locomotion, motor coordination on an accelerating rotarod, spatial learning in the Morris water maze, and depression-like behavior during the forced swimming test were also studied. In the behavioral tests, males (but not females) showed decrements in performance related to MeHg exposure. For the locomotor and rotarod tests, there was no difference in performance between MeHg-exposed mice and controls during either testing period (Onishchenko et al., 2007).

In Intelli-Cage behavior during the 5-15 week period, MeHg-exposed mice were much slower in exploring their new "novel-enriched" environment ($p < 0.05$). The activity

during 12-hour day-night cycles was also different between the two groups. While peak activity periods were similar, the MeHg-exposed mice were less active during the dark periods ($p < 0.05$). Differences in patrolling behavior in a changed environment were also observed in the Intelli-Cage. The MeHg mice were significantly slower to correctly seek-out and learn reward areas (containing water, for example) ($p < 0.05$), and unlike controls, the exposed mice did not improve during subsequent testing of the same lay-out ($p < 0.05$). However, no such differences between MeHg exposed and control mice were seen during the 26-36 week observation period (Onishchenko et al. 2007).

The forced swimming test evaluates an animal's response to an inescapable adverse situation (such as being placed in a beaker of water). In such a situation, an animal typically exhibits either an active (swimming, climbing on the walls) or inactive (floating) behavior. The floating can be interpreted as a measure of depressive-like behavior (Porsolt et al., 1977). In the Onishchenko et al. (2007) experiment, the MeHg mice displayed significantly ($p < 0.05$) longer immobility time (passive floating without limb movements) than control mice during both the 5-15 week and 26-36 week testing intervals. In contrast, there was no difference between MeHg-exposed mice and control mice in spatial learning in the Morris Swim Maze (Onishchenko et al. 2007).

Onishchenko et al. (2007) concluded that low-level exposure to MeHg during development induces alterations in learning and depression-like behavior. Further, they stated that their results provided evidence that developmental exposure to MeHg can affect not only cognitive function, but also motivation-driven behaviors.

Sakamoto et al. (2002) examined the effects of MeHg on neurobehavioral performance tests using studied changes in brain mercury concentration of rat pups exposed to MeHg throughout embryonic development, during breast-feeding, and after weaning. In this study, adult female rats were given a diet containing 5 ppm mercury (as MeHg) for 8 weeks prior to mating, with no apparent adverse effects. This diet was continued throughout gestation and after parturition. Newborn offspring were weaned at day 30 of life and then placed on the same diet as the mothers. On the day of birth, rat pups had

blood mercury concentrations significantly higher ($p < 0.01$) than that of the mother (*i.e.*, about 1.5 times that of the mother on the same day). Rat pups sacrificed at birth were found to have brain mercury concentrations 1.5 times that of the mothers. This concentration declined during the suckling period to just 1/5 of that measured in the rats sacrificed at birth. Sakamoto et al. (2002) stated that this might be explained by the limited MeHg transfer from milk and the rapid increase in the brain and body volumes. Once the weaned pups were placed on a MeHg-contaminated diet, the brain mercury concentration began to gradually rise again. When behavioral tests were conducted during the 5th and 6th weeks of life, exposed rats showed a significant decrement in performance on the rotarod motor coordination test, as well as decreased learning ability in the passive avoidance response test, compared to controls. Focal cerebellar dysplasia, including heterotopic location of Purkinje cells and granule cells was observed post-mortem.

Kakita et al. (2002) studied the effects of fetal MeHg exposure on neuronal migration in the developing cerebral cortex. To accomplish this, pregnant rats were administered both MeHg and 5-bromo-2-deoxyuridine (BrdU) on gestational days 11, 13, 16, or 21. Histopathological examination of offspring sacrificed on postnatal day 28 revealed no apparent cytoarchitectural abnormalities in either the primary motor or primary somatosensory areas of the cerebrum. Further, morphometric analysis of these areas revealed no differences in total neuron population, nor were there any differences in subpopulations of any of the cortical layers, when compared to controls. However, BrdU immunohistochemistry revealed an abnormally widespread distribution of the labeled cells throughout cortical layers II-VI of offspring exposed to MeHg on gestational days 16 and 21, indicating disruption of the inside-out pattern of neuronal migration (Kakita et al., 2002).

Newland and Reile (1999) exposed groups of 10 female rats each to either 0, 0.5, or 6 ppm Hg (as MeHg chloride) in drinking water. For half of the rats in each exposure group, treatment was begun 4 weeks prior to mating, with the remainder of the test animals exposed for 7 weeks before mating. Maternal exposure continued to 16 days

postpartum. All mating males were unexposed. Levels of mercury in blood taken from pups on the date of birth and again on the day of weaning (21 days postpartum), as well as brain mercury levels, were found to be unrelated to the duration of maternal exposure before mating. (Reproductive success, however, was found to be related to the duration of maternal pre-mating exposure.) Pup brain and blood mercury levels were found to be dependent on maternal consumption during gestation. Post-partum brain mercury levels in the pups decreased from 0.49 to 0.045 ppm in the low-dose rats and from 9.8 to 0.53 ppm in the high-dose group animals, leading to the conclusion that maternal exposure during lactation apparently did not result in significant exposure of nursing pups. ;Brain Hg concentrations at weaning were approximately 10-fold lower than the concentrations at birth in the 0.5 ppm group, but were about 20-fold lower in the 6 ppm group. This may be in part due to an average 5.5-fold increase ($p < 0.0001$) in brain size between birth and weaning and the apparent lack of significant transfer of mercury from the dams to the pups during the lactation period.

In another study, Watanabe et al. (1999a) examined the effects of prenatal MeHg exposure on the fetal brains of mice born to exposed dams. Injections of 3 mg Hg/kg as MeHg were administered to pregnant mice on gestational days 12 through 14, inclusive. Postmortem analysis of fetal brains collected on gestational days 14 or 17 revealed significantly elevated levels of glutathione (reduced, GSH). Thiobarbituric acid-reactive substances (TBARS) in fetal brain tissue showed a similar, but non-significant, trend.

The potential for low-dose exposure of pregnant rats to MeHg to result in ictal effects in offspring was investigated by Szasz et al. (1999). Eighteen four-week-old offspring of dams exposed to MeHg (equivalent to 0.375 mg/kg/day) during gestation and 12 controls were examined for possible MeHg-induced changes in epileptogenic activity produced by 3-aminopyridine in the neocortex. Epileptogenicity was found to be significantly increased (*p* value not provided) in offspring of mercury-treated animals when compared with controls. This activity was characterized by an increase in the frequency of periodic ictal activity, facilitated propagation of epileptiform discharges, and a strong tendency toward generalization. In addition, the amplitude of seizure discharges was significantly

lower in MeHg-treated animals than in controls, possibly due to a loss of cortical neurons. The authors concluded that the synaptic and membrane mechanisms responsible for the initiation and propagation of paroxysmal activity were probably facilitated as a result of maternal MeHg exposure, while the efficacy of cortical inhibition in preventing initiation and the spread of epileptiform discharges was reduced by exposure of the developing nervous system to mercury.

Pregnant BALB/c, C57BL/6J, and C57BL/6Cr mice were administered oral doses of 0 or 3 mg MeHg/kg/day (as MeHg chloride) during gestational days 12-14 (Kim et al. 2000). The behavior of male offspring in open field, in their home cage, and in a Morris water maze was subsequently evaluated. While treated BALB/c and C57BL/6Cr offspring exhibited less total locomotor activity than did their respective controls, no significant difference was observed between the C57BL/6J treated and control groups. In BALB/c male offspring, the MeHg-treated mice exhibited significantly more central locomotion, but less peripheral locomotion, than did their control group. With respect to spontaneous home cage activity, all but the BALB/c MeHg-treated offspring moved more actively in the dark phase than in the light phase. BALB/c activity was the same in both light and dark phases, suggesting a possible disturbance of nocturnal rhythm of spontaneous activity. In the Morris water maze, prenatal exposure to MeHg resulted in significantly prolonged latency in the C57BL/6J and C57BL/6Cr mice, but not in the BALB/c strain. Kim et al. (2000) concluded that their study demonstrated that intraspecies differences in neurobehavioral performance can occur in mice treated with MeHg.

In another study (Dore et al. 2001), groups of pregnant C57BL/6 mice were administered daily oral doses of 4 or 6 mg MeHg/kg by peroral injection during gestational days 7-9 or 12-14. Female offspring 6 to 16 weeks of age were subsequently tested on a variety of behavioral tasks, including motor coordination, visual discrimination, open-field behavior, and spatial alternation training. This study found that, overall, more detrimental effects were observed in the female offspring of animals administered MeHg on gestational days 12-14 than among those being dosed on days 7-9 of gestation. Among the most remarkable effects were a reduction in locomotor activity and impaired

reference memory for egocentric (location from the perspective of the subject/observer) and allocentric (location of one object relative to the location of another object) spatial information, and working memory for places.

Cooper and Kusnecov (2007) studied the effects of MeHg exposure on exploratory behavior in C57BL/6J mice. Six-week-old male mice were administered 0, 2, 4, or 8 mg/kg doses of MeHg (administered as MeHg chloride) by *i.p.* injection and subsequently exposed either to an open field (OF, with or without a novel object) or allowed to remain in its home cage (HC). Controls received injections of saline. OF exposure occurred one hour after injection and lasted for 15 minutes. For the first 10 minutes, the animals were allowed to freely explore the field. A novel object (metal cylinder) was then placed in the center of the field for the remaining five minutes. The OF was divided into zones for purposes of evaluating preference for location. During the 10-minute period, there were two zones (outer perimeter and inner zones). For the last five minutes of OF exploration, there were three zones (outer, inner, and centered novel object zones). In a later part of the experiment, 2 or 4 mg/kg MeHg or saline was administered *i.p.* every third day over a 15-day period. One hour after the final injection, mice were either examined in the OF or allowed to remain in the HC.

Cooper and Kusnecov (2007) found that regardless of treatment, the time spent in the outer zone increased significantly ($p < 0.001$) after the novel object was introduced. Without the novel object, there was a dose-dependent increase ($p < 0.001$) in time spent in the outer zone following treatment with MeHg. The 4 mg/kg and 8 mg/kg groups showed the longest duration of time in the outer zone, compared with controls, with statistical significance levels of $p < 0.001$ and $p < 0.0001$, respectively. However, this significant trend was not seen with the repeated treatment animals, with or without the introduction of the novel object. Exploration of the inner zone was dependent on the presence of the novel object. In the absence of the novel object, the animals spent a significantly higher ($p < 0.0001$) percentage of time in the inner zone, with time spent in that zone being inversely related to the magnitude of MeHg dosage. However, the total distance traveled in the OF without the presence of the novel object was found to

decrease in a dose-dependent fashion ($p < 0.0001$), with no significance being seen in the 2 mg/kg group compared with controls ($p = 0.055$). When the novel object was introduced into the OF, there was a significant reduction in inner zone exploration for all groups, with the 4 and 8 mg/kg groups showing the greatest reduction in exploration ($p = 0.03$ and $p = 0.04$, respectively). Time actually spent in the novel object zone showed no significant differences among treatment groups. In the case of repeated exposures over a 15-day period, there were no significant differences in time spent in the inner or novel object zones, compared to controls.

In a separate part of their study, Cooper and Kusnecov (2007) sacrificed animals repeatedly exposed to MeHg and removed their brains for c-Fos immunochemical testing. Stressor, dosing regimen, and dose-dependent changes in neuronal c-Fos were observed in stress-associated regions of the brain. When compared with control and HC mice, marked increases were seen in the number of c-Fos positive immunoreactive cells in MeHg-exposed mice the thalamus ($p < 0.0001$), hypothalamus ($p < 0.001$), hippocampus ($p < 0.0001$), central amygdaloid nucleus ($p < 0.0001$), lateral septum ($p < 0.0001$), bed nucleus of the stria terminalis ($p < 0.001$), and locus coeruleus ($p < 0.0001$). The c-Fos response was not permanently altered within these stress-associated nuclei, however, as the c-Fos response was back down to control levels just 3 days after the last repeated MeHg dose.

In a study using mouse neural crest (NC) cells, Carey and Matsumoto (1999) reported that up to 50% of the cultured NC cells exhibited calcium transients during the period of neuronal differentiation. As neurogenic activity declined, the percentage of active NC-derived cells and their calcium spiking frequency also declined. Thimerosal (containing EtHg) was found to increase the frequency of oscillations in active NC-derived cells and induce them in a subpopulation of quiescent cells. As neurogenesis ended, NC-derived cells became nonresponsive to thimerosal.

Effects on Special Senses (vision, hearing, touch: humans)

Takaoka et al. (2004) measured tactile sensation in subjects believed living around the MeHg-polluted area of Minamata City, Japan. The study subjects were either certified Minamata disease patients or other outpatients of Minamata Kyoritsu Hospital. The study subjects were over 60 years-of-age, since the most serious pollution occurred in the 1950s. Control subjects ranged in age from 20-79 years and had denied any exposure to MeHg. The older control subjects were selected because tactile sensation is known to diminish with increasing age. The exposed subjects were divided into two groups: 42 subjects with distal numbness of all four limbs and 17 subjects without limb numbness. Fifteen of those with distal numbness had been officially certified as having Minamata disease, and all of the 17 without numbness were presumed to have been exposed to MeHg. A history of MeHg exposure was taken and a neurological examination was performed for all patients. Hair samples were also taken to assess recent Hg exposures. The stimuli used in the fine-surface texture discrimination tests, one sub-modality of tactile sensation, were aluminum-oxide abrasive papers of differing grit texture. The grit values of the paper corresponded to average particle sixes of 30, 12, 9, 5, 3, or 1 μm. In the actual tests, subjects were seated and blindfolded to prevent any visual inspection of the test materials. Test subjects touched two stimuli with the index finger of the dominant hand and determined which abrasive paper felt rougher. Each subject completed 10 discrimination trials for each of six stimulus combinations.

Results of the conventional neurologic tests conducted prior to the texture tests revealed that all subjects having reported limb numbness showed sensory disturbance, as identified by the conventional pin and hair method, compared with none of the test cohort that had no numbness (Takaoka et al. 2004). Visual constriction and ataxia were frequent in the subjects with numbness. Only one of the subjects without numbness showed signs of ataxia, and none of them had visual constriction. Hair mercury was higher among 60-79 year-olds in both control and test subjects with numbness, compared with younger subjects in both groups and with test subjects without numbness in the 60-79 year age range, but no subject had a hair Hg concentration above 10 ppm. In the fine-surface texture discrimination tests, the discrimination difference threshold was 6.3 μm in

subjects with sensory symptoms, compared with 4.9 μm for exposed subjects without sensory symptoms and 2.7 μm for controls. Thus, acuity of fine-surface-texture discrimination was disturbed not only in subjects with clinical complaints, but also in subjects without hand numbness who lived in the district where MeHg exposure occurred. Takaoka et al. (2004) concluded that their results suggest that the number of individuals affected by MeHg exposure in the polluted area was greater than previously reported.

To assess the impact of chronic exposure to PCBs and MeHg, Saint-Amour et al. (2006) studied visual evoked potentials (VEPs) in 110 children with an average age of 5.44 years (range: 5.07 to 5.81) for their study. The children were from mother-infant pairs who had participated in the Nunavik Cord Blood Monitoring Program, in which Hg and other persistent pollutants were measured in umbilical cord blood. Blood and hair samples were collected at the time of testing to determine levels of total Hg, PCBs, and other environmental chemicals. After testing, adequate electrophysiological data were available for 78 children. Following adjusting for possible confounders, including PCBs, blood Hg was associated with shorter latencies of the early N75 VEP component at the 95% and 30% contrasts and the P100 component at 95% contrast ($p \leq 0.001$) and 30% contrast ($p \leq 0.01$). Overall, Saint-Amour et al. (2006) found that an increase in mercury concentration of one unit of natural logarithm (*i.e.*, by a factor of ~2.7) was associated with a decrease in latency in the order of 3-4 ms. While a decreased latency is certainly an effect, it is not considered to be a negative physiologic effect.

Murata et al. (2004) studied the latency period for evoked auditory potentials in 859 14-year-olds born in the Faroe Islands during a 21-month period in the years 1986 and 1987. Most of the examinations used for this study were conducted at the National Hospital in Torshavn, the capitol of the Faroes; examinations were also conducted in Odense, Denmark in the cases of families that had moved since the birth of the children. All audiometric tests were performed by a trained nurse in a sound-insulated room. Cord blood samples (reported in Grandjean et al. 1997) were used to assess the impact of *in utero* exposure to MeHg. Hair samples were taken to estimate recent MeHg exposure.

Geometric mean hair Hg concentrations were found to have increased significantly ($p <$ 0.001) from an average of 0.60 ug/g (ppm) (range: 0.34 – 1.24) to 0.96 ppm (range: 0.45 – 2.29) over a seven year period since the last testing of auditory evoked potentials (Murata et al. 1999). In the 2004 study, the latency of brainstem evoked potential peaks I, III, and V at 20 Hz and 40 Hz represented the outcome variables.

Murata et al. (2004) found that the latencies of peaks III and V increased ~0.012 ms when the cord blood Hg concentration doubled, the same as seen at 7 years (Murata et al. 1999). In the 2004 study, however, regression coefficients suggested an effect of recent MeHg exposure on the III-V peak interval ($p = 0.056$) at 20 Hz and showed a statistically significant effect of recent exposure on the III-V peak at 40 Hz ($p = 0.028$). Murata et al. (2004) concluded that the persistence of prolonged I-III inter-peak intervals seen at both 7-year and 14-year testing indicates that some neurotoxic effects from intrauterine MeHg exposure are (likely) irreversible. They also concluded that a change in vulnerability to MeHg toxicity was suggested by the apparent sensitivity of the peak III-V component to recent MeHg exposure.

Effects on Special Senses (vision, hearing, touch: non-human animals)

Rice and Hayward (1999) assessed the visual function during adulthood in monkeys exposed to 50 µg MeHg/kg/day from birth to 7 years, as well as in monkeys exposed to 10, 25, or 50 µg MeHg/kg/day throughout gestation, and up to 4 years of age. Age-related decrements were observed on both spatial and temporal visual function. Treatment-related effects were observed in the monkeys exposed to MeHg *in utero* and postnatally during the first assessment period, but not during aging. Four of ten MeHg-treated monkeys exhibited slight constriction of visual fields at the second assessment that had not been present earlier. These results were determined to support previous findings showing evidence of delayed neurotoxicity in the somatosensory and auditory systems following MeHg exposure.

Burbacher et al. (2005a) assessed visual function of 21 adult female monkeys (*Macaca fascicularis*) who were exposed to MeHg doses of 0, 50, 70, or 90 µg/kg/day prior to, and throughout, pregnancy. The method of administration was ingestion of MeHg hydroxide mixed with apple juice. The dosages were based on individual body weights of the females prior to conception, and were administered seven days per week. The ages of the offspring at testing were between 11 and 14.5 years-of-age. Visual function of the offspring monkeys was assessed by administration of spatial visual contrast sensitivity tests, consisting of stimuli of alternating black and white sinusoidal gratings that varied in spatial frequency and contrast. Contrast sensitivity threshold values were plotted for each animal across all test frequencies. Those threshold plots for MeHg-exposed animals indicated that there were two distinct groups: those with threshold values within normal limits ($n = 6$) and those with low threshold values at all frequencies ($n = 6$). To determine whether or not the contrast sensitivity thresholds varied significantly due to MeHg exposure, spatial frequency, or the interaction of both, a repeated measures analysis of variance test was used. Then, log-transformed data were used to compare the four experimental thresholds of the MeHg-exposed group ($n = 12$) with the control group ($n = 9$). The results showed statistically significant main effects of MeHg exposure ($p < 0.03$), frequency ($p < 0.01$), and exposure by frequency interaction ($p < 0.04$) suggesting that *in utero* exposure to MeHg may have long-term effects on visual contrast sensitivity thresholds.

As a follow-up to a previously reported study that showed high-frequency hearing loss in monkeys given MeHg from birth to age seven and tested at 14 years (Rice and Gilbert 1992), Rice (1998) exposed *Macaca fascicularis* monkeys to MeHg throughout gestation and postnatally until approximately age 4 (approximate age of puberty); actual ages of the animals ranged from 3.5 to 4.5 years. Prior to pregnancy, the dams were dosed three times per week at dosage levels equivalent to 0, 10, 25, and 50 µg Hg/kg/day as $MeHgCl_2$ added to a small amount of juice. When at least 90% of the estimated blood equilibrium value (based on a one-compartment model) was reached, those females were bred to untreated males. At birth, infants were separated from their mothers and dosed with the same nominal doses that their mothers had received, only the dosing was extended to five

days per week. Of the test monkeys, five were born in the high-dose group, two in the middle-dose group, and one at the low-dose. By the time testing was begun at 11 years, only two of the high-dose monkeys were still alive, but all three of those born to mothers in the two lower dosage groups were still alive. Similar testing was performed at 19 years-of-age. Pure-tone detection thresholds were measured for six frequencies between 0.125 and 31.5 MHz.

When tested at 11 years-of-age, the two control monkeys had elevated detection thresholds at the lowest and highest frequencies, but were generally unchanged between those frequencies (Rice 1998). Upon retesting at 19 years, one of the controls had slightly-to-moderately elevated thresholds in the right ear at all frequencies from about 3 to 17 dB. In contrast, the other control exhibited an 8.5 dB elevation in threshold at 1 kHz, but lower thresholds for most other frequencies.

When compared with controls, the two high-dose monkeys showed an elevation of hearing thresholds when tested at 11 years-of-age, with one high-dose animal having an elevation in threshold in both ears at all but the highest frequency. In the mid-dose group, one monkey had normal thresholds in the right ear, but showing elevated thresholds at the three lowest frequencies in the left ear. The other mid-dose animal had an elevated threshold at 10 kHz in the left ear and a highly elevated threshold at 25 kHz in the right ear. (The right ear was not tested at 31.5 kHz because the threshold was so elevated at 25 kHz.) For the low-dose monkey, the three threshold values collected were within the range of control values (Rice 1998).

At 19 years-of-age, all five monkeys exhibited elevated pure-tone thresholds compared with controls (Rice 1998). One of the high-dose monkeys had extremely elevated thresholds in both ears at all frequencies at 19 years, while the other showed elevated thresholds at the three higher frequencies in both ears compared with the 11-year measurements. In the mid-dose animals, the monkey that had normal thresholds in the right ear at 11 years showed elevated thresholds in that ear in all but the highest frequency compared with controls at 19 years. The other mid-dose monkey showed even

higher elevations in the right ear than seen at 11years. The low-dose monkey exhibited elevated thresholds in both ears at 19-years-of-age, with a greater impairment being seen in the right ear than the left. The author noted that the findings in this study extended previous data from that laboratory in which delayed manifestation of overt toxicity was observed in another cohort of MeHg-exposed monkeys and were consistent with findings in humans with Minamata disease (Rice 1998).

The mouse is particularly well-suited as a model of hearing loss in humans (Zheng et al. 1999), and various strains of mice have been studied as models of hearing loss through examination of auditory brainstem response (ABR) thresholds to acoustic stimuli. Chuu et al. (2001) investigated the oto-neurotoxicity of MeHg in male mice. The test animals (ten per group) were administered MeHg doses of 0, 0.2, 2.0, or 10 mg/kg/day for seven consecutive days. The 10 mg/kg dosage subsequently proved fatal to all mice in that group, although data was collected during tests immediately following the cessation of treatment. Animals in the other two treatment groups were sacrificed by pentobarbital injection at 0, 5, and 11 weeks following cessation of treatment. Tests of ABR were conducted to evaluate possible MeHg-induced compromise of hearing. Stimulus intensity was varied in 5-db stepwise increments until hearing thresholds were obtained. Absolute latencies and inter-wave latencies of ABR waveforms were recorded at an 85 db signal intensity for all mice, including controls, under anesthetic condition following *i.p.* injection of pentobarbitol (60 mg/kg). Absolute latencies and inter-wave latencies were recorded for all five waveforms (I, II, III, IV, and V).

At the end of the 7-day treatment period, ototoxicity in the form of significant elevations in ABR threshold was observed in all exposed groups ($p < 0.05$), but not in vehicle controls (Chuu et al. 2001). In the 2.0 and 10 mg/kg/day groups, the elevation in hearing was more severe than in the 0.2 mg/kg/day animals. At 11 weeks after the dosing period, persistent bilateral hearing loss was seen in the 2.0 mg/kg/day group animals, but not in mice in the 0.2 mg/kg/day group. Significant ($p < 0.05$) prolongation of the absolute latency of wave V was seen immediately following cessation of dosing, as well as in animals sacrificed at five and 11 weeks post-treatment in 2 mg/kg-treated mice. Mice

receiving the 0.2 mg/kg daily dose of MeHg and sacrificed immediately subsequent to the cessation of dosing also showed a significant ($p < 0.05$) prolongation of the absolute frequency of wave V compared with controls, but recovery from this latency had occurred by the time of the testing five weeks following the termination of MeHg treatment (no latency also at 11 weeks). Prolongation of the absolute latency of wave IV was also seen in the 2.0 mg/kg group in mice sacrificed five and 11 weeks post-treatment, but not in mice sacrificed immediately following the cessation of dosing. Prolongation of inter-wave latencies I-V and III-V was also seen immediately following the cessation of MeHg dosing in the 0.2 and 2 mg/kg/day groups. However, restoration of normal latencies was observed in 0.2 mg/kg animals tested five and 11 weeks post-treatment (Chuu et al. 2001).

Other Neurological Effects (humans)

Through sural nerve biopsy and autopsy examination of a 64 year-old male fisherman, Eto et al. (2002) examined the effects of MeHg consumption on peripheral nerves. The sural nerve (from dorsal side of calf) biopsy was performed approximately one month before the man's death. This biopsy showed a decrease in myelinated nerve fibers and an increase in small axons with attendant proliferation of fibroblasts and Schwann cells. Electron microscopic examination showed morphological changes of the sural nerve that included irregular Schwann cells and the appearance of fibroblasts with an increase of collagen fibers. Regressive changes were seen in the form of degeneration resulting in swollen myelin, wavy degeneration of myelin with extremely thin and electron-dense axons, incomplete regeneration including abnormally small axons, and incomplete myelination and absence of myelin. Autopsy examination of the dorsal roots and sural nerve revealed the presence of endoneurial fibrosis, loss of nerve fibers, and bands of Bungner (denervated Schwann cell bands due to the loss of myelinated axons). Autopsy examination of the spinal cord showed Wallerian degeneration of the fasciculus gracilis (*a.k.a.* tract of Goll: a bundle of axon fibers in the dorsomedial spinal cord that carries sensory input regarding touch, vibration, and proprioception from the lower part of the body to the brain stem), with relative preservation of neurons in sensory ganglia. Eto et

al. (2002) concluded that their findings supported the contention that peripheral nerve generation also occurs in Minamata disease due to injury caused by MeHg.

Chang et al. (2008) investigated the correlation between MeHg exposure and neurologic outcomes among residents living in the neighborhoods around a deserted chloralkali plant in Taiwan. Blood total Hg and MeHg were evaluated from samples collected prior to neurobehavioral testing, and a questionnaire was administered to evaluate personal characteristics (sex, age, medical history, etc.) and lifestyle (including alcohol and tobacco use and eating/dietary habits). The Mini-Mental State Examination (MMSE) was administered by trained technicians blind to the subject's blood Hg levels and dietary history. Study subjects were also administered the Cognitive Abilities Screening Instrument (CASI) test, which measures nine cognitive domains: remote memory, recent memory, attention, mental manipulation, orientation, abstract thinking, language, drawing, and verbal fluency.

In all, 141 men and 99 women, comprising 12.6% of the people living in the target area around the deserted plant, were eventually recruited for this study. The average length of residence of all participants in the target area was 49.3 years. The average fish and other seafood consumption for the entire study cohort was 5.82 kg/month. Blood MeHg levels were significantly different ($p = 0.025$) in different age groups, but differences in the number of subjects in age group categories made a strict age-dependent comparison difficult. The highest blood MeHg average level (19.4 +/- 2.8 µg/L) was in the < 40 age group, which had only 3 individuals. All other age groups had from 50-70 subjects. Blood MeHg levels in the other age groups were 12.0 +/1 6.6 µg/L for the 4-49 year group, 18.1 +/- 12.5 µg/L for the 50-59 year group, 15.4 +/- 8.1 µg/L for the 60-69 year group, and 14.6 +/- 7.1 for the > 70 year-old group. Blood MeHg levels were also significantly higher ($p = 0.005$) in men (16.6 µg/L) compared with women (13.5 µg/L). Total Hg in blood was found to consist of ~ 90% MeHg. After adjusting for gender and age, blood MeHg concentrations increased significantly with the level of consumption of fish and other seafood, indicating that these were the primary source of MeHg consumption among the study participants. (Methylation of inorganic Hg does not occur

in vivo to any measurable significant extent, if typically at all.) Further, men were found to consume more fish than women, accounting at least in part in the differences in blood Hg seen between the sexes. Since there was no control population in this study, the Hg exposed individuals were broken down into high (26.4 +/- 10.0 µg/L; $n = 60$) and low (11.6 +/- 5 .0 µg/L; $n = 180$) MeHg groups. Mean CASI-MMSE scores in the areas of remote memory ($p < 0.01$), recent memory ($p < 0.05$), and mental manipulation ($p < 0.01$) were significantly lower in the high-MeHg than in the low-MeHg group. The total scores of CASI and MMSE in the high-MeHg group were both significantly lower than in the low-MeHg group ($p = 0.003$ and $p = 0.001$, respectively). When normalized for age and education to obliterate the influence of these two variables on cognitive decline, abnormal rates of recent memory, orientation, abstract thinking, and drawing were the top four cognitive domains in both the high-MeHg and low-MeHg groups. With adjustment for age and education, abnormal rates of remote memory, mental manipulation, and orientation were significantly higher in the high-MeHg group ($p < 0.05$). Verbal fluency was also statistically different between the high and low-MeHg groups ($p = 0.05$).

Cao et al. (2010) examined postnatal, background MeHg exposure and cognition and behavior in 780 children enrolled in the Treatment of Lead (Pb)-exposed Children clinical trial (TLC). IQ and neurobehavioral performance were tested at ages 2, 5, and 7 years. These authors reported weak, but consistent, positive associations between blood MeHg and IQ test scores in stratified, spline regression and generalized linear model data analyses, even after adjustment for Pb exposure; however, these associations were not statistically significant. Behavioral problem scores were either constant or decreased slightly with increasing MeHg concentrations. Cao et al. (2001) thus concluded that at the present postnatal MeHg exposure levels of U.S. children, any adverse effects on children's IQ and behavior are not detectable.

In a study conducted as a follow-up to the Cord Blood Monitoring Program in Nunavik of Northern Quebec, Canada, Despres et al. (2005) looked at the effects of prenatal and postnatal chronic exposure to a variety of chemicals, including Hg. This phase of the study involved 110 mother-infant pairs, whose umbilical cords were analyzed for Hg.

Blood Hg concentrations were measured at both the time of birth and the time of testing of the children. The source of any postnatal exposure to Hg and other chemicals studied was consumption of traditional Inuit foods. This study reported no adverse effects of prenatal exposure on either gross motor function of neurologic status among the Inuit preschool children examined. The only MeHg-related effect was on hand tremor. This effect, postural hand tremor, was measured using the Catsys system. Postural hand tremor was measured during an 8-second period in which children held a light stylus at ~ 10 cm in front of their navel, with the elbow joint bent at a right angle and without body contact. During testing, the subjects were asked to look at the tip of the stylus, breathe normally, and relax. After adjustment for confounders, an increase in tremor during pointing movements ($p < 0.001$) was associated with postnatal exposure only.

Other Neurological Effects (non-human animals)

Vilagi et al. (2000) investigated the effect of MeHg on the excitability of developing rat cortical neurons. Pregnant Wistar dams were administered MeHg at a reported dose of 0.375 mg/kg bw/day in drinking water from the day of mating through the suckling period. Control rats received normal drinking water for the same period of time. At three weeks of age, the young rats received normal drinking water. At four weeks of age, rats of both sexes (eight animals total) were sacrificed and cortical slices from both treated and control pups were prepared for use in electrophysiological experiments one week later. Some rat pups were allowed to live and were paired for mating after sexual maturation and provided only normal water for drinking. The offspring of these animals were later investigated at four weeks of age using the same procedure. Vilagi et al. (2000) found that resting neuronal membrane potentials were nearly the same in the control and second generation untreated groups, but averaged 6% lower in the MeHg-treated animals. The amplitude of evoked spikes was also very similar in controls and second generation pups, whereas the evoked potential was 13% smaller in the treated rats. The threshold stimulus for evoking excitatory postsynaptic potentials (EPSPs) was

also lower (about 15%) in MeHg-treated animals. All second generation untreated animals showed normal neuronal characteristics (Vilagi et al., 2000).

Weiss et al. (2005) studied the effects of perinatal and lifetime MeHg exposure on behavioral performance in adult mice at different ages. Mice were chosen as subjects for this study because their brain-blood ratios are closer to those seen in primates than are rat brain-blood ratios. One hundred female mice were assigned to one of three dosing groups: 0, 1, or 3 ppm MeHg in drinking water. The mice were paired and bred four weeks after initiation of dosing. MeHg litters were then divided in two subgroups, one exposed through post-natal day 13 and the other exposed to the original dosage throughout lifetime. Thus, five groups were studied: control, 1 ppm perinatal, 1 ppm lifetime, 3 ppm perinatal, and 3 ppm lifetime. For each group, tests of memory, operant behavior, and motor function were conducted. Delayed spatial alteration (a test of memory) and running in a wheel to earn food pellets (schedule-controlled operant behavior) tests were conducted at 5 and 15 months of age. Hindlimb splay (a measure of motor function) was tested at 5, 15, and 26 months of age.

In the delayed spatial alteration test (Weiss et al. 2005), the mouse was required to correctly respond to one of two locations in a strictly alternating sequence within a specific time period, and being rewarded with food for a correct choice. The initial delay between choices was 1 second, with the delay eventually building up to a 10 seconds. The measure of success was the number of subjects reaching the delayed alternation criterion of 10 seconds within 55 sessions of training. Other than the mice in the 1 ppm perinatal group, all treatment groups failed to meet the criterion at longer delay values. In the "running in a wheel to earn food" test, a decreased response occurred only in the 3 ppm lifetime group. There was a significant ($p = 0.03$) dose x age interaction in the 3 ppm lifetime group at one year-of-age, but not at 2 years-of-age.

Weiss et al. (2005) also found that MeHg exposure altered hindlimb splay distance compared to controls. Splay distance differed significantly between the 1ppm lifetime and 3 ppm lifetime groups ($p < 0.05$). Significant effects on hindlimb splay distance

were seen for dose ($p < 0.01$), age ($p < 0.01$), and trial number ($p < 0.01$), but not for duration of exposure ($p = 0.62$). There was also a significant age-by-dose interaction ($p = 0.03$) when compared to the control mice. Weiss et al. (2005) concluded that exposure to low levels of MeHg produces behavioral effects that depend on the test procedure, the dose, the duration of exposure, and the age of the organism as testing.

Schionning and Danscher (1999) used an autometallographic silver-enhancement technique to trace inorganic mercury bound to sulphide or selenide in sections of dorsal root ganglia and dorsal nerve roots taken from rats treated with 2 mg organic mercury/kg body weight for 19 days. In the dorsal roots, inorganic mercury-sulphide/selenide complexes were observed in only a few macrophages. At the ultrastructural level, however, such mercury complexes were observed within lysosomes of target cells. The authors concluded that the inorganic mercury complexes were located primarily within glial cells, and that the pattern of deposition was the same as that seen in morphological changes in rats intoxicated with organomercurials (Schionning and Danscher 1999).

Eto et al. (2001) reported that they had previously noted histopathological similarities between Minamata disease and anoxic–ischemic encephalopathy. This, they believed, pointed to the possibility of a vascular factor in the development of cerebral lesions. This vascular hypothesis of the mechanism for the selective vulnerability of the brain to MeHg postulates that brain lesions seen with severe MeHg poisoning are the result of ischemia secondary to compression of sulcal arteries from MeHg-induced cerebral edema. Eto et al. (2001) tested this hypothesis using four 4-year-old male marmoset monkeys and conducting MRI analysis, assays of tissue specimens, and histologic and histochemical studies of their brains. Marmosets were used because the cerebrum of these monkeys has two distinct deep sulci, namely the calcarine and Sylvian fissures. Two groups of two monkeys received MeHg in their drinking water at an Hg concentration of 5 μg/ml (equivalent to 5 ppm). Four control monkeys received normal drinking water. Blood total Hg in the two MeHg-exposed groups was measured once weekly. Both animals in the first group were sacrificed 37 days after the start of exposure, when their blood Hg concentrations reached 8 μg/ml, and before the appearance of any clinical sign of Hg

poisoning. Exposure of the other two monkeys to MeHg was terminated when the blood levels approached 10 μg/ml, since preliminary studies had shown that blood Hg concentrations above 10 μg/ml in common marmosets had resulted in severe clinical signs of MeHg poisoning. The animals were allowed to live another 205 days before being sacrificed. Just before termination of the experiment, these monkeys appeared restless, irritable, and showed a mild ataxia of the hind limbs compared to controls.

The levels of total mercury in all organs examined were high compared to controls. The ratio of MeHg to total Hg was reported to be high in the cerebrum, cerebellum, liver, and kidneys of treated animals. The ratio of gray-to-white matter was measured in three MeHg-exposed animals and two controls. A statistically significant decrease ($p < 0.001$) in this ratio was observed in treated animals, compared to the control animals. The most affected area of the cerebrum was the occipital lobe. Edematous white matter was observed in the cerebrum. Eto et al. (2001) concluded that their results suggest that edema in the white matter near the calcarine fissure may contribute to the selective damage of the calcarine cortex caused by MeHg.

In a study of the effects of transplacentally administered MeHg on the rat fetal brain, Kakita et al. (2000) administered adult female Wistar rats (six animals per treatment group) oral doses of 1, 2, or 3 mg/kg/day via a stainless steel catheter for either 5 or 12 days prior to mating. After mating, the pregnant females were then administered MeHg in the same manner during pregnancy days 1-19. Five female rats serving as controls were treated with equivalent doses of cysteine only for 12 days of the pre-gestational period and from pregnancy days 1-19. Neurologic symptoms, such as unsteadiness and flexion of the hind limps appeared on pregnancy days 15 or 16 in animals receiving 3 mg/kg/day for five days before pregnancy and for 19 days after conception (total dose of 72 MeHgCl). Rats receiving 2 mg/kg/day for 12 days before pregnancy and 19 days following conception (total dose of 62 mg/kg) showed a slowness of movement from mid-gestation onward. No such effects were seen in rats administered 2 mg/kg/day for 5 days prior to pregnancy and 19 days of pregnancy (total dose of 48 mg/kg), 1 mg/kg for five days before pregnancy and 19 days following conception (total dose of 24 mg/kg), or

1 mg/kg for 12 days prior to pregnancy and 19 days after conception (total dose of 31 mg/kg).

In the high-dose group, Kakita et al. (2000) found the most significant histological change in the brain stem was widespread neuronal degeneration, manifested by shrinkage and chromatolysis of the neuronal perikaryon with eosinophilic changes in the pontine reticular nucleus, inferior olivary nucleus, and gigantocellular reticular nuclei of the medulla oblongata. The red nucleus had a smaller proportion of degenerative neurons. Damage to the cerebrum was remarkable in the cingulate cortex, thalamus, and cerebral basal area, including the hypothalamus, with just a few degenerative neurons observed in the hippocampus and amygdaloid nucleus. In the areas of those lesions, variable degrees of astrocytosis were observed. In contrast, neurons in the cerebral neocortex, striatum, cerebellar cortex, spinal cord, and dorsal root ganglia were reported to be well-preserved in those animals. Degenerative changes similar to those observed in the high-dose group were also observed in the four lower treatment groups, but to a lesser degree, and in accordance with the total administered dosage of MeHgCl. Since the distribution of neuronal damage seen in the pups exposed *in utero* was different from damage seen in postnatally exposed or adult rats, the authors concluded that their findings suggest that distinctive pathomechanisms operate in the fetal brain exposed to MeHg.

Franco et al. (2006) examined the exclusive contribution of MeHg exposure through maternal milk on biochemical parameters related to the thiol status in the cerebellums of suckling mice. The thiol status was determined by glutathione (GSH) levels and glutathione peroxidase (GP*x*) and glutathione reductase (GR) activity. In this study, 14 Swiss albino mice dams were randomly assigned to one of two groups of seven females each (one treatment and one control group). Pups (eight per litter) were maintained with their mothers, half of which were immediately exposed to MeHg (10 mg/L) in drinking water (treatment group) or to MeHg-free tap water *ad libitum* (control group). The exclusive route of MeHg exposure for the treated offspring was maternal milk.

Franco et al. (2006) found that mercury levels in the cerebellums of MeHg-exposed dams were about 9-fold higher than in control dams, and the lactationally-exposed pups had cerebellum mercury levels about 4.5 times higher than control pups. Two-way NOVA analysis showed a significant ($p < 0.001$) interaction between treatment (control *vs.* MeHg) and ontogenetic status (dams *vs.* pups) for cerebellar mercury. The GSH level in the cerebellum was significantly higher ($p < 0.05$) in the MeHg-exposed dams than in the control rats. In the exposed pups, however, the response was completely the opposite, with MeHg exposure through breast milk resulting in a significant decrease in GSH levels ($p < 0.05$). Cerebellar GR activity was also significantly higher ($p < 0.05$) in MeHg exposed dams than in controls, but this increase was not seen in exposed pups. A two-tailed Pearson correlation test revealed a significant positive correlation between cerebellar GR activity and cerebellar GSH in mothers ($p < 0.001$). MeHg exposure did not affect GP*x*.

Using a one-way ANOVA, Franco et al. (2006) found that pups lactationally exposed to MeHg showed significant impairment in motor performance, as measured in rotarod tests ($p < 0.01$). MeHg-exposed pups also showed reduced locomotor activity in the open-field test, however the reduction was not statistically significant. Those authors concluded that their results indicate that exposure of lactating mice to MeHg causes significant impairment in motor performance in the offspring, and that this may be related to alterations in the cerebellar thiol status.

The effect of MeHg on suckling mouse pups exposed exclusively through breastmilk consumption was studied by Manfroi et al. (2004). On the first day following parturition (PND 1), 14 Swiss albino dams and their pups (8 per litter) were assigned to either the MeHg-treatment or control groups (7 per group). MeHg was provided to the treatment group in drinking water, which was available for consumption by the dams *ad libitum*. The total exposure period was 21 days. On day 21, two weaning mice from each litter were randomly selected for behavioral testing using the SHIPRA protocol, which provides a behavioral and functional profile by observational assessment. When the mice were weighed, it was found that the MeHg-exposed pups had significantly ($p < 0.05$)

lower average body weights than controls (11.4 +/-0.7 for exposed pups *vs.* 14.3 +/- 0.7 for controls). In the behavioral testing, three significant ($p < 0.05$) differences were found between the MeHg-treated pups and controls. The MeHg-exposed group had a higher incidence of tremors, increased peripheral analgesia in the toe pinch test (consisting of a gentle lateral compression of the mid-digit of a hind foot with fine forceps), and decreased ability to grasp with the hind limbs (Wire Maneuver test).

Consistent with the lower body weight in MeHg-treated animals reported by Manfroi et al. (2004), Farina et al. (2003a) reported a significant ($p < 0.05$) decrease in body weight gain in Swiss albino mice given a MeHg dose equivalent to 40 mg/kg/day for 15 days, when compared with controls. No such effect was seen at a MeHg dose of 10 mg/kg/day.

The long-term effect of prenatal MeHg exposure on the stress response during active learning was investigated by Carratu et al. (2008) using rats as the test organism. Primiparous Sprague-Dawley females were mated with untreated males and given either a saline control or 8 mg/kg MeHg by gavage (in a 1 ml/kg bw solution) on day 15 of gestation. One male pup per litter was used from each of control and MeHg-treated groups. In the first test, 10 90-day old offspring rats from both control and MeHg-treated groups were subjected to an acoustic active avoidance test. Rats were subjected to 100 trial sessions (4 blocks of 25 trials each), with a 50-sec. inter-trial interval. The conditioning tone was an 80-dB tone lasting for 10 sec. The unconditioned stimulus was a 2-sec. positive half-wave constant current with an intensity of 0.5 mA.

Carratu et al. (2008) found that MeHg-exposed rats exhibited impaired active learning, when compared with controls. An overall two-way repeated measures ANOVA for conditioned avoidance responses revealed significant differences between treatments ($p < 0.005$), between blocks ($p < 0.0001$), and between treatments x blocks ($p < 0.05$). There was also a difference in the ability of the treated vs. control rats to progressively avoid electrical shock during training sessions. Within comparison groups, Dennett's multiple comparison test showed a progressive improvement in the ability of control rats to avoid electrical shock (third block: $p < 0.05$; fourth block: $p < 0.01$). In contrast the MeHg-

treated rats failed in this task, considered a demonstration of impaired learning ability. In addition, Tukey's multiple comparison test revealed significant impairment in MeHg-exposed offspring in learning the active avoidance tasks (third block: $p < 0.05$; fourth block: $p < 0.01$). Since there was no difference between MeHg-exposed and control rats in response to a hot plate test, the authors concluded that the learning deficit could not be attributed to sensory dysfunction, and motor disability was also ruled out. Carratu et al. (2008) further dismissed any possibility of impaired task acquisition due to auditory loss, since another study from that lab had recently demonstrated that the acoustic startle response is not altered by prenatal MeHg exposure (Carratu et al. 2006). Carratu et al. (2008) concluded that their study demonstrated that prenatal MeHg exposure affects the animals' ability to develop adaptive behavior.

Plasma corticosterone levels were also determined for six rats sacrificed following the last trial session (Carratu et al. 2008). MeHg-exposed rats were reported to exhibit an abnormal increase in plasma corticosterone concentrations during active learning. An overall two-way ANOVA revealed significant differences between treatments ($p < 0.05$), between behavioral challenge ($p < 0.0001$), and between treatments x behavioral challenge ($p < 0.01$). Further, Tukey's multiple comparison test showed that within each treatment group, plasma corticosterone levels were significantly increased in active avoidance-experienced rats. Surprisingly, MeHg-exposed rats showed a much higher increase ($p < 0.001$) than did the saline controls ($p < 0.05$). Carratu et al. (2008) stated that their findings highlight the impact of MeHg on the adrenocortical component of the stress response, which could contribute to the poor performance on the treated rats in the learning task.

Gao et al. (2008) investigated the effects of MeHg on postnatal neurobehavioral development in mice. Primiparous 10-week-old female ICR mice were mated with males of the same strain. Each resulting litter was culled to four males and four females on postnatal day four. One male and one female from each litter were assigned to a high, mid, or low dose group or to the control group. In all, a total of 80 pups from 10 liters were used in the study. All pups remained on milk from their mothers before weaning.

Mice from the MeHg treatment groups were given *i.p.* injections of 0.1, 1, or 3 mg/kg MeHg chloride from postnatal days 15 through 17, whereas control mice were injected with sterile saline. On postnatal day 45, the mice were tested using the Morris water maze to evaluate spatial learning and memory. Following four days of acquisition training, significant differences were seen between 1 mg/kg and 3 mg/kg groups and controls with regard to two measured parameters. Both the 1 mg/kg and 3 mg/kg groups had significantly ($p = 0.020$) longer latencies prior to finding the partially-submerged platform. The 3 mg/kg group also exhibited longer swim distances ($p = 0.009$) before finding the platform. The 0.1 mg/kg group did not differ significantly from controls on either parameter.

Mori et al. (2007) administered MeHg chloride in solution to 9-week-old male Wistar rats. The test animals were given daily gavage doses equivalent to 10 mg/kg/day for five days, and observed for another 14 days following the cessation of treatment --- then sacrificed. The MeHg-treated rats were reported to display grossly visible neurotoxic signs, including crossing of hind limbs and ataxia. On the day of sacrifice, the MeHg-treated rats had body weights only 55% of controls.

Ou et al. (1999a) exposed 6-week-old C57B/6 female mice to concentrations of 0, 3, or 10 ppm MeHg (as MeHgOH) in drinking water for 4 weeks. The animals were weighed and water consumption recorded twice weekly. After the treatment period, the animals were sacrificed and brain, liver, and kidney tissue were dissected and prepared for analysis. Animals in the 3 ppm MeHg group showed no overt signs of toxicity. In the 10 ppm treatment group, however, three of five animals showed overt toxicity, including hyperactivity, scoliosis, pacing deficits, posterior paresis, and tremor. On post-mortem tissue examination, the amount of inorganic Hg in the cerebral cortex was less than 5% of the total Hg present in that tissue. There was also evidence of disruption of the cell cycle, supported by the increased expression of the p21 cell cycle regulatory gene, which is associated with terminal differentiation of many types of cells. Average p21 mRNA increases of 6.8 (+/- 1.4)-fold, 12.9 (+/- 21.)-fold, and 13.7 (+/- -.3)-fold over controls were reported for brain, liver, and kidney tissue, respectively. No other signs of organ

damage were reported in this study, which was designed to identify sub-cellular/molecular mechanisms of MeHg-induced toxicity.

2.2.2.5 Reproductive Effects

Methylmercury

The possible association of preterm delivery in Michigan women exposed to MeHg during pregnancy was investigated by Xue et al. (2007). As part of the Pregnancy Outcomes and Community Health (POUCH) study, 1,024 pregnant women from 52 prenatal clinics in five Michigan communities were enrolled in the present study. All enrollees were in their 15^{th} to 27^{th} week of pregnancy. At the time of enrollment, information was collected regarding the type and amount of fish consumed during the current pregnancy, and a hair sample was obtained and analyzed for total Hg and MeHg. The maternal hair mercury levels at mid-pregnancy were ultimately compared with the gestational week at the time of delivery. The mean number of fish meals for six different categories of fish were 19.6 (SD +/- 28.2; range 0–214.5) for total fish meals, 3.7 (SD +/- 10.1; range 0–182.5) for shellfish, 8.5 (SD +/- 16.5; range 0–182.5) for canned fish, 6.3 (SD +/- 18.5; range 0-182.5) for bought fish, 0.7 (SD +/- 4.9; range 0-90) for sport-caught fish, and 0.4 (SD +/- 6.0; range 0-182.5) for other fish. In this study, a full-term pregnancy was defined as ≥ 37 weeks. A total gestation time of 35-36 weeks at delivery was defined as moderately preterm, and < 35 weeks was called very preterm. The results of the data analysis indicated that women who delivered very preterm were more likely to have had hair Hg levels at or above the 90^{th} percentile (0.55 to 2.5 μg/g) than women who delivered at term.

In a study of the effects of organic mercury on reproductive parameters, semi-domesticated female mink (*Mustela vison*) were fed daily fish-based diets containing 0.1, 0.5, or 1.0 ppm total mercury (Dansereau et al. 1999). In this study, 20-month-old

females exposed to the experimental diets for 400 days, as well as their 10-month-old offspring that were exposed to mercury for approximately 300 days, were all mated to 10-month-old males who had been fed the 0.1 ppm diet for 60 days prior to the mating season. In this study, the proportion of females giving birth was low for all treatment groups, except the parental general females fed the 0.1 ppm diet. Mercury exposure did not influence the survival or growth of neonatal kits.

In investigating the possible factors associated with male reproductive dysfunction following MeHg exposure, Dufresne and Cyr (1999) found that exposure of adult rats to MeHg can modulate metalothionine mRNA levels in both the testis and epididymis. Further, changes in metalothionine mRNA levels following MeHg exposure differed between epididymal segments, suggesting either differences in MeHg accumulation or differences in metalothionine modulation.

Newland and Reile (1999) exposed groups of 10 nulliparous female Long-Evans rats to MeHg (as $MeHgCl_2$) in drinking water at concentrations of 0, 0.5, or 6.4 ppm. Within each exposure group, five rats began treatment 28 days prior to mating and the other five began treatment 49 days before mating. All females were mated with unexposed males. In this study, a breeding failure was considered to be a mating in which a sperm plug was noted, but the female did not give birth. The authors reported one breeding failure in each the control and high-dose group, and three failures in the low-dose group. The three failures in the low-dose rats occurred in females exposed for 49 days prior to mating and who mated with male rats that had successfully sired offspring with other females. Thus, the failures could not be attributed to male reproductive problems. The single failure in the control group occurred with a male that had previously sired a litter of 14 pups with another dam. The cause of the high-dose failure was not as clear, because the involved male had previously sired a litter of only two pups with another female. The high-dose failure dam had also been exposed to MeHg for 49 days prior to mating. Chi square analysis resulted in a *p* value of 0.058, which Newland and Reile stated should be interpreted cautiously due to the small sample size.

Ethylmercury

Ethylmercury-containing thimerosal has been shown to be a potent activator of intracellular calcium release in pig oocytes. Such activation mimics the effects of sperm-induced release of intracellular calcium, as well as other activation events that occur in pig oocytes (Machaty et al. 1999).

Wang et al. (1999) examined the temporal relationship between intracellular calcium transients, cortical granule exocytosis, and the zone reaction induced by thimerosal. These researchers found that thimerosal induced the same degree of exocytosis in oocytes that was caused by sperm penetration. Further, the zona block to sperm penetration in thimerosal-treated oocytes occurred within 35 minutes of cortical granule exocytosis and within 40 minutes of the first calcium transient. Machaty et al. (1999) found that the thimerosal-induced Ca^{++} release did not require the formation of IP_3. In addition, thimerosal destroyed the meiotic spindle, preventing further development.

2.3 TOXICOKINETICS

2.3.2 Distribution

2.3.2.2 Oral Exposure (Methylmercury)

Ortega et al. (1997) examined the effects and tissue distribution of four different forms of MeHg in male Sprague-Dawley rats. Groups of six animals were given either tap water (controls) or water containing 5 or 500 µg of MeHg/L in the form of $MeHgS^-$, $(MeHg)_2S$, $(MeHg)_3S^+$, or MeHgCl for a period of eight weeks. The amount of water ingested by each animal was recorded daily. At the end of the exposure period, all animals were sacrificed by lethal anesthetic injection, and liver, brain, kidney, spleen, and testis tissue samples were collected and analyzed for mercury content. The largest mercury concentrations were observed in the kidney, followed by the spleen, testes, brain, and liver, respectively. The distribution of mercury was reported to be consistently low in all tissue from 5 µg/L group, with the controls showing non-detectable mercury tissue levels.

Of the animals exposed to 5 µg/L MeHg in water, only the $(MeHg)_2S$ and $(MeHg)_3S^+$ consistently showed higher levels of mercury than controls in all tissues analyzed. In the high-dose $(MeHg)_3S^+$ and MeHgCl groups, the tissue mercury levels were significantly higher than in the corresponding low-dose groups (Ortega et al., 1997). The authors concluded that organ distribution of mercury may vary according to the chemical structure and concentration of the specific compound to which the organism is exposed.

Sundenberg et al. (1999) studied the concentration of mercury in milk and the distribution pattern in the suckling pup following administration of a single *i.v.* injection to lactating mice. A single dose of 0.5 mg/kg bw of either ^{203}Hg-labeled $MeHgCl_2$ or $HgCl_2$ was injected into the mothers on day 10 of lactation. Mercury concentrations in milk and in whole body, blood, plasma, gastrointestinal (GI) tract, liver, kidneys, and brain of the offspring were followed up to 11 days after the beginning of dosing. Lactational exposure following a maternal MeHg or inorganic Hg dose resulted in almost similar mercury concentrations in liver, kidneys, and plasma of the suckling pup; however, the MeHg group had twice the body Hg burden of the inorganic mercury group and higher concentrations in the brain (up to 14 times). The authors concluded that differences in kinetics indicate that lactational exposure to MeHg is a greater hazard for the breast-fed infant than is inorganic Hg.

Sakamoto et al. (2002) studied changes in brain mercury concentration of rat pups exposed to MeHg throughout embryonic development, during breast-feeding, and after weaning. Adult female rats were fed a diet containing 5 ppm mercury (as MeHg) for 8 weeks prior to mating, with no apparent adverse effects. This diet was continued throughout gestation and after parturition. Newborn offspring were weaned at day 30 of life, and then placed on the same diet as the mothers. On the day of birth, rat pups had blood mercury concentrations significantly higher ($p < 0.01$) than that of the mother (*i.e.*, about 1.5 times that of the mother on the same day). This concentration declined during the suckling period to just 1/5 of that measured in the rats sacrificed at birth, suggesting limited transfer of mercury by the milk during a period of rapid growth of the brain and body. At weaning on postnatal day 30, Hg concentrations in the brains of mothers were

significantly higher ($p < 0.01$) than at parturition, indicating continued distribution to the brain with continued dosing.

Newland and Reile (1999) exposed groups of 10 nulliparous female Long-Evans rats to MeHg (as $MeHgCl_2$) in drinking water at concentrations of 0, 0.5, or 6.4 ppm (nominal concentration of 6 ppm). Within each exposure group, five rats began treatment 28 days prior to mating and the other five began treatment 49 days before mating. All females were mated with unexposed males. From these matings, 88 pups were born. Treatment of the dams continued until postnatal day 16, when the pups were old enough to reach the water bottles themselves. Blood and whole-brain mercury concentrations were determined in pups on postnatal day 0 and at weaning (postnatal day 21). Newland and Reile (1999) found that the blood mercury concentration increased with increased levels of MeHg in the drinking water ($p < 0.001$). The blood Hg level in the high-dose group was approximately 20-fold higher than in the low-dose group. Blood Hg was also higher in neonates than weanlings ($p < 0.001$) and decreased about 20- to 30-fold between birth and weaning. Brain Hg also increased significantly with increasing MeHg concentration in the drinking water ($p < 0.001$), and was 20-fold higher in high-dose animals. As with blood concentration, brain Hg concentration also decreased with age ($p < 0.001$). Brain Hg concentrations were approximately 10-fold lower at birth in the 0.5 ppm pups and 20-fold lower in 6 ppm-exposed animals. The authors noted that the decrease in brain Hg occurred despite continued maternal consumption throughout most of the test period; thus, loss of Hg from the brain occurred, even though there was some replenishment of Hg from milk.

The temporal variation in the distribution of mercury during pregnancy was investigated by Morrissette et al. (2004). In their study, 159 pregnant women were recruited from two prenatal clinics in Southwest Quebec, Canada. All enrollees completed two detailed questionnaires regarding their consumption of fish prior to and during pregnancy. Items queried included socio-demographic information, as well as the specific fish species consumed and the frequency of fish consumption. Blood samples were collected during all three trimesters of pregnancy in women recruited during the first trimester and

between the 14th and 24th gestational weeks for women recruited during their second trimester. In addition to maternal blood analysis, a hair sample was obtained two weeks after delivery and analyzed for Hg. At delivery, the authors were able to collect blood from 101 mothers, along with 92 cord blood samples. The survey of fish-consumption habits showed that over 80% of the women ate at least one fish meal per month prior to pregnancy. After becoming pregnant (or becoming aware of their pregnancy), the large majority of women continued to eat at least one fish meal per month, although there was a 7% reduction in both the number of fish consumers and a significant reduction in the frequency of fish consumption ($p < 0.001$).

Significant decreases in hair Hg ($p < 0.0001$), total blood Hg ($p = 0.04$), and blood organic Hg ($p < 0.01$) were seen between the second and third trimester measurements (Morrissette et al. 2004). In addition, paired analyses revealed significant reductions in total and organic Hg between the second trimester and delivery (total Hg, $p < 0.01$; organic Hg, $p = 0.02$). When compared with a maternal blood sample collected at parturition, significantly elevated levels of total Hg ($p = 0.03$) and organic Hg ($p < 0.001$) were seen in cord blood. For organic Hg levels, cord blood was an average of 1.7-fold higher than third trimester maternal blood ($p < 0.001$). In contrast, inorganic Hg in maternal blood was higher in the third trimester than in cord blood ($p < 0.001$). The decrease in total hair Hg was more pronounced during the last three months of pregnancy.

Morrissette et al. (2004) concluded that Hg in the newborns was strongly correlated with the frequency of maternal fish consumption before and during pregnancy. Further, maternal hair Hg was correlated with blood Hg and was highly predictive of the organic fraction of cord blood. Those authors also noted that the consumption of fresh, canned, and/or frozen market fish were more important sources of Hg than were fish from the St. Lawrence River.

2.3.2.3 (Ethylmercury)

Oral exposure to EtHg

In a review of the mechanisms of mercury disposition in the body, Clarkson et al. (2007) note that, like MeHg, EtHg moves freely throughout the body and is probably transported as complexes with small molecular weight thiol compounds on endogenous carriers.

Parenteral exposure to EtHg

To compare the pharmacokinetics of EtHg (as thimerosal) with MeHg, Zareba et al. (2007) administered a single *i.m.* injection of either 1.4 mg/kg EtHg or MeHg to newborn ICR mice on postnatal day (PND) 10. PND 10 was selected for the injections because it temporally coincides with the last phase of mouse brain development and approximates a similar stage of human brain development that corresponds with the timing of vaccine administration to infants (*i.e.*, 0 to 6 months of age) (Ball et al. 2001, Bayer et al. 1993). Pups were selected from the litters of timed-pregnant dams. A total of 12 litters, each of which had at least four males and four females, were produced (five litters for EtHg exposure and seven for MeHg exposure). Two additional litters, each having less than four females and/or less than four males, were used for controls. On PNDs 11, 12, 13, and 14, one male and one female pup from each litter was sacrificed, and total Hg and inorganic Hg were measured in brain, kidney, liver, and muscle (application site) tissue. In addition, blood and hair were analyzed for total Hg.

For both species of organic Hg, Zareba et al. (2007) found that the mercury was rapidly absorbed from the injection site. Only 3% of the injected thimerosal and 4% of the injected MeHg remained at the injection site after four days. Organic Hg levels were significantly lower (~3- to 4-fold) in the thimerosal group than in MeHg-exposed mice ($p < 0.001$). Inorganic Hg levels were reported to be comparable in both groups, except for the first day post-exposure. However, the authors found that inorganic Hg accounted for a higher fraction (12-22%) of total Hg in the thimerosal group, *vs.* 10% or less in the MeHg-exposed group. In the kidneys, there were lower organic Hg levels in the thimerosal group at each day of the 4-day observation period. Further, inorganic Hg

levels in the thimerosal group increased more than 2.5-fold (from 17% to 44%) of total mercury during the 4-day period, compared with only a slight increase (from 8% to 12%) for MeHg-treated animals. In contrast to the kidney and brain, both organic and inorganic Hg levels were significantly higher in the livers of thimerosal-exposed animals ($p < 0.001$ for each of days 2, 3, and 4 post-treatment). For inorganic Hg, the thimerosal group demonstrated a much higher deposition of inorganic Hg in the liver than did the MeHg group throughout the observation period ($p < 0.001$). For blood, there was a significant difference in total Hg only on day one, with a similar decline in blood Hg level roughly similar for both the thimerosal and MeHg mice being seen over the next three days. The total Hg concentration measured in hair was approximately two times higher in the MeHg group than in the thimerosal-treated group ($p < 0.001$ at day four). This was the first published study showing that EtHg incorporates into growing hair in a manner similar to MeHg. Zareba et al. (2007) concluded that their data showed significant differences in tissue distribution and metabolism of EtHg and MeHg and challenge the assumption that EtHg is toxicologically identical to MeHg, particularly with regard to Hg levels in the two primary target organs, the brain and kidney.

Burbacher et al. (2005b) compared the pharmacokinetics of parenterally administered EtHg (as thimerosal) with orally-administered MeHg in 41 infant *Macaca fascicularis* monkeys. Seventeen monkeys were assigned to each the thimerosal and MeHg groups, with thimerosal being administered by *i.m.* injection and MeHg by oral gavage. The total Hg dose administered in the vaccine injections was 20 μg/kg on days 0, 7, 14, and 21 days of age. The MeHg was administered as MeHg hydroxide dissolved in water, with a dose of 20 μg/kg administered on the day of birth and at 7, 14, and 21 days of age. Seven infant monkeys were assigned as controls and received no gavage or *i.m.* injection treatments, whatsoever. Blood was drawn at birth (prior to Hg administration) and on days 2, 4, and 7 after the initial Hg exposure on day 0. Animals were sacrificed 2, 4, 7, or 28 days after their last exposure on day 21. Depending on the sacrifice group, blood was drawn up to 28 days after the final exposure on day 21.

Burbacher et al. (2005b) found no significant differences in weight gain, brain weights at sacrifice, and brain-to-body-weight ratios across the three groups ($p > 0.10$). For monkeys exposed orally to MeHg, total blood Hg peaked at 2 days after the first dose, with progressive Hg accumulation over the 3 subsequent doses (~ 3-fold higher after the fourth dose). The half-life of total Hg in the blood was 6.9 +/- 1.7 days for thimerosal-exposed animals, compared with a half-life of 21.5 days for MeHg-treated monkeys. The decrease in organic Hg in the brain was not statistically significant ($p = 0.17$). The half-life of total Hg in the brain was 59.5 +/- 24.1 days, which was significantly ($p < 0.05$) longer than the half-life of blood Hg in the MeHg-exposed animals. For the thimerosal-treated monkeys, blood Hg concentrations declined by more than 50% between doses. There was a significant ($p < 0.01$) decrease in total Hg in the brain over time, with an apparent brain half-life of 24.2 +/- 7.4 days in those monkeys. The concentration of total Hg was about 3-fold lower in thimerosal-treated monkeys than in those receiving MeHg orally, but the average ratio of brain-to-blood Hg concentration was slightly higher in the thimerosal group (3.5 +/- 0.5 *vs*. 2.5 +/- 0.3). Burbacher et al. (2005b) concluded that the key findings of their study were the differences in disposition kinetics and demethylation rates of EtHg (as thimerosal) and MeHg. Further, **these differences make MeHg unsuitable for use as a surrogate of EtHg in risk assessment.**

Jin et al. (2007) divided young male Sprague Dawley rats into five different diet groups (based on type of dietary fat) of 18 animals per group. Semi-purified casein-based diets containing one of five fat types were provided to the rats for 28 days. On day 29, rats were administered daily doses of 0, 1, or 3 mg MeHg/kg/ bw by gavage for 14 days. On day 43 of the study, the rats were sacrificed and blood and selected organs were harvested for examination. Total mercury content in brain, liver, and blood all increased in a linear and dose-dependent fashion with MeHg treatment, regardless of the diet used. In all diet groups, total Hg was higher in the blood than in the liver, and the lowest total Hg concentration was found in the brain. In that organ, over 75% of Hg was in the form of MeHg. Jin et al. (2007) also reported that MeHg disposition in brain may differ in rats fed different dietary fats.

2.3.4 Elimination and Excretion

Hashemi and Young (2003) used neuronal network modeling of pharmacokinetic data regarding MeHg from over three dozen peer-reviewed, published articles. The data collected were for humans and a variety of non-human animals, including mice, hamsters, Guinea pigs, monkeys, goats, pigs, and sheep. Artificial neuronal networks constitute an approach to predicting human pharmacokinetic parameters from animal data. These networks provide a means to formulate a model expression of pattern recognition based on the animal data that can, in turn, be applied to the human test data. Using the raw half-life data from the published literature, total Hg half-lives ranged from 3 days to 76 days, depending on the species, route of MeHg administration (oral, *s.c.*, or *i.p.*), and frequency of administration. The longest half-lives were for humans (72-76 days), and the shortest half-lives were seen in rodents (mice: 3-10.04 days; hamsters: 5-7.7 days). (A half-life of 41.3 days was reported for mice in a single study in which mice received one oral dose of 5 mg/kg MeHg, but this is not consistent with other studies using even higher doses (up to 25 mg/kg) and a variety of exposure methods.) Total Hg blood half-lives ranged from 3.81 to 79 days, with the highest being again in humans and the lowest in mice. Using modeled animal data, the closest match to human data was obtained from the allometric relationship for MeHg elimination half-life based on total Hg in blood and animal weight, termed the category-thermometer-reduced data neural network model. This model predicted half lives for humans (based on animal data) ranging from 55 to 75 days for blood (vs. 42-79 for actual measured human blood half-lives) and 75 for whole-body half-life (vs. 72-76 days actually measured in humans).

2.4 MECHANISMS OF ACTION

2.4.1 *Methylmercury*

As an introduction to this section, it is necessary to point out that mercury has a strong affinity for sulfur, a fact known since at the days of alchemy (Hughes 1957). Mercury in

any form will bind with sulfhydryl (-SH) groups in thiols or proteins. Sulfur is present in the amino acids cysteine, cysteine, and methionine, as well as the cysteine-derivative taurine, which is plentiful in the bile. Since receptors in/on cell membranes are all constructed from proteins, those receptors are susceptible to attack by various forms of mercury. And when mercury binds to one of the amino acid residues in receptor proteins, it reduces that protein molecule's range of availability for normal metabolic function. When the effected protein is normally used to transport a substance, such as calcium ion (Ca^{++}), across the membrane of a cell or sub-cellular organelle, the selective permeability of that membrane and the proper physiologic function of that cell or organelle is compromised.

The strong affinity of sulfhydryl (-SH) groups for mercury has often been considered to be the mechanism of toxic action of mercury and its compounds. In a review of the mechanisms of mercury disposition in the body, Clarkson et al. (2007) wrote that the high mobility of MeHg in the body is due to the formation of small molecular weight thiol complexes that are readily transported across cell membranes. However, the effects that occur following, or as a result of, entry into the cell are key to understanding the ultimate mechanism or mechanisms of toxic action of mercury. Since these effects occur at both the cellular and sub-cellular levels, *in vitro* and *in situ* studies are necessary to examine the chemical mechanisms occurring at these levels. Thus, *in vitro* and *in situ* studies have provided the most valuable evidence and explanations of exactly why and how mercury and its organic compounds cause damage to nervous tissue.

A number of recent studies have examined the sub-cellular mechanism of the neurotoxicity of MeHg. Impaired calcium homeostasis (Dreiem and Seegal 2007; Sirois and Atchison 2000), oxidative stress (Garg and Chang 2006, Mori et al. 2007, Yin et al. 2007), and the alteration of glutamate homeostasis (Farina et al. 2003 a,b; Ou et al. 1999a, Yin et al. 2007) have all been suggested as possible mechanisms contributing to neurotoxicity. Those and other mechanisms are discussed in this section.

Intracellular Calcium Homeostasis/Altered Membrane Permeability

In a review of possible mechanisms for the cytotoxic action of MeHg, Limke et al. (2004a) noted that intracellular calcium $[Ca^{++}]_i$ undergoes cyclic changes in concentration during normal neurologic function, and that a large concentration gradient of calcium ions typically exists across the neuronal membrane. Because of these, the sustained elevation of $[Ca^{++}]_i$ can be deleterious in two major ways: (1) a rundown of energy reserves resulting from excessive employment of ATP-dependent Ca^{++} pumps to restore resting membrane potential; and (2) the activation of catabolic functions; both of these can contribute to Ca^{++}-mediated cell death (Limke et al., 2004a). Further, Peng et al. (2002) pointed out that MeHg disrupts normal Ca^{++} channel functions, as seen in studies measuring the influx of radiolabeled calcium in synaptosomes, ligand binding, and studies employing electrophysiological techniques.

Hare et al. (1993) used single NG108-15 cells preloaded with the fluorescent dye fura-2 to evaluate whether MeHg caused an increase in $[Ca^{++}]_i$. Whereas 0.5 μM MeHg had no effect, both 2 and 5 μM MeHg produced a biphasic increase in fluorescence. The initial increase in fluorescence was sustained, with the time to onset being concentration-dependent. The maximum increase, however, was not found to be dependent on the MeHg concentration. The initial phase was considered to likely be the result of an increase in Ca^{++} from both intra- and extra-cellular sources, since removal of Ca^{++} from the extracellular medium reduced, but did not eliminate, the increase. The time to the onset of the second phase was also concentration dependent. Thus, MeHg was found to alter the measured fura-2 fluorescence in the cells in both a concentration- and time-dependent manner. Hare et al. (1993) also concluded that the initial effect involved alterations in intracellular cation buffering, as well as an increase in the permeability of the plasma membrane to Ca^{++}.

Sirois and Atchison (2000) used whole-cell patch clamp techniques to investigate the ability of MeHg to block Ca^{++} channel currents in cultures of neonatal cerebellar granule cells taken from 7-day-old rat pups of either gender. This cell type was chosen for this study due to their diversity of Ca^{++} channels, as initially reported by Randall and Tsien

(1995). To determine whether MeHg is specific for one or more of the known sub-types of Ca^{++} channel receptor, Sirois and Atchison (2000) used a number or putative Ca^{++} channel antagonists (ω -conotoxin GVIA, ω-conotoxin MVIIC, ω-agatoxin IVA, calcicludine, and nimodipine), each with specific blocking characteristics.

To eliminate the possibility of mixing the release of intracellular calcium with the influx of extracellular calcium through calcium channels, Sirois and Atchison (2000) used the radiolabeled divalent bromine ion (Br^{++}) instead of Ca^{++} in the bathing medium. While Br^{++} is not normally a constituent of extracellular fluid bathing the neurons, the gated calcium channels are nonetheless highly permeable to Br^{++}, making Br^{++} a logical surrogate for extracellular Ca^{++}. In this study, the authors found that acute exposure to sub-micromolar concentrations of MeHg can block Ba^{++} currents carried through multiple Ca^{++} channel subtypes. They further reported that the channel-blocking effect of MeHg was seen well within concentrations seen during episodes of MeHg intoxication. And while the role that MeHg-induced calcium block plays in MeHg neurotoxicity remains to determined fully, the low concentration at which these effects were seen in this study makes it likely that the effects on Ca^{++} channels at least contribute to the pathological damage observed in MeHg poisoning (Sirois and Atchison 2000). These authors further postulated that the blockage of Ca^{++} channels could possibly contribute to ultimate neuronal death in the cerebellum through the disruption of neurotransmitter release, cell growth and differentiation, protein synthesis, maintenance of the membrane potential, and regulation of $[Ca^{++}]_i$.

Additional support for the ability of MeHg to block Ca^{++} channels comes from work in that same lab in the form of a study found that MeHg blocked Ca^{++} currents, although not completely, in human embryonic kidney cells in culture (Peng et al. 2002).

Altering intracellular calcium levels in brain capillary endothelial cells has a direct effect on blood-brain barrier permeability and transport (Paemeleire et al. 1999). Thus, substances such as organic mercury compounds, which cause the release of intracellularly

bound Ca^{++}, might not only have a direct affect on neuronal function, but may also increase the availability of mercury, and possibly other neurotoxicants, in the CNS.

Oxidative Stress/Reactive Oxygen Species (ROS)

Active transport processes essential to the maintenance of the neuronal resting membrane potential require a great amount of the energy-carrying molecule adenosine triphosphate (ATP), which is produced in the mitochondria within the neurons through the oxidative metabolism of glucose. The effect of MeHg on mitochondrial metabolic function was examined by Dreiem and Seegal (2007) using rat striatal synaptosomes (pinched-off nerve endings). Specifically, the study was designed to determine whether MeHg-induced elevations in reactive oxygen species (ROS) or alterations in intracellular calcium were the cause of compromised mitochondrial function. The formation of ROS was assessed through the use of DCFH-DA, a non-fluorescent, cell-permeable compound to form 2'7'-dichlorolfluorescein (DCF) (Myhre et al., 2003). Mitochondrial metabolic function was assessed by the ability to convert the dye methyl-thiazoletetrazolium to formazan. The authors reported that MeHg increased ROS levels, decreased mitochondrial function, and caused an increase in both cytosolic and mitochondrial calcium levels in the synaptosomes. When the synaptosomes-MeHg preparation was co-incubated with Trolox, an antioxidant, the MeHg-induced ROS level was significantly reduced; however, the Trolox failed to restore mitochondrial function. The elevation in calcium levels was found to be independent of extra-synaptosomal calcium. The authors concluded that the MeHg-induced mitochondrial dysfunction is not the result of increased ROS levels, but instead the ROS increase is a secondary event in MeHg toxicity. Further, they suggested that MeHg-induced elevations in mitochondrial calcium are responsible for the mitochondrial damage caused by MeHg (Dreiem and Seegal, 2007).

Minnema et al. (1987) also found evidence of MeHg-induced release of mitochondrial calcium from rat brain synaptosomes. In a multi-faceted experiment, Minnema and his co-investigators found that MeHg produced large effluxes of calcium from isolated

mitochondria preloaded with radio-labeled calcium, but not from synaptosomes preloaded with radiolabeled calcium. They also found that 0.5 to 5.0 μm MeHg caused a concentration dependent increase in the spontaneous release of tritiated dopamine from striatal synaptosomes, gamma-aminobutyric acid (GABA) from cerebellar cortical synaptosomes, and acetylcholine from hippocampal synaptosomes. These releases were determined to not be attributable to a MeHg-increase in calcium permeability of the synaptosomal membrane, since these increases persisted in the absence of extra-synaptosomal calcium (Minnema et al., 1987).

One of the functions of the smooth endoplasmic reticulum (SER) is to serve as a storage site for $[Ca^{++}]_i$. As such, Ca^{++} in the cytosol must be actively transported against its concentration gradient into the SER, a process requiring ATP. Bearss et al. (2001) reported that the application of thapsigargin, an inhibitor of smooth endoplasmic reticulum Ca-ATPase activity, reduced the amplitude of MeHg-induced increase in $[Ca^{++}]_i$ by 30% in rat cerebellar granule cells in primary culture.

In a follow-up paper from the same laboratory, Limke et al. (2004b) examined the role of muscarinic receptors in MeHg-induced dysregulation in rat cerebellar granule cells *in vitro* through the use of fura-2 single-cell microfluorimetry. When atropine, a non-specific muscarine receptor antagonist, was added to the preparation, the onset of MeHg-induced Ca^{++} release into the cytosol was significantly delayed. In addition, depletion of smooth endoplasmic reticulum (SER) Ca^{++} with thapsigargin or the down-regulation of muscarinic receptors and inositol-1,3,4-triphosphate (IP_3) receptors with bethanechol caused similar reductions in the amplitude of the MeHg-induced Ca^{++} increase. Collectively, these suggest that MeHg interacts with muscarinic receptors to cause Ca^{++}release from the SER through activation of IP_3 receptors.

Limke et al. (2004b) concluded that the results of their experiments suggested that interactions of MeHg with muscarinic receptors and resultant perturbation of Ca^{++} regulation within the SER may contribute to the selective vulnerability of cerebellar granule cells. Further, the importance of the SER as a MeHg target may lie in the effect

of the released Ca^{++} on nearby mitochondria, and the localization of specific muscarinic receptor subtypes may contribute to the regional selectivity of MeHg toxicity within the CNS. These conclusions are supported by previous work in the same laboratory (Limke et al., 2003; 2002), which suggested that the SER contributes Ca^{++} to the observed mitochondrial dysregulation and subsequent neuronal death via an MTP-dependent pathway.

In order to investigate the mechanism of selective neurotoxicity of MeHg in the nervous system, Mori et al. (2007) measured the oxygen consumption levels, the production of reactive oxygen species (ROS), and several antioxidant levels in mitochondria in the cerebrum, cerebellum, and liver of rats. In this study, 9-week-old male Wistar rats were administered MeHg chloride (equivalent to 10 mg/kg/day) in solution by gavage for five days. On the 14^{th} day following the cessation of treatment, the animals were sacrificed and subjected to tissue analysis, including evaluation of changes in sub-cellular activity in the mitochondria.

Mitochondrial oxygen consumption was found to be higher ($p < 0.05$) in the cerebrum and cerebellum of controls than in the livers of controls, and oxygen consumption was significantly higher ($p < 0.05$) in the cerebellum of controls than in the cerebrum of that same group. This was not unexpected because of the high energy needs of the active transport processes in the brain requiring a greater amount of aerobic respiration. However, Mori et al. (2007) pointed out that about 1-5% of the oxygen consumed by mitochondria is converted to ROS, such as O^{2-} and H_2O_2. Further, the brain is particularly sensitive to oxidative injury, in large part due to its high rate of oxidative metabolism (Halliwell 1992).

In the cellular cytosol, GSH serves as the major antioxidant, helping to scavenge ROS. Mori et al. (2007) point out that the function of mitochondrial GSH would be expected to be essentially the same as cytosolic GSH with respect to MeHg toxicity. In this study, both whole tissue GSH and mitochondrial GSH were lower in the rat brains than in the livers, and the same was true of two other antioxidant enzymes, superoxide dismutase

(SOD) and glutathione peroxidase (GPX). This occurred despite the higher oxygen consumption and subsequent ROS production in the brain. The authors suggested that the brain mitochondria might therefore function with marginal stability against oxidative stress. Further, the mitochondria of the cerebellum might be particularly sensitive to this, because of their higher oxygen consumption and ROS production than the cerebrum. In fact, the GSH levels measured in mitochondria in the cerebellum of intact rats was as low as one-third that or cerebral mitochondria. Mori et al. (2007) noted that the higher susceptibility to MeHg-induced oxidative damage in brain mitochondria (particularly in the cerebellum) compared with the liver mitochondria might account, at least in part, the selective toxicity of MeHg. In summarizing, Mori et al. (2007) concluded that their data suggest that the high MeHg-induced activity in mitochondrial ROS generation and low activity in the brain's defense system, particularly in the cerebellum, would easily cause a critical imbalance in ROS production and at least partially account for the selective neurotoxicity of MeHg.

Effects on Receptor Binding/Neurotransmitter Release

The muscarinic ACh (mACh) receptor has five different isotopes that regulate a diverse number of motor, sensory, and cognitive neurobehaviors (Basu et al., 2008). And while it is recognized that MeHg can affect the general population of mACh receptors and alter receptor protein levels, relatively little is known about the interaction of MeHg with specific isoforms of that receptor. Therefore, Basu et al. (2008) investigated the effects of MeHg on muscarinic cholinergic receptor subtypes M1 and M2 using a combination of *in vitro* competitive binding assays and examination of tissues from MeHg-exposed mink. In this study, juvenile male mink (9 per treatment group) were fed diets containing MeHg concentrations of 0, 0.1, 0.5, 1, or 2 μg/g diet (ppm) for 89 days. Animals were then sacrificed and the entire brain was extracted from each skull, after which the occipital cortex and brain stem were dissected from the right hemisphere. Pirenzepine, a selective antimuscarinic agent, was used in tritiated form to block M1 muscarinic ACh receptors, and [^{3}H]-AFDX-384 was used as an M2 blocker.

In the *in vitro* tests, MeHg inhibited the binding of radioligands to the M1 and M2 receptors in the occipital cortex and brain stem of the mink. Whereas MeHg inhibition of [^{3}H]-pirenzepine binding to M1 receptors was observed in both the cortex and brain stem, the effect was more pronounced in the cortex. Similarly, [^{3}H]-pirenzepine *in vitro* binding results showed that MeHg was more potent at inhibiting ligand binding in the occipital cortex than in the brain stem (Basu et al. 2008).

Conversely, *in vivo* exposure of the mink to dietary MeHg resulted in greater binding of radioligands to both M1 and M2 receptors (Basu et al. 2008). While an exposure-dependent increase in [^{3}H]-pirenzepine binding was measured in both the occipital cortex and brain stem, only the increase in the cortex was statistically significant ($p < 0.01$, vs. p = 0.2 in the brain stem). In the case of [^{3}H]-AFDX-384, a MeHg-dependent increase in binding was observed in the occipital cortex of mink exposed to concentrations of 0.5, 1, and 2 ppm MeHg ($p < 0.01$), but the increase in binding in the brain stem was non-significant ($p = 0.1$). The authors explained this difference in direction of binding change between *in vitro* and *in vivo* studies as being logical. They pointed out that previous neuropharmacologic studies have established that exposure of animals to the muscarinic agonist atropine results in the up-regulation of receptor protein (Wall et al. 1992). The relevance to the Basu et al. (2008) study is straight-forward, as the receptor binding inhibition outside of a whole-body situation could not result in a compensatory increase in protein necessary to make new receptors. However, the response to blockage of receptors *in vivo* over a period of time would be the compensatory up-regulation of receptors.

In another study, adult female Sprague-Dawley rats were provided MeHg in drinking water at nominal concentrations of 0, 2.5, or 10 µg/L for 16 consecutive days (Coccini et al. 2000). Water consumption and body weights were obtained daily and used to determine daily intakes of 0.5 or 2.0 mg MeHg/kg/day for the 2.5 and 10 µg/L concentrations, respectively. The rats were decapitated either immediately following the dosing period or 14 days after the cessation of MeHg administration. The cerebral

cortex, cerebellum, and hippocampus were dissected out and examined for muscarinic receptors radiolabeled with [^{3}H]quinuclidinyl benzilate. The authors found evidence of up-regulation of muscarinic ACh receptors. The density of these receptors was increased in only the cerebellum and hippocampus, but receptor affinity remained unaltered in all three brain areas. While an increase in muscarinic ACh receptors was not initially seen in the cerebral tissue, it was demonstrable in the cerebrum of animals examined two weeks after the termination of treatment.

Coccini et al. (2000) concluded that prolonged ingestion of low doses of MeHg by rats causes subtle molecular changes, with adaptive imbalance of muscarinic ACh receptors in the hippocampus and cerebellum. These authors also noted that because cholinergic systems play an important role in learning and memory, as reported by Mash et al. (1985), the increased ACh receptor density caused by MeHg may be a deleterious process.

Yuan and Atchison (2003) conducted experiments using whole-cell recording techniques to examine why cerebellar granule cells are much more sensitive to MeHg than are their neighboring cerebellar Purkinje cells. A specific area of interest was whether the expression of phenotypically different $GABA_A$ receptor alpha subunits plays a role in this differential sensitivity. The premise was that if so, the responses of $GABA_A$ receptor-mediated inhibitory postsynaptic currents (IPSCs) in Purkinje and granule cells should differ in their response to MeHg. Despite the fact that a range of MeHg bath concentrations was used, the pattern of response was similar. Biphasic changes were observed in frequency and amplitude of both spontaneous IPSCs and miniature IPSCs recorded from Purkinje and granule cells in a concentration and time-dependent fashion. However, the magnitude of the changes in frequency or amplitude of postsynaptic current was independent of the concentration of MeHg, suggesting that either a fairly constant series of events is initiated once an effective MeHg concentration is achieved, or that the concentrations tested were all at the high end of the concentration-response relationship. To use lower MeHg concentrations, however, the authors felt would compromise the high

quality of the continuous whole-cell recordings due to the concentration-dependent latent period preceding the time of onset of the response.

While thus unable to prove their hypothesis, Yuan and Atchison (2003) did establish that MeHg acts at both pre- and post-synaptic sites to alter $GABA_A$ receptor-mediated inhibitory synaptic transmission. And while the general patterns of effects on the two cell types were similar, $GABA_A$ receptors in granule cells did appear to be more sensitive to block by MeHg than are those in Purkinje cells, as spontaneous GABAergic currents in granule cells were blocked at an earlier time than were those in Purkinje cells.

To determine whether MeHg interacts specifically with the $GABA_A$ receptor, Fonfria et al. (2001) used intact mouse cerebellar granule cells as an *in vitro* model of neuronal selectivity of that organomercurial. In this study, the authors investigated whether MeHg had an effect on granular cells pretreated for 30 min. with the radioligand [^{3}H]flunitrazepam, and found that binding was increased in a dose-dependent fashion. They further found that this increased was completely blocked by bicuculline and picrotoxinin, both $GABA_A$ receptor antagonists, and by the organochlorines pesticide gamma-endosulfan as well. It was also found that the increase in [^{3}H]flunitrazepam binding in the presence of MeHg was independent of intracellular events, such as $[Ca^{++}]_i$, kinase activation/inactivation, or antioxidant conditions. Fonfria et al. (2001) concluded that MeHg interacts with the $GABA_A$ receptor by the way of alkylation of SH groups of cysteinyl residues found in $GABA_A$ receptor subunit sequences.

The effects of 40 μM, 400 μM, or 4 mM MeHg on the dopaminergic system of the rat striatum in conscious, freely moving animals were studied by Faro et al. (2000). All doses increased dopamine (DA) release (907 +/- 31%, 2324 +/- 156%, and 9032 +/- 70%, for low, middle, and high dose concentrations, respectively). High-dose exposure also caused significant decreases in extracellular levels of the DA metabolites dihydroxyphenylacetic acid (DOPAC) and homovanillinic acid (AV). The authors attributed these effects to the MeHg-stimulated DA release, decreased DA intra-neuronal degradation, or both.

Gao et al. (2008) investigated the effects of MeHg on postnatal neurobehavioral development in mice. Mice from the MeHg treatment groups were given intraperitoneal injections of 0.1, 1, or 3 mg/kg MeHg chloride from postnatal days 15 through 17, while control mice were injected with sterile saline. On postnatal day 45, the mice were tested using the Morris water maze to evaluate spatial learning and memory. Significant differences were seen between 1 mg/kg and 3 mg/kg groups and controls with regard to two measured parameters (described previously in this addendum). Twenty-four hours after the final tests, three mice from each group were sacrificed and the hippocampus removed. Increases in NR1, NR2A, and NR2B subunits of the NMDA dopamine receptor were expressed in the hippocampus, relative to controls. Expressed as a percentage of control values, subunit NR1 (NMDAR1) was increased by 10% in the 0.1 mg/kg group, 378% in the 1 mg/kg group, and 314% in the 3 mg/kg group mice. NR2A expression was increased by 21% in 0.1 mg/kg mice, 256% in 1 mg/kg mice, and 670% in 3 mg/kg mice, while NR2B expression was enhanced by 59%, 168%, and 252% in the 0.1 mg/kg, 1 mg/kg, and 3 mg/kg groups, respectively. Gao et al. concluded that the MeHg-induced subtle, but persistent, learning deficits and neurobehavioral abnormalities seen in the mice might be ascribed to alteration of the gene expression of specific NMDA receptor subunits in the hippocampus.

To investigate the role of the dopamine transporter in MeHg-induced dopamine release, Faro et al. (2002) administered adult female Sprague-Dawley rats MeHg dissolved in perfusion fluid and applied locally into the striatum via a dialysis probe. Intrastriatal infusion of 400 uM MeHg increased the extracellular dopamine levels to 1941% of baseline levels (+/- 199%). The MeHg-induced release of dopamine was not attenuated in the total absence of calcium in the bathing Ringer's solution, nor was it attenuated after *i.p.* pre-treatment with reserpine (a depletor of catecholamine stores in brain tissue) or the sodium channel blocker tetrodotoxin (TTX), suggesting that the dopamine release was independent of calcium and vesicular stores, as well as not affected by the blockade of voltage-sensitive sodium channels. Following infusion of KCl (75 mM) through the dialysis probe, Faro et al. (2002) found that MeHg caused a decrease in the KCl-evoked

release of dopamine. The authors concluded that collectively, their experiments suggest that MeHg induces the release of dopamine via a transport-dependent mechanism. The MeHg-induced release of dopamine was also independent of calcium and vesicular stores.

A study by Faro et al. (2005) investigated the effect of pretreatment with glutathione (GSH), cysteine, or methionine on MeHg-induced dopamine release from the rat striatum. Adult female Sprague-Dawley rats were administered a perfusion fluid containing either MeHg, MeHg following GSH pretreatment, MeHg following cysteine pretreatment, or MeHg following methionine pretreatment via a dialysis probe inserted directly into the striatum. The individual concentration of each of the four substances in the perfusion fluid was 400 μM. Infusion of 400 μM MeHg alone resulted in a 1,941% (+/- 199%) increase in extracellular dopamine levels (vs. basal levels). Infusion of 400 uM following GSH pretreatment resulted in an increase in extracellular dopamine of only 465% (+/- 104%), or 76% less increase than that caused by MeHg alone. MeHg infusion following pretreatment with cysteine resulted in only an increase of 740% (+/- 149%), or only 62% lower than that induced by MeHg alone. Treatment with MeHg following methionine pretreatment resulted in no significant difference from MeHg alone.

Faro et al. (2005) pointed out that MeHg promotes a decrease in intracellular GSH levels, as previously demonstrated by Choi et al. (1996). So pretreatment with GSH would to some extent ameliorate the impact of MeHg on existing cellular GSH, which is what happened in this study. They also noted that the amino acid-cysteine has a free -SH group, whereas the amino acid methionine has a sulfur atom without the capacity to form -SH groups. Therefore, it is not surprising that the -SH group available in the cysteine pretreated group would bind with MeHg and serve to decrease dopamine release. But since the sulfur atom in methionine was unavailable for binding to MeHg, pretreatment with that amino acid had no affect on MeHg-induced dopamine release (Faro et al. 2005).

Bemis and Seegal (1999) investigated whether effects seen in the rat brain in the presence of both MeHg and PCBs were due to the presence of MeHg, PCBs, or a combined effect

of both. Their results showed *in vitro* exposure of striatal tissue to MeHg had no significant affect on tissue dopamine or medium dopamine levels. In contrast, exposure to PCBs alone reduced tissue dopamine (DA) and elevated media DA in a dose-dependent fashion. However, when striatal tissue was exposed simultaneously to both PCBs and MeHg, there were significantly greater decreases in tissue DA concentrations and elevations in media DA than those caused by PCBs alone. This led the authors to suggest that the significant interaction between these two toxicants may be due to a common site of action that influences DA function (such as toxicant-induced increases in intracellular calcium and changes in second messenger systems).

Effects on Cell Cycle/Cell Division

Castoldi et al. (2000) exposed *in vitro* cultures of rat cerebellar granule cells to MeHg concentrations of 0.5-1 μM or 5-10 μM. One hour of exposure to the higher concentration resulted in impairment of mitochondrial function and plasma membrane lysis, resulting in cell death. While the lower (0.5-1 μM) concentrations did not compromise cell viability or mitochondrial function at early time points, neuronal network fragmentation and depolymerization of microtubules were observed within 1.5 hours. This damage continued to progress over time, and complete dissolution of microtubules and neuronal processes was seen after 18 hours. The authors postulated that cytoskeletal breakdown and deprivation of neurotrophic support may play a role in delayed toxicity following MeHg exposure. Similarly, Miura et al. (1999) studied the relationship between changes in the cell cycle and the induction of apoptosis caused by MeHg in cultured mammalian cells, and reported that GM2/M-phase arrest through the disruption of microtubules is an important event in the development of apoptosis by MeHg. Consistent with altered mitotic division, Bahia et al. (1999) observed a reduction in the frequency of mitotic divisions following *in vitro* exposure of human lymphoblastoid (TK6) cells to MeHg.

To investigate the molecular mechanisms underlying the ability of MeHg to inhibit cell cycle processes, Ou et al. (1999b) studied the activity of cell cycle regulatory genes in the presence of MeHg. Two phases of the study were conducted: an *in vitro* cell culture phase and an *in vivo* exposure phase using C57BL/6 mice. In the cell culture experiment, gravid uteri were removed from pregnant Sprague-Dawley rats 12.5 days post-coitum, and embryonic midbrain and limb bud cells were dissected-out and dissociated into single-cell suspensions. A MeHgOH solution (1 μM and 2 μM MeHg) was applied to the culture medium 24 hours following plating and continued over a 5-day (total) period, during which CNS and limb bud cells undergo differentiation. A gradual decrease in the expression of p21 mRNA (p21 being associated with terminal differentiation of many cell types) was consistent from days 1-5 of treatment, but failed to reach statistical significance. For CNS cells, scanning dosimetry measurements from three separate experiments revealed that the average mRNA expression level of p21 on day 5 was 60% (+/- 12%) of those on day 1. For limb bud cultures, however, the average expression of p21 on days 1-5 was significantly increased ($p < 0.05$), with levels of 900% (+/- 230%) at day 3 and 580% (+/- 130%) at day 5 of treatment, compared with day 1 values. With regard to stages of the cell cycle (in sequence: G_1, or growth 1; S, or DNA synthesis; G_2, or growth 2; and M, or mitosis), 2 μM MeHg exposure for 30 minutes resulted in a 215% (+/- 12%) increase in the proportion of G2-M phase cells and a corresponding decrease of 40% (+/- 3%) in the S phase population, indicating the inhibition of DNA synthesis. Also at an exposure level of 2 μM, average increases in p21 mRNA expression of 2.0 +/- 40.4-fold and 2.6 +/- 0.5-fold were reported for CNS and LB cells, respectively, compared to untreated controls.

Dose-dependent decreases in the number of viable cells and cell cycling rates were observed following 24 and 48 hours of MeHg exposure (Ou et al. 1999b). After 24 and 48 hours of exposure to 2 μM MeHg, the percentage of cells successfully completing one cell division were only 25 and 13%, respectively, of concurrent untreated controls. The percentage of viable cells was 77% of controls at the 24 hour measurement and only 33% of the control values after 48 hours of MeHg exposure. When compared with cells undergoing treatment with the known microtubule disruptor colchicine, MeHg was found

to be the more powerful inhibitor of the cell cycle, with colchicine reducing the number of viable cells to 46% of controls, *vs.* 33% with MeHg.

In the *in vivo* experiments (referred to as Study A and Study B in the report), 6-week-old C57B/6 female mice were arbitrarily separated into dose groups of 0, 3, or 10 ppm MeHg (as MeHgOH) in drinking water (Ou et al. 1999b). After 4 weeks of treatment, animals were sacrificed and brain, liver, and kidney tissue were dissected and prepared for analysis. In Study A, none of those organs showed any gross abnormalities; however, the induction of p21 mRNA expression was observed, but only at the 10 ppm MeHg treatment level and only in animals that showed signs of toxicity. An estimation of 6.8 (+/- 1.4)-fold increase (over controls) in p21 mRNA was observed in the brain ($p < 0.05$). In Study B, symptomatic animals receiving the 10 ppm MeHg water, 4.5 (+/- 0.1)-fold and 5.5 (+/- 0.6)-fold increases in p21 induction were observed in the cerebellum and cortex, respectively.

Effects on GSH Activity

Franco et al. (2006) examined the exclusive contribution of MeHg exposure through maternal milk on biochemical parameters related to the thiol status in the cerebellums of suckling mice. The thiol status was determined by glutathione (GSH) levels and glutathione peroxidase (GP*x*) and glutathione reductase (GR) activity. In this study, 14 Swiss albino mice dams were randomly assigned to one of two groups of seven females each (one treatment and one control group). Pups (eight per litter) were maintained with their mothers, half of which were immediately exposed to MeHg (10 mg/L) in drinking water (treatment group) or to MeHg-free tap water (control group) *ad libitum*. The exclusive route of MeHg exposure for the treated offspring was maternal milk.

Franco et al. (2006) found that the GSH level in the cerebellum was significantly higher ($p < 0.05$) in the MeHg-exposed dams than in the control rats. In the exposed pups, however, the response was completely the opposite, with MeHg exposure through breast

milk resulting in a significant decrease in GSH levels ($p < 0.05$). Cerebellar GR activity was also significantly higher ($p < 0.05$) in MeHg exposed dams than in controls, but this increase was not seen in exposed pups. A two-tailed Pearson correlation test revealed a significant positive correlation between cerebellar GR activity and cerebellar GSH in mothers ($p < 0.001$). MeHg exposure did not affect GP*x*. Franco et al. (2006) concluded that decreased motor performance in the MeHg-exposed pups (reported elsewhere in this profile addendum) may be related to alterations in the cerebellar thiol status. They suggest that the increases in GSH levels and GR activity in the cerebellums of MeHg-exposed dams could represent a compensatory response to the oxidative effects of MeHg toward endogenous GSH. Franco et al. (2006) also suggest that the inability of the pups to perform this compensatory response is probably due to the immaturity of the CNS in the pups, making them more susceptible to the oxidative effects of MeHg on cerebellar thiol status.

In investigating the possibility that intracellular glutathione GSH synthesis may determine sensitivity to MeHg exposure, Ou et al. (1999a) found that while oxidative stress may mediate aspects of MeHg toxicity, disruption of GSH homeostasis alone is not responsible for the sensitivity of embryonic CNS cells to MeHg. In a separate study, Ou et al. (1999b) reported that the activation of cell cycle regulatory genes may be one mechanism by which MeHg interferes with the cell cycle in both adult and developing organisms.

Kim et al. (2007) used SH-SY5Y neuroblastoma cells to investigate whether or not MeHg alters the activity of regulatory proteins involved in the cell cycle. All-*trans*-retinoic acid (ATRA) was used in this study to induce differentiation of the neuroblastoma cells. The existence of retinoid receptors and related cytoplasmic binding proteins has previously been demonstrated in mammalian models (Maden et al. 1990; Ruberte et al. 1993); and it has been suggested that retinoids have a fundamental morphological action in the mammalian nervous system (Perez-Castro et al. 1989; Maden and Holder 1991). ATRA has been associated with several fundamental aspects of CNS development, including axonal growth (through modulation of nerve growth factor, or

NGF), migration of elements of the neural crest (Perez-Castro 1989, Maden and Holder 1991), and specifying the rostrocaudal position of the forebrain, midbrain, hindbrain, and spinal cord in the developing CNS (Maden and Holder 1992). Two days after ATRA-stimulated differentiation of neuroblastoma cells, Kim et al. (2007) performed cell cycle analysis using flow cytometry. It was found that MeHg treatment caused a significant change ($p < 0.05$) in the SH-SY5Y cell cycle. The G_1 phase was reduced in duration, and arrest of the S phase was reported.

The effects of MeHg on microglia were examined using the murine N9 microglial cell line (Garg and Chang 2006). Microglia are macrophage-like cells that make up ~ 15% of the cell population within the CNS. As such, they are responsible for removal of invading pathogens and are critical to the survival and maintenance of CNS neurons. They also secrete a number of proteins, such as interleukin-6 (IL-6), which can be either beneficial or detrimental to neuronal cells, depending on the internal conditions. To test for cytotoxicity, the cells were treated with various concentrations of MeHg for one day, and the viability of each treatment subsequently determined. Cytotoxicity was found to appear in a rapid and irreversible manner. Under the conditions of this experiment, MeHg at concentrations of 4, 8, 12, or 16 μM reduced cell viability by 12%, 59%, 85%, and 95%, respectively.

Results from the Garg and Chang (2006) study indicated that MeHg caused an increase in reactive oxygen species (ROS) in a dose- and time-dependent manner. However, only a modest increase in the generation of ROS was seen at 10 μM or lower MeHg concentrations, and the increase was statistically significant (compared with controls) only at MeHg concentrations of 20 μM or greater. Significant depolarization of the mitochondrial membrane was also observed with increasing MeHg concentrations up to 20 μM ($p < 0.001$). Aconitase activity was also impaired by MeHg, with activity dropping by 14% at 5 μM, 53% at 10 μM, and 90% at a MeHg concentration of 20 μM. Aconitase is an enzyme catalyzing the conversion of citrate to isocitrate in the tricarboxylic acid (or citric acid/Krebs) cycle, and is thus crucial to the production of the energy-carrying molecule adenosine triphosphate (ATP) from glucose. At concentrations

of 10 μM, MeHg also caused a significant increase in IL-6 production, despite the fact that this concentration greatly inhibited protein synthesis. The authors noted, however, that the role(s) of IL-6 in neurotoxicity is still unknown. Overall, Garg and Chang (2006) concluded that their study demonstrated that exposure to MeHg caused microglial cellular oxidative stress as determined by ROS generation, in addition to changes in mitochondrial membrane potential and aconitase activity.

Involvement with Nitric Oxide Synthetase

Shinyashiki et al. (1998) examined the effects of MeHg on cerebral and cerebellar neuronal nitric oxide synthase (nNOS) isoforms in rat brain *in vivo* and *in vitro*. Eight week-old male Wistar rats were given subcutaneous doses of 10 mg MeHg/kg/day for eight days. *In vivo* manifestation of neurotoxicity was considered to be hind limb crossing, which was evaluated as an indication of paralysis. Five animals were sacrificed each at 1, 2, 5, and 8 days after the first injection and when paralysis was achieved, and the cerebrum and cerebellum of each was removed and examined for MeHg levels. Effects on enzyme activity, interaction of NOS with mercuric compounds via thiols, and involvement of SH groups in MeHg-induced enzyme loss were examined *in vitro*.

Total Hg concentrations in blood, cerebrum, and cerebellum increased progressively through eight days of exposure (Shinyashiki et al. 1998). The first hind limb crossing was seen 14.5 days after the first injection. The activity of nNOS increased in both the cerebrum and cerebellum, but in a different manner. In the cerebrum, nNOS activity increased significantly with time and peaked 5 days following the first injection ($p < 0.01$); it then declined slightly at 8 days ($p < 0.01$). NOS activity in the cerebellum, however, was significantly increased only after 8 days ($p < 0.05$). In contrast to the *in vivo* results, *in vitro* tests showed a decrease in cerebellar nNOS activity in a concentration-dependent fashion following MeHg-induced covalent modification of thiol groups. Shinyashiki et al. (1998) suggested MeHg causes not only an increase in intracellular calcium, but also in nNOS protein, bringing about overproduction of NO.

Chuu et al. (2001) administered male mice oral gavage MeHg doses of 0.2 or 2.0 mg/kg/day for seven consecutive days. The animals were sacrificed by pentobarbital injection at 0, 5, and 11 weeks following cessation of treatment. Brainstem tissue was assayed for Na^+/K^+-ATPase activity immediately following the cessation of treatment and from animals sacrificed at five and 11 weeks post-treatment. Brainstem tissue and whole blood were also assayed using a nitric oxide (NO)/ozone chemiluminescence assay method. Their tests revealed MeHg-related inhibition of Na^+/K^+-ATPase activity in the brainstems of mice treated with 0.2 and 2.0 mg/kg MeHg , but this was observable only in those animals sacrificed immediately following treatment cessation; no such effect was seen in animals sacrificed at five or 11 weeks post-treatment. A significant ($p < 0.05$) and irreversible increase in brainstem NO level was seen at all times during the experimental course (0, 5, or 11 weeks post-treatment) at 2.0 mg/kg/day, compared to vehicle controls. No discernible change in nitric oxide level was seen at any post-treatment analysis period in animals receiving the 0.2 mg/kg dose. The authors concluded that high-dose MeHg intoxication is associated with a decrease in functional Na^+/K^+-ATPase activity in the brainstem of affected animals, secondary to excessive production of NO, leading to oto-toxicity and hearing loss (Chuu et al. 2001). (This study also examined MeHg-induced hearing loss, described separately in this addendum.)

Glutate uptake/Effects on Glial Cells

Of the non-neuronal support cells in the CNS, astrocytes are the most abundant. They participate in neurotransmitter synthesis, help to maintain K^+ balance, assist in neuronal migration during development, play a prominent role in glutamate-glutamine homeostasis, and are a structural component of the blood-brain barrier. To identify the mechanisms involved in astrocytes damage due to MeHg exposure, Yin et al. (2007) examined the effects of MeHg on oxidative injury, mitochondrial inner membrane potential, glutamine uptake, and expression of glutamine transporters in primary astrocytes cultures. The ability of MeHg to induce oxidative stress in astrocytes was

assessed by measuring levels of F_2-IsoPs, a lipid peroxidation biomarker of oxidative injury. Primary astrocytes exposed to MeHg concentrations of 1μM, 5 μM, or 10 μM for one or six hours showed a statistically significant increase ($p < 0.05$) in F_2-IsoPs levels compared to controls. The maximum effect was seen following a 6-hour exposure to 5 μM MeHg. Higher concentrations did not further increase the F_2-IsoPs level. No sign of oxidative stress was seen at 1 μM MeHg.

To investigate whether mitochondrial dysfunction is involved in MeHg-induced neurotoxicity, astrocytes were exposed MeHg at the same concentrations and for the same time intervals as above (Yin et al. 2007). Mitochondrial membrane potential was measured by quantification of TMRE fluorescence. The mitochondrial membrane potential was significantly reduced both at 1 hour and 6 hour measurements at all three MeHg concentrations. After just one hour of exposure, 1 μM, 5 μM, and 10 μM MeHg concentrations all resulted in a significant ($p < 0.001$) decrease in membrane potential, compared to controls. Pretreatment with MeHg for 30 minutes resulted in significant ($p < 0.001$) inhibition of glutamine uptake at one and five minute measurements at all three concentrations, compared to controls. The degree of inhibition was concentration, but not time, dependent. Differences in glutamate uptake with 10 μM MeHg was significantly higher than uptake with 1 μM ($p < 0.001$) and 5 μM ($p < 0.05$) at both 1 and 5 minutes.

To evaluate the effects of *in vivo* MeHg exposure on glutamate release from synaptosomes and glutamate uptake by brain cortical slices, Farina et al. (2003b) divided 48 suckling Wistar rat pups into two groups of equal size. The pups received daily *s.c.* injections of MeHg dissolved in a 25 μM NaHCO solution beginning on PND 3. The 24 MeHg-treated rat pups were divided in three groups of 8 pups each that were sacrificed on PNDs 10, 17, and 24 for preparation of synaptosomal and cortical preparations. The 2 mg/kg dosage was based on a prior study by Miyamoto et al. (2001), in which the vulnerability of developing cortical neurons to MeHg was examined. Control rats ($n = 24$) received daily *s.c.* injections of a 25 μM NaHCO solution.

Farina et al. (2003b) found that glutamate release increased with age in both control and treated animals. The control pups sacrificed on PND 24 had significantly ($p < 0.05$) elevated glutamate release levels as compared to the 10- and 17-day sacrificed animals. The 24-day-old MeHg-treated pups had glutamate release levels significantly higher than the 24-day controls ($p < 0.05$) and the 10- and 17-PND-sacrificed MeHg-treated pups ($p < 0.05$). In contrast with the increase in glutamate with age and MeHg treatment, glutamate uptake by cortical neurons was found to decrease with age in both controls and MeHg-treated animals. In cortical slices from pups sacrificed on PND 24, there was a significant ($p < 0.05$) increase (56%) compared with age-matched controls. These authors concluded that their data suggest that the effect of MeHg on glutamate release from presynaptic nerve terminals could be involved in its neurotoxicity in suckling rats, and that the observed increase in glutamate uptake could correspond to a pathologic response to MeHg. Farina et al. (2003b) went on to extrapolate it is possible that the neurologic deficits seen in MeHg-exposed children might be related, at least in part, to a MeHg-induced disturbance of glutamate homeostasis.

In another paper, Farina et al. (2003a) examined the effects of MeHg on glutamate uptake by brain cortical slices. Two-month-old male Swiss Albino mice were given MeHg chloride equivalent to doses of 0, 10, or 40 mg/kg MeHg chloride in drinking water for 15 days and then sacrificed and the cerebral cortex removed for experimentation. The 40 mg/kg equivalent dosage, but not the 10 mg/kg dose, significantly ($p = 0.013$) decreased glutamate uptake in the cortical slices.

The mechanism(s) associated with the observed effects on glutamine uptake were assessed by measuring the astrocytic amino acid transporter mRNAs by RT-PCR (Yin et al. 2007). Glutamine uptake is dependent upon several sodium-dependent transporters, including SNAT1, SNAT3, and ASCT2. MeHg treatment at 10 μM MeHg (but not at 1 μM or 5 μM) significantly ($p < 0.05$) reduced the mRNA expression of SNAT3 and ASCT2, but not SNAT1, when compared to controls. Yin et al. (2007) concluded that their data, when taken collectively, demonstrate an association between MeHg exposure and increased mitochondrial membrane permeability, alterations in glutamine/glutamate

cycling, and oxidative injury resulting from increased ROS formation. They further suggested that the ultimate effect of MeHg is the initiation of multiple additive or synergistic mechanisms of a disruptive nature that lead to cellular dysfunction and cell death.

The effects of MeHg from maternal milk consumption on suckling Swiss albino mouse pups were investigated by Manfroi et al. (2004). On the first day following parturition (PND 1), 14 dams were assigned to either the MeHg-treatment or control groups (7 per group). MeHg was provided to the treatment group in drinking water, which was available for consumption by the dams *ad libitum*. On day 21, two weaning mice from each litter were randomly selected for sacrifice and subsequent dissection of the cerebellum. The cerebellum was chosen for this study because of its known affinity of cerebellar granule cells for MeHg. The right cerebellar hemisphere was homogenated and used for the study of the activity of antioxidant enzymes and the levels of non-protein hydroperoxide and sulfhydryl groups. The left hemisphere was used for the preparation of cerebellar slices for measurement of glutamate uptake. Whereas no differences in glutamate uptake were found for the dams, the MeHg-exposed offspring showed lower glutamate uptake values than controls ($p < 0.05$). No change in either the levels of total or non-protein sulfhydryl groups was found in either the mothers or their offspring. However an increase in hydroperoxide levels in MeHg-treated mice relative to controls was observed ($p = 0.025$). In addition, a negative correlation ($p = 0.025$) between hydroperoxide levels and glutamate uptake was seen in exposed pups *vs*. control pups; but this effect was not seen in mothers. Manfroi et al. (2004) concluded that their results indicate that exposure of lactating mice to MeHg causes inhibition of glutamate uptake by cerebellar slices in the offspring, and that this inhibition seems to be related to increased levels of hydroperoxide.

Other possible mechanisms of toxicity

Targets of developmental MeHg exposure include neural cell adhesion molecules (NCAMs), which are sialoglycoconjugates whose proper temporal and spatial expression

is important at all stages of neurodevelopment, especially during the formation of synapses. Dey et al. (1999) dosed rat pups subcutaneously with 7.0 mg/kg on every other day from postnatal days 3-13, and investigated the effects of MeHg on the temporal expression of NCAM during development. Postmortem examination of whole-cerebellum homogenates, cerebellar synaptosomes, and isolated cerebellar growth cones collected at postpartum days 15, 30, and 60 was conducted. Golgi sialytransferase activity analysis revealed significant reductions in samples collected at postnatal day 15; however, no such changes were found at postnatal days 30 or 60. *In vitro* studies revealed decreasing MeHg sensitivity of cerebellar sialytransferases with increasing developmental age. The authors concluded that MeHg-induced perturbation of the developmentally regulated expression of polysialylated NCAM during brain formation may disturb the stereotypic formation of neuronal contacts and contribute to the behavioral and morphologic disturbances seen following MeHg poisoning (Dey et al. 1999).

In a study of the *in vivo* degenerative effects of methylmercuric chloride on rat brain and cranial nerves, Kinoshita et al. (1999) demonstrated a disturbance in the integrity of microtubules and neurofilaments in the rat nervous system, particularly in the optic nerves. Specifically, electron microscopic examination revealed a marked decrease in microtubules and a moderate decrease of neurofilaments in the myelinated fibers of optic nerves in treated animals.

2.4.2 *Ethylmercury*

Thimerosal is a preservative used in some multi-dose vials of vaccine. Its active metabolite ~~i~~s ethylmercury (EtHg), which constitutes approximately 49% of the parent thimerosal volume. Thimerosal has been shown to be a versatile sulfhydryl reagent, a mobilizer of intracellular calcium, and a modulator of cell function (Elferink, 1999). Thimerosal has been shown to induce the release of intracellular calcium stores in many cell types. This mobilization of calcium can in turn modulate a number of cell functions.

Tornquist et al. (1999) found that thimerosal mobilized sequestered calcium and evoked modest store-dependent calcium entry in thyroid FRTL-5 cells. The mechanism of action was suggested to be mediated via activation of protein kinase C, as thimerosal potently stimulated the bonding of [^{3}H]phorbol 12,13-dibutyrate and was without effect on store-operated calcium entry in cells treated with staurosporine or in cells with down-regulated protein kinase C. Whole-cell patch clamping experiments revealed that thimerosal did not depolarize the membrane potential. It was concluded that thimerosal attenuates any increase in internal calcium ion concentration, probably by activating a plasma membrane Ca^{++}-ATPase (Tornquist et al., 1999).

Further evidence of the mobilization of intracellular Ca^{++} stores comes from a study by Jan et al. (2003). In this study, the effect of thimerosal on free cytoplasmic Ca^{++} levels in canine kidney cells was investigated using the Ca^{++}-sensitive dye fura-2. It was found that thimerosal increased intracellular Ca^{++} levels in a concentration-dependent manner. Thapsigargin is a chemical known to raise the cytosolic calcium concentration by blocking the ability of the cell to pump calcium into the endoplasmic reticulum. This, in turn, causes these stores to become depleted. The depletion of intracellular Ca^{++} stores can secondarily activate calcium channels in the plasma membrane, thereby allowing an flux of extracellular Ca^{++} into the cytosol. Jan et al. (2003) found that in the presence of thapsigargin and a Ca^{++}-free extracellular bathing medium, the effect of thimerosal on raising intracellular Ca^{++} levels was completely inhibited. In addition, the increase in intracellular Ca++ caused by thimerosal (5 μM) was not affected by the inhibition of phospholipase C. The authors concluded that thimerosal induces an increase in $[Ca^{++}]_i$ by causing the release of Ca^{++} stores from the endoplasmic reticulum in a manner not dependent on phospholipase C activity.

As previously noted in this chapter, inositol 1,4,5-triphosphate (IP_3) is involved in intracellular calcium homeostasis; and the binding of this compound to the IPR receptor is modulated by a number of compounds, including MeHg. Vanlingen et al. (1999) reported that the binding of IP_3 to its membrane receptors can also be differentially modulated by thimerosal. Using a preparation of cerebellar microsomes, these

researchers found that thimerosal had a stimulatory effect on the binding of IP_3 to IP_3R1 receptors. Green et al. (1999) reported that the sensitivity of intracellular calcium stores to IP_3 increases the affinity of the IP_3 receptor in rat hepatocytes; and thimerosal was further shown to enhance agonist-specific differences in the oscillation of intracellular calcium in rat hepatocytes. Mason and Mahaut-Smith (2001) also reported voltage-dependent Ca^{++} release in rat megakaryocytes following sensitization of IP_3 receptors with thimerosal.

Bultynck et al. (2004) compared the functional and molecular effects of thimerosal on IP_3R1 and IP_3R3 receptors. Using a culture of A7r5 embryonic rat aorta cells, which express primarily the IP_3R1 receptor isoform, they found that thimerosal produced a modulated biphasic effect on the IP_3-induced Ca^{++} release and IP_3-binding activity of IP_3R1 receptors. Thimerosal (1 μM) was found to strongly potentiate the IP_3-induced Ca^{++} release, and this was additive to the potentiation by the Ca^{++} itself. The authors stated that the additive effect of thimerosal and Ca^{++} may indicate that both agents cooperate to induce a conformational change to the IP_3R1 receptor that is much more sensitive to activation by IP_3. Bultynck et al. (2004) also studied the effects of thimerosal on R23-11 cells in culture. R23-11 cells are IP_3R knockout cells derived from DT40 chicken B lymphoma cells by homologous recombination. These latter experiments showed that thimerosal potentiated IP_3-induced Ca^{++} release and the IP_3 binding activity of IP_3R1 in triple IP_3R-knockout R23-11 cells, but not in the IP_3R3 receptor isoform, which lacks the N-terminal suppressor domain. Bultynck et al. (2004) concluded that, collectively, their data revealed a thimerosal-dependent intramolecular interaction within the N-terminal domain of IP_3R1, and that this may be at least a part of the conformation changes occurring during the desensitization of IP_3R1 by thimerosal. Further, IP_3 may induce opening of the channel pore by modifying the interaction of the C-terminal channel domain with the N-terminal IP_3-binding domain.

Ethylmercury-containing thimerosal has been shown to be a potent activator of intracellular calcium release in pig oocytes. Such activation mimics the effects of sperm-induced release of intracellular calcium, as well as other activation events that occur in

pig oocytes (Machaty et al. 1999). Wang et al. (1999) examined the temporal relationship between intracellular calcium transients, cortical granule exocytosis, and the zone reaction induced by thimerosal. These researchers found that thimerosal induced the same degree of exocytosis in oocytes that was caused by sperm penetration. Further, the zona block to sperm penetration in thimerosal-treated oocytes occurred within 35 minutes of cortical granule exocytosis and within 40 minutes of the first calcium transient. Machaty et al. (1999) found that the thimerosal-induced Ca^{++} release did not require the formation of IP_3. In addition, thimerosal destroyed the meiotic spindle, preventing further development.

Calcium channels in skeletal muscle, cardiac muscle, and certain nerve fibers have a high affinity for the plant alkaloid ryanodine (Sitsapesan and Williams 2000). The receptors for which ryanodine has this particular affinity are known as ryanodine receptors (RyR). Eager and Dulhunty (1999) found that thimerosal reacts with specific cysteine residues on RyR, contributing to either activation or inhibition of the channel, depending on the domain and particular class of cysteine associated with that receptor.

Using whole-cell patch clamping to study the effects of thimerosal on tetrodotoxin (TTX)-sensitive and TTX-resistant sodium channels in dorsal root ganglion neurons, Song et al. (2000) found that thimerosal blocked the two channel types in a dose-dependent fashion. The inhibition was considerably more pronounced in the TTX-resistant channels, but the effect was not reversed in either case with washing with thimerosal-free solution. The thimerosal-induced inactivation of both types of sodium channels would serve to diminish neuronal activity.

2.5 RELEVANCE TO PUBLIC HEALTH

Neurodevelopmental Effects. The Seychellois diet contains around 10 times more ocean fish than the typical U.S. diet (Davidson et al. 2006). Myers et al. (2003) reported the

results of the Seychelles Child Development Study (SCDS) neurobehavioral testing of the 107-month-old cohort. Pre-natal maternal hair mercury levels ranged from <3 ppm to > 12 ppm, with an average maternal mercury concentration of 6.9 ppm for the overall test cohort. Pre-natal hair mercury concentration was determined from maternal hair growing during pregnancy. The mean age of children at testing was 107 months. A broad range of global and domain-specific tests were conducted to assess neurocognitive, language, memory, motor perceptual-motor, and behavioral functions. Nearly all of the tests that were reported to show significant association with pre-natal exposure in the Faroes were included in this test battery. Statistically significant associations between pre-natal MeHg exposure and performance were found for only two of 21 measured endpoints. There was a significant decrease in performance on the grooved pegboard time for the non-dominant hand in males, and a significant improvement of the hyperactivity index of the Connor's teacher-rating scale as prenatal exposure increased. The authors pointed out that for these two endpoints, there were three outliers in the grooved pegboard non-dominant hand test and two outliers in the Connor's teacher-rating scale test, all of which had pre-natal MeHg concentrations of 7.5 ppm or less and low performance on those tests. Interestingly, the Boston Naming Test, which was used as the most critical test in the derivation of EPA's RfD for MeHg, showed no statistically significant association with pre-natal MeHg exposure on this round of testing in the 107-month Seychelles cohort. It was pointed out that the Wexler's intelligence scale for children III full scale IQ (WISC III) and Boston Naming Tests were both affected by cultural variation, with lower means for the Seychellois children than for US controls. The Faroes study did not obtain a HOME score or report SES, variables previously suggested to affect child development.

The data from the testing of the 107-month (Myers et al., 2003) was used in a benchmark dose analysis reported by van Wijngaarden et al. (2006). The average 95% lower confidence limit of the BMD (BMDL) across all 26 neurobehavioral endpoints measured varied slightly among the three models used (*k*-power, Weibull, and logistic). The choice of statistical model was reported to not greatly affect the BMDL estimates. The lowest BMDL of 20.1 ppm (range: 17.2 – 22.5) was calculated using the logistic model, while

the highest BMDL calculated, 20.4 ppm (range: 17.9 - 23.0) was determined using the *k*-power model. The lowest individual BMDLs determined in this study were 17.2 ppm for the logistic model with the BMR set at 10% and 15.5 ppm for the *k*-power model with the BMR set at 5%. The study authors recommend presenting an average BMDL and its corresponding range based on all available evidence to provide an indication of the exposure limits within which the true BMDL is likely to fall.

Further testing of the 107-month cohort in the Seychelles Child Development Study was reported by Myers et al. (2004). The Child Behavior Checklist (CBCL) was administered to 643 members of the original cohort (enrolled in 1989-1990) in this prospective, longitudinal, double-blind study. The CBCL measures behavior in eight domains, and provides an overall behavioral index and 10 sub-scale indices. The pre-natal and post-natal measures of exposure were total mercury in maternal and child hair samples, respectively. Pre-natal exposure was determined in the longest available segment of maternal hair representing growth during pregnancy, assuming a growth rate of 1.1 cm per month. Post-natal exposure was measured in the centimeter segment of the child's hair closest to the scalp that was taken at the time of the 107-month evaluation.

For pre-natal exposure, there were two sub-scales that showed significant or marginal *p* values and negative coefficients: Social Problems and Somatic Complaints. The pre-natal association with the Social Problems sub-scale was not significant with outliers ($p = 0.121$), but was without outliers ($p = 0.032$). The pre-natal association for the Somatic Complaints sub-scale was not significant with outliers ($p = 0.31$), but was called "marginally significant" ($p = 0.067$) without outliers (Myers et al., 2004). The authors concluded that the (pre-natal exposure) effect on the Social Problems sub-scales was beneficial, while the (post-natal exposure) effect on the Thought Problems sub-scale that appeared to begin around 8 ppm was considered an adverse association. No other associations with mercury exposure were apparent among the 22 behavioral endpoints studied.

For post-natal exposure, the Poisson model for the Thought Problems sub-scale showed an association with post-natal both with ($p = 0.011$) and without outliers ($p = 0.013$). Using a semi-parametric additive model, some evidence for a non-linear association between post-natal exposure and the Thought Problems subscale was seen ($p = 0.01$). No effects were seen below approximately 8 ppm hair mercury, but an increasing trend (adverse effect) was observed above that level. The non-linear relationships between pre-natal exposure and the Social Problems and Somatic Complaints sub-scales were not statistically significant.

While Myers et al. (2004) concluded that their data showed no "clear pattern of adverse associations between behaviors measured by the CBCL and either pre- or post-natal exposure, they also stated that their data suggest that fish consumption may pose both risk and benefits.

In a subsequent review of previously reported 66-month and 107-month data and examination of new data, Myers et al. (2009) focused on post-natal methylmercury exposure and any possible adverse health effects. In this study, three types of alternative post-natal exposure metrics were used to examine their association with the children's intelligence quotient (IQ) at 107 months of age. Myers et al. (2009) found four endpoints adversely associated with post-natal exposure in one or both sexes. Post-natal mercury exposure was adversely associated with the Connor's Teacher rating Scale ADHD Index in both sexes. The WISC III FS IQ test, grooved Pegboard with the non-dominant hand test, and the Connor's Continuous Performance Task Risk Taking test revealed adverse post-natal mercury associations in girls, only.

These authors pointed out that some associations in their primary analysis were in the direction of declining performance as post-natal exposure increase, while others were in the opposite direction. They did note, however, a reversal of associations between post-natal exposure and endpoints from improved performance to deteriorating performance between the 66- and 107-month exams, which they felt were unexpected and of unclear significance. Although a number of adverse associations were found, Myers et al.

(2009) noted that the results of their testing varied across ages and psychological domains. For example, in evaluating associations with mercury exposure and test performance, Myers et al. (2009) looked at all similar tests and pointed out that the grooved pegboard is a test of fine motor coordination, and that two other tests that also measure fine motor coordination (Finger Tapping and Trail Making A and B) showed no association with recent post-natal exposure. Thus, they state that no consistent or clear pattern of associations has emerged, and that their findings do not provide clear evidence for an adverse association between the levels of post-natal mercury exposure in this cohort. In their summary, Myers et al. (2009) do note that their findings raise intriguing possibilities and suggest that post-natal exposure should be studied prospectively.

The average post-natal hair mercury concentrations in the Seychelles cohort ranged from 6.6 ppm at 6 months of age to 4.8 ppm at 66 months and 6.9 ppm at 107 months. Thus, it is clear that post natal exposure has occurred on a relatively consistent basis since infancy in this fish-eating cohort. When the hair sample was taken for the post-natal measurements, it was taken from the 1cm segment nearest the scalp. As such, those measurements only provided information on the most recent month, and not on continuous mercury intake. It may be that relatively continuous exposure to small amounts of MeHg from eating fish has resulted in the test scores seen in the 107-month cohort. Future testing should provide more information.

Grandjean et al. (1997) reported another epidemiologic study of MeHg exposure for a population in the Faroe Islands. Although the Faroese are a fishing culture, the major source of MeHg exposure for this population is pilot whale meat, which is intermittently consumed (1-2 meals/week) as part of the cultural tradition. The initial study cohort consisted of 1,022 singleton births occurring in a 21-month window during 1986-1987. At approximately 7 years of age, neurobehavioral testing was conducted on 917 of the remaining cohort members. No abnormalities attributable to mercury were found during clinical examinations or neurophysiologic testing. A neuropsychologic test battery was also conducted to evaluate possible effects on specific domains of brain function. The neuropsychologic testing indicated mercury-related dysfunction in the domains of

language, attention, memory, and visuospatial and motor function (to a lesser extent), which the authors considered to remain after the children of women with maternal hair mercury concentrations above 10 μg/g (10 ppm) were excluded. A follow-up paper on this population suggested that the time-dependent susceptibility may vary for different brain functions (Grandjean et al., 1999). These researchers reported that the greatest susceptibility to MeHg neurotoxicity occurs during late gestation, while early postnatal vulnerability is less.

Crump et al. (1998) conducted benchmark dose (BMD) analysis and further regression analyses of data collected in a study in which a series of scholastic and psychological tests were administered to children whose mothers had been exposed to MeHg during pregnancy. Hair samples were collected from 10,970 new mothers in New Zealand in 1977 and 1978. High hair mercury levels were considered to be those over 6 ppm, which was the hair level predicted to result at steady state from consumption of mercury at the WHO/FAO Provisional Tolerable Weekly Intake of 0.3 mg total mercury/week and 0.2 mg MeHg/week. By this criterion, 73 of approximately 1,000 mothers who had consumed fish more than 3 times/week during pregnancy were determined to have high hair mercury levels. In 1985, when the children were 6 to 7 years of age, 61 children (including one set of twins) of the 73 mothers in the high hair mercury group were located; these children constituted the high exposure group, which was matched with three control groups [one with 3-6 ppm (μg/g) maternal hair mercury levels, one with 0-3 ppm whose mothers had been identified as "high fish consumers," and one with 0-3 ppm whose mothers had not been high fish consumers]. The entire study cohort consisted of 237 children. A battery of 26 psychological and scholastic tests was administered to the children at school during the year 1985. Mothers were interviewed at the time of test administration to obtain additional data on social and environmental factors. In the high exposure group, one boy's mother had a hair mercury level of 86 ppm, which was more than four times higher than the next highest hair mercury level of 20 ppm. BMDs (10% response rate) calculated from five tests ranged from 32 to 73 ppm, when the child of the mother with the hair mercury level of 86 ppm was included. This corresponded to a benchmark dose level (BMDL) range of 17 to 24 ppm. The BMDL is a modeled number

considered to correspond to an experimental no-observed-adverse-effect-level (NOAEL). Although none of the test scores of the child whose mother had the hair mercury level of 86 ppm were outliers according to the definition used in the analyses, his scores were significantly influential in the analyses. When this child was omitted from the analyses, BMDs ranged from 13 to 21, with corresponding BMDLs of 7.4 to 10 ppm. According to this most conservative interpretation of the New Zealand data, no neuro-psychological effects would be seen (and were not seen) in the offspring of women with hair mercury levels at or below the bottom of this BMDL range (i.e., 7.4 ppm maternal hair mercury level).

Following the publication of the results of the Faroes (Grandjean et al., 1997) and New Zealand (Crump et al., 1998) studies, Palumbo et al. (2000) conducted a reanalysis of the possible association of MeHg with performance on the McCarthy Scales of Children's Abilities (MSCA) in the 66-month Seychellois cohort. Since no association between MeHg exposure and performance on the MCSA General Cognitive Index had been found previously in this cohort (Davidson et al., 1998), Palumbo and co-workers conducted further analyses to determine whether associations on specific subscales of the MSCA could be identified. After analysis of standard MSCA subscales, more specific subscales of the MSCA were defined and analyzed using a neuropsychological approach. In this process, subscales were recombined to approximate the domains of cognitive functioning evaluated in the Faroes and New Zealand studies. Palumbo et al. (2000) found that analyses of both the standard and recombined MSCA subscales showed no adverse associations with MeHg exposure and the neuropsychological endpoints examined.

Debes et al. (2006) reported the results of neuropsychological testing of the Faroes child cohort at 14 years of age. In this paper, 878 members of the initial 1022 mother-child pair cohort underwent detailed neurobehavioral examination. Eighteen of the participating children were excluded due to existing neurological disorders. The neuropsychological test battery used was based on the same criteria as applied for previous testing at 7 years of age. Debes et al. (2006) reported that higher prenatal MeHg exposure, as indicated by cord blood mercury concentration, was associated with lower

finger tapping scores, increased reaction time, and lower cued naming scores. Maternal hair mercury concentrations showed significant or near-significant associations with deficits only on three conditions of finger tapping, and the two measures of reaction time. Yet another measure of exposure, cord tissue mercury, showed associations only with deficits on the naming and verbal learning results. As with the Seychelles testing, not all associations with pre-natal mercury exposure were negative. Higher MeHg exposure was associated with better scores on the WMS-II Spatial Span test in this Faroes cohort.

Debes et al. (2006) also reported that prenatal MeHg exposure seemed a less important predictor of neuropsychological performance at age 14 than at age 7 years; however, they noted that this risk factor (prenatal exposure) appeared to represent about the same proportion of the total variance explained by the regression model at the two testing occasions.

Post-natal exposures were, in general, reported to be only weakly related to cognitive test scores at 14 years, with the only statistically significant association being the NES2 finger tapping score for the preferred hand (Debes et al. 2006). Likewise, PCB exposure, although only available for half of the subjects, was reported to show only weak associations with the outcomes, none of them reaching statistical significance.

Jedrychowski et al. (2006) evaluated the effects of prenatal MeHg exposure through maternal fish consumption on cognitive and motor function in 1-year-old infants in Poland. The study cohort consisted of 233 infants born between January 2001 and March 2003. The *in utero* period of the infants ranged from 33 to 42 weeks, with 91% being born after 38 weeks of gestation. The maternal population consisted of residents of Krakow, who attended ambulatory prenatal clinics in the first and second trimesters of pregnancy and who had singleton births. Those women ranged in age from 18 to 35 years, and all were non-smokers. Maternal blood (30 to 35 ml) was collected at delivery, as was a comparable amount from venal cord umbilical blood. The geometric mean blood mercury level was 0.55 μg/L (range 0.10 to 3.40 μg/L) for mothers at delivery. Of these subjects, 75% had whole blood mercury levels <1 μg/L; and 90% had levels not

greater than 2 μg/L. The geometric mean of cord blood mercury was 0.88 μg/L (range 0.10 to 5.00 μg/L). Of the infants, 60% had levels <1 μg/L, and 90% had cord blood mercury levels below 2 μg/L.

The Bayley Scales of Infant Development II (BSID-II) tests were conducted to assess infant mental and motor function at approximately one year of age (Jedrychowski et al. 2006). On testing, 197 infants had normal performance on the Motor and Mental scales of the BSID-II, while 36 infants showed delayed motor and/or psychomotor performance on these tests. Of the infants with normal test performance, the geometric mean maternal mercury blood level was 0.52 μg/L, whereas the mothers of infants showing delayed performance had blood mercury levels with a geometric mean value of 0.75 μg/L. The difference in these two values was statistically significant at the $p < 0.01$ level. In contrast, the difference between geometric mean cord blood levels in the normal-result and delayed performance infant groups was not statistically significant ($p = 0.07$). The geometric mean cord blood values were 0.85 μg/L for the normal group and 1.05 μg/L for the delayed performance group. In the Jedrychowski et al. (2006) study, however, blood mercury levels and test performance data were not reported for individual mother-infant pairs.

Review of Epidemiologic Studies by Expert Bodies

The epidemiological studies of the Faroes and Seychelles mother-child pair cohorts were examined by panels of independent experts in 1998 and 2003. The 1998 review consisted of a workshop sponsored by the White House Office of Science and Technology Policy (OSTP) of the Committee on Environment and Natural Resources (CENR), and the second review was sponsored by the National Academy of Science in 2000. The organizing committee for the OSTP workshop was chaired by the National Institute of Environmental Health Sciences (NIEHS) and included representatives from the Department of Health and Human Services (DHHS) Office of the Assistant Secretary for Planning and Evaluation, the Centers for Disease Control and Prevention (CDC), the ATSDR, the U.S. Food and Drug Administration (FDA), the U.S. Environmental

Protection Agency (EPA), the National Oceanographic and Atmospheric Administration (NOAA), OSTP, and the Office of Management and Budget (OMB). The purpose of the workshop was to discuss and evaluate the major epidemiologic studies associating MeHg exposure with an array of developmental measures in children.

The workshop was structured around the deliberations of five panels. The panel members were selected by the organizing committee for their national and international recognition as subject matter experts in subject areas of the five panels. The panels were the Exposure Panel, the Neurobehavioral Endpoints Panel, the Confounders and Variables Panel, the Design and Statistics Panel, and the Experimental (animal) Panel. The 21 members of these panels can be found in NIEHS (1998). While the workshop did not attempt to derive a risk assessment for MeHg, the product was intended to facilitate agreement on risk assessment issues primarily related to the Seychelles and Faroes studies through expert examination and discussion of all the data existing at the time of the workshop. A number of findings and recommendations were produced that praised both the Seychelles and Faroes study teams, while noting some shortcomings of those studies.

The NRC (2000) review was the result of a Congressional direction in the appropriations report for EPA's fiscal 1999 funding that required EPA to request that the National Academy of Sciences perform an independent study on the toxicological effects of MeHg and prepare recommendations on the establishment of a scientifically appropriate reference dose (RfD) for exposure to MeHg. Upon request by EPA, the National Research Council (NRC) of the National Academies of Sciences and Engineering convened the Committee on Toxicological Effects of Methylmercury. There were ten non-NAS members on this external committee, with expertise in the fields of toxicology, pharmacology, medicine, epidemiology, neurophysiology, developmental psychology, public health, nutrition, statistics, exposure assessment, and risk assessment. Unlike the OSTP workshop charge, the NAS committee was specifically charged with evaluating the existing EPA RfD of 0.1 μg/kg/day based on an accidental poisoning incident that occurred in Iraq. And whereas the committee was not charged with calculating an RfD

for MeHg, it did provide scientific guidance to EPA on the development of an appropriate RfD for MeHg. In the analysis of existing data and considerations for ensuring the protection of the most sensitive individuals within the human population, the committee did in fact derive an RfD for MeHg that numerically matched the existing EPA RfD for MeHg, but based it on a different study and critical effect.

Some of the findings of the 1998 OSTP-sponsored Workshop on Scientific Issues Relevant to Assessment of Health Effects from Exposure to Methylmercury and the EPA-sponsored NAS committee report differed significantly.

At the 1998 OSTP-sponsored workshop, the Confounders and Variables Panel found that "Although in most tissues, PCBs are measured most accurately on a lipid-adjusted basis, the lipid adjustment for cord tissue measures (as done in the Faroes) is not useful." In the Faroes study, prenatal PCB exposure was associated with four of the same outcomes as Hg exposure. Regarding these, the Confounders and Variables Panel reported that "These outcomes related primarily to verbal and memory performance, the domains found in prior studies to be associated with PCB exposure. When PCBs and Hg are included together in the model, one of the outcomes is specifically related to Hg exposure. For the other three (including the Boston Naming Test), however, both the PCB and Hg effects fall short of conventional levels of statistical significance."

The following information (Table 2) was provided by Grandjean et al. (1997) regarding the results of the Boston Naming Test.

Table 2. Boston Naming Test *p* Values.

Before PCB wet weight adjustment	After PCB wet weight adjustment
MeHg	MeHg
w/o cues: $p = 0.04$	$p = 0.21$
w/ cues: $p = 0.007$	$p = 0.10$

Source: Grandjean et al., 1997

Table 2 clearly shows that when properly adjusted for PCB content, the Boston Naming Test loses statistical significance for both the "with cues" and "without cues" categories. It further illustrates that when properly measured, PCBs played an important role in the test results and that the contribution of MeHg was merely to enhance the ability of PCBs to evoke the response on the Boston Naming Test, and not itself the sole or primary cause of the reported neuropsychological deficit in the test population. This hypothesis is supported by the findings of Grandjean et al. (2001), who found that cord PCB concentration was significantly associated with deficits on the Boston Naming Test, without cues ($p = 0.03$), and that wet-weight PCB concentration appeared to be a better predictor of neurobehavioral deficits. This is consistent with the findings of Stewart et al. (2000), who also found a decrease in performance in some portions of the Neonatal Behavioral Assessment Scale (NBAS) tests among babies born to women who consumed Lake Ontario fish contaminated with PCBs. Stewart et al. (2000) also found that the decreased NBAS performance was unrelated to concomitant exposure to MeHg, lead, or pesticides.

The conclusion reached by Grandjean et al. (2001) that the limited PCB-related neurotoxicity in this study cohort appears to be affected by concomitant MeHg exposure could as justifiably be restated to indicate that the limited MeHg-related neurotoxicity (both prenatally and postnatally) in this study cohort appears to be affected by concomitant PCB exposure. Based on the data published to date, it appears that that both MeHg and PCBs may be jointly responsible for the effects reported in the Faroes (Risher et al., 2002). Dourson et al. (2001) also provide an excellent discussion of the relationship between the results reported in the Faeroes and the potential impact of mixed chemical exposures in that population.

Cardiovascular Effects. Guallar et al. (2002) examined a population of 684 males 70 years-of-age or younger from eight European countries and Israel. These individuals were identified from coronary care units of various hospitals as having had their first myocardial infarction (MI), as confirmed by characteristic electrocardiographic changes and elevated enzyme levels. A control population of 724 men without a history of MI was selected as being representative of the same populations. Toenail samples were used as an indicator of mercury exposure, and docosohexaenoic acid (DHA) levels were determined from samples of adipose tissue. After adjustment for DHA levels and coronary risk factors, mercury levels in the study population were 15% higher than in controls. It was concluded that total toenail mercury levels were directly associated with the risk of MI, while the adipose tissue DHA level was inversely associated with risk.

Yoshizawa et al. (2002) studied the correlation between toenail mercury levels and MIs in male health professionals who had not previously had an MI and who were in the age range 40 to 75 years in 1986. In 1987, toenail clippings were collected from 33,737 cohort members. In the ensuing 5-year follow-up period, 470 cases of coronary disease were documented. A matched control subject from whom toenail clippings had been collected in 1987 and who was still alive at the time the MI was confirmed and a matched counterpart was selected for comparison. Among the study participants, dentists and those who consumed more fish had significantly higher levels of mercury in their toenails. However, when compared against the matched controls, the authors concluded that the study data did not support an association between mercury levels and an increased risk of coronary heart disease. When dentists were excluded, there was a positive, but not statistically significant, association between mercury and coronary heart disease.

Yaginuma-Sakurai et al. (2009) studied the effects of MeHg intake through fish consumption at the Japanese provisional tolerable weekly intake (PTWI) level of 3.4 μg/kg bw/week on the resting heart rate. Fifty-four healthy volunteers who were either students or graduates of universities in Sendai, Japan were enrolled in the study. The experimental and control groups each consisted of 14 men and 13 women. Subjects in the experimental group were instructed to consume a specific amount of bigeye tuna and

swordfish during a 14-week period. The actual amount of fish consumption was calculated for each subject in the experimental group based on their individual body weights. Consumption of the tuna and swordfish was terminated after 14 weeks, and restricted (presumed to mean forbidden) during the following 15-week period. Intake of both bigeye tuna and swordfish was restricted for the entire 29-week period in the control group. Subjects in the control group were instructed to continue their usual diets during the 29-week period. The levels of MeHg were determined from two bigeye tuna and one swordfish purchased from a nearby wholesale fish firm. Total mercury concentrations in these fish were determined from 20 samples to be 1.08 (+/- 0.07) µg/g (ppm) for the tuna and 1.008 (+/- 0.11) ug/g for 10 samples from the swordfish. MeHg concentration was assumed to be 94% of the total mercury content. Hair mercury concentration was considered to be the indicator of MeHg exposure. Hair of all subjects grew at an average of 1.3 (+/- 0.2) cm every four weeks, and hair samples were thus collected from the 1.3 cm nearest the scalp at 4-week intervals. Heart rate variability (HRV) was measured in all subjects three times: before exposure, after MeHg exposure for 14 weeks, and after the 15-week "wash-out" period following cessation of exposure. Subjects were also asked their health condition, such as the presence of tremor or finger numbness every four weeks at hair sampling, but no such findings were reported in the study. Yaginuma-Sakurai et al. (2009) reported that experimental and control hair mercury levels differed significantly ($p < 0.001$) at 9, 13, 17, 21, 25, and 29 weeks.

A number of parameters were used by Yaginuma-Sakurai et al. (2009) to evaluate HRV, with low frequency component variability ($CCV_{LF)}$ and power spectral density (PSD_{LF}) used to evaluate sympathetic cardiac activity and high frequency component variability ($CCV_{HF)}$ and power spectral density (PSD_{HF}) used to evaluate parasympathetic cardiac activity. The measurement protocol consisted of the subject resting in a supine position for 10 minutes, followed by the measurement of 300 R-R intervals of the ECG. Consecutive 100 R-R intervals with a minimal SD were then automatically extracted by computer to minimize measurement bias. While several parameters indicated an increase in sympathetic activity after 14 weeks of exposure, heart rate remained unchanged; and only CCV_{LF} was statistically significant compared to total hair mercury. All such effects

had disappeared by week 29. The authors concluded that MeHg consumption in their study induced a temporary sympathodominant state, and that long-term exposure to MeHg may pose a potential risk for cardiac events involving sympathovagal imbalance in fish-consuming populations. The levels of polyunsaturated fatty acids in plasma were found to be reduced in the experimental population.

As part of the Shiwha-Banwol Environmental Health Study in Korea, Lim et al. (2010) investigated the association between mercury and heart rate variability in 1,589 study participants with an average age of 33 years (range 5 to 83 years of age). Hair mercury levels ranged from 0.01 to 13.36 ppm, with an arithmetic mean of 1.02 ppm (S.D. +/- 0.77) and a geometric mean of 0.83 ppm (S.D. +/- 1.80). Heart rate variability was assessed in both time and frequency domains using standardized methods of the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology (1996). High frequency (HF), low frequency (LF), very low frequency (VLF), total power (TP), LF/HF ratio, LF norm (LF/LF+HF) and JF norm (HF/LF+HF) were used to evaluate the frequency domains. Standard deviation of the NN intervals (SDNN), which is the standard deviation of the average R-R intervals of the EKG calculated over a 24-hour period, was used as the index of time domain measure. SDNN is considered to reflect both sympathetic and parasympathetic influence on heart rate variability.

Lim et al. (2010) found that the hair mercury concentration negatively affected both the time and frequency measures in general, and particularly the HF parameter. Using multiple linear regression and adjusting for selected variables, heart rate variability was found to decrease by 8.4% for each 1 ppm increase in hair mercury concentration. Hair mercury concentration was significantly higher ($p < 0.0001$) in those eating fish one or more times a week than in those consuming less than one fish meal per week. The authors pointed out that their results suggested that low levels of mercury intake may cause parasympathetic dysfunction, and that their findings were consistent with the findings of Sorensen et al. (1999), Grandjean et al. (2004), and Murata et al. (2006).

In a study of 7-year-old Japanese children exposed to MeHg *in utero* through maternal fish consumption, Murata et al. (2006) assessed cardiac autonomic function in 136 subjects for whom umbilical cords has been preserved. MeHg in cord tissue was used as the biomarker of prenatal exposure. Of the maternal population, 15 women (11%) reported some drinking during pregnancy, and another 10 (7.4%) reported smoking during pregnancy. No mothers ate shark or whale during pregnancy.

Immediately prior to testing, the children remained quietly in a supine position for five minutes (Murata et al. 2006). Then, 300 R-R intervals were measured, and 100 consecutive R-R intervals with the minimal standard deviation were automatically extracted by computer to avoid measurement error. No abnormal ECG findings were reported in the 7-year-olds, but cord tissue MeHg was correlated in a negative fashion with CCV_{HF} and PSD_{HF} ($p = 0.027$), and positively with the LF/HF ratio and %LF ($p = 0.016$), indicating a negative parasympathetic effect and positive sympathetic on cardiac activity. Test results and cord MeHg for individual children were not reported, so it is not possible to determine the precise level at which an adverse effect might have been seen. Likewise, it was not indicated whether there was a higher prevalence of autonomic effects in children of drinking mothers than in the children of mothers who did not report alcohol consumption during pregnancy. It has been previously reported that CCV_{LF} and PSD_{LF} seem to be reduced by chronic high-level exposures to alcohol, but be unaffected by low-level alcohol exposures during pregnancy (Murata and Araki, 1995). The authors also calculated a maternal hair mercury equivalent concentration using the equation of Akagi et al. (1998): maternal hair Hg (in µg/g) at parturition = 25.24 x cord-tissue concentration (in µg/g dry weight). Murata et al. (2006) thereby estimated a median maternal hair Hg concentration of 2.4 µg/g (range: 0.43 to 9.26 µg/g), and suggested that a maternal hair concentration at parturition may be associated with reduced parasympathetic activity and/or sympathovagal shift.

As part of the Kuopio Ischemic Heart Disease Risk Factor Study, Rissanen et al. (2000) randomly selected 1,871 men aged 42-60 who had no clinical coronary heart disease as baseline examination. After an average follow-up time of 10 years, 194 coronary events

were reported. Of these, 100 were definite (fatal and non-fatal) and 60 were probable MIs; 34 were typical episodes of acute chest pain. At follow-up in the 194 subjects with coronary events, serum fatty acids were measured, and the percent proportion of the sum of docosohexaenoic acid (DHA) and docosapentaenoic acid (DPA) was calculated. Men in the highest fifth of DHA + DPA in all fatty acids had a 44% reduced risk of acute coronary events. Men in the highest fifth of DHA + DPA who had low hair mercury 2 ppm or lower had a 67% reduced risk of acute coronary events, compared with men in the lowest fifth DHA + DPA with a hair mercury level above 2 ppm. The authors of that study concluded that their data provided support for the concept that fish oil-derived fatty acids reduce the risk of acute coronary events (Rissenen et al., 2000).

Sorensen et al. (1999) evaluated the cardiovascular effects of mercury on approximately 1,000 7-year-old children in the Faroes Islands. These study subjects were part of the 1,022 cohort of children born to mothers who had ingested fish, pilot whale meat, and pilot whale blubber during pregnancy. Heart rate in the children was found to decrease with increasing body weight, height, and body mass index (BMI). Although heart rate itself was found not to be associated with mercury exposure, the variability in heart rate did decrease slightly with mercury exposure, particularly in boys. Increased heart rate was found to be associated with increased diastolic and systolic blood pressures. After adjustment for body weight, blood pressure underwent an increase that reached a plateau as a function of prenatal MeHg exposure. However, the magnitude of this effect was not dose-dependent, with the greatest mercury-associated changes in blood pressure occurring in the low-level exposure range. Adjusted diastolic and systolic blood pressures decreased by 13.9 mmHg and 14.6 mmHg, respectively, when the cord blood mercury concentration increased from 1 to 10 μg/L. Above the 10 μg/L cutoff, blood pressures were not associated with mercury exposure (Sorensen et al., 1999). The authors concluded that their findings suggest that prenatal exposure to MeHg may affect the development of cardiovascular homeostasis.

There are differences of opinion regarding just how to interpret the collective volume of all published studies regarding the possible causal relationship of MeHg to the effects observed. Mozaffarian (2009) and Stern (2005) both addressed this in their respective reviews of the current evidence on this subject. Mozaffarian (2009) pointed out that the conflicting results of prior studies of mercury and cardiovascular disease are not dose-related, with some studies with relatively low exposures reporting a positive association and other studies finding no significant associations at similar or higher exposures. Mozaffarian also notes that growing evidence suggests that fish consumption has benefits for health outcomes beyond CHD mortality, including non-fatal cardiovascular events. The positive effects of polyunsaturated fatty acids (PUFA), including omega-3 fatty acids, appeared to provide an overall positive effect of fish consumption. Stern (2005), however, after reviewing the available literature on the same subject, arrived at the conclusion that a statement indicating that the benefits of fish intake exceed the potential risks is unsupported by the available data. He strongly felt that the data support the existence of a net negative effect of fish consumption on cardiovascular health.

The above studies considerably strengthen the data base on cardiovascular effects of MeHg exposure; however, the there is no clear preponderance of data to unequivocally determine that MeHg exposure as a result of consuming contaminated fish can present significant risk of adverse cardiovascular effects. The data previously reported in the 1999 Toxicological Profile for Mercury do indicate that effects might occur at exceptionally high dosages, but not for levels of MeHg seen in most fish.

2.7 BIOMARKERS OF EXPOSURE AND EFFECT

The abrupt increase of Minamata disease in 1952 was reportedly preceded by the beginning of the MeHg discharge from the Chisso Company's factory in Minamata, Japan two years earlier. To examine the burden of MeHg in umbilical cords of adversely effected individuals, Harada et al. (1999) collected 151 preserved cord specimens from the period 1950-1969, inclusive, from the Minamata area of Japan and examined them for MeHg content. Of the 151 cords collected, 25 were from 25 confirmed cases of

Minamata disease, 13 from acquired Minamata disease, 20 from cases of mental retardation, 16 from other effects (Down's syndrome, Werdnig-Hoffmann syndrome, after-effects of encephalitis, muscular dystrophy, etc.), and 77 with no symptoms. The umbilical cords from 24 Tokyo residents collected prior to this study (Nishigaki and Harada, 1975) were used as controls (cord MeHg average: 0.11 +/- 0.03 ppm).

Harada et al. (1999) considered the 2-year delay between the known onset of the pollution of Minamata Bay with MeHg to likely correspond to the mother's term of exposure prior to pregnancy, plus the length of pregnancy. All five groups had cord MeHg levels significantly higher than the Tokyo controls: congenital Minamata disease group --- $p < 0.01$; all other groups --- $p < 0.05$. Mean values for cord MeHg and corresponding ranges for the test groups were as follows: congenital Minamata disease (1.60 +/- 1.00 ppm; range 0.15-4.65); acquired Minamata disease group (0.72 +/- 0.65 ppm; range 0.04-1.81); mental retardation group (0.74 +/- 0.64 ppm; range 0.13-1.96); others group (0.22 +/- 0.15 ppm; range 0.02-0.57); no symptom group (0.28 +/- 0.20 ppm; range 0.02-0.95).

Huel et al. (2008) investigated the relationship between hair Hg and erythrocyte Ca^{++} pump activity in full-term human newborns and their mothers at delivery. In this study, 98 mother-newborn pairs were recruited at the Robert Debre Maternity Hospital in Paris, France. A standardized questionnaire containing questions regarding smoking, daily fluid intake, alcohol and caffeine consumption, and other items was administered to the mothers by the same observer on the third day following delivery. Cord blood at delivery and maternal hair samples were collected and analyzed. The mean maternal age at delivery was 29.7 +/- 4.6 years, and the mean gestational age was 39.8 +/- 1.2 weeks. The authors found highly significant, positive correlations between basal and stimulated erythrocyte Ca^{++} pump activities in both mothers ($p < 0.0001$) and newborns ($p < 0.0001$). Using simple regression analysis, maternal hair Hg level was found to negatively correlate with maternal erythrocyte Ca^{++} pump activity ($p = 0.001$ for basal activity; $p = 0.002$ for stimulated activity). Cord blood Hg level was found to correlate negatively with neonatal Ca++ pump activity ($p = 0.0002$ for basal activity; $p = 0.01$ for stimulated activity). Huel et al. (2008) concluded that their study indicated that a

variation in Ca^{++} pump activity exists in relation to very low doses of exposure to chemicals (e.g., Hg) that interfere with Ca^{++} homeostasis. They also suggested the need for further investigation into the relationship between peripheral biomarkers, such as Ca^{++} pump activity, and neurodevelopmental skills.

Factors Mitigating Toxicity

Dietary intakes of both selenium and long chain polyunsaturated fatty acids (LCPUFAs) have been suggested to prevent or otherwise mitigate MeHg toxicity in general and cardiovascular and neurologic effects in particular. There have been a number of studies to investigate these possibilities. Some of the studies report positive correlations, while others have found no correlations. This section of the addendum will present key studies in this area by the study outcomes.

LCPUFA: positive correlation

Docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) are long chain polyunsaturated fatty acids found in fish, as well as some other dietary products. The omega-3 LCPUFA DHA and the omega-6 LCPUFA arachidonic acid (AA) are important structural components of the human central nervous system (Innis 2005). And since the growing fetus is dependent upon the accumulation of these fatty acids in the brain for proper neurologic development, dietary intake of LCPUFAs by the mother and their subsequent transfer across the placenta is essential (Innis 2005). A number of studies have investigated the importance of the intake of LCPUFAs during fetal development to neurologic performance in infants and toddlers exposed to the neurotoxicant MeHg.

Guallar et al. (2002) examined a population of 684 males 70 years-of-age or younger from eight European countries and Israel. These individuals were identified from coronary care units of various hospitals as having had their first myocardial infarction (MI), as

confirmed by characteristic electrocardiographic changes and elevated enzyme levels. A control population of 724 men without a history of MI was selected as being representative of the same populations. Toenail samples were used as an indicator of mercury exposure, and docosohexaenoic acid (DHA) levels were determined from samples of adipose tissue. After adjustment for DHA levels and coronary risk factors, mercury levels in the study population were 15% higher than in controls. It was concluded that total toenail mercury levels were directly associated with the risk of MI, while the adipose tissue DHA level was inversely associated with risk.

As part of the Kuopio Ischemic Heart Disease Risk Factor Study, Rissanen et al. (2000) randomly selected 1,871 men aged 42-60 who had no clinical coronary heart disease as baseline examination. After an average follow-up time of 10 years, 194 coronary events were reported. Of these, 100 were definite (fatal and non-fatal) and 60 were probable MIs; 34 were typical episodes of acute chest pain. At follow-up in the 194 subjects with coronary events, serum fatty acids were measured, and the percent proportion of the sum of docosohexaenoic acid (DHA) and docosapentaenoic acid (DPA) was calculated. Men in the highest fifth of DHA + DPA in all fatty acids had a 44% reduced risk of acute coronary events. Men in the highest fifth of DHA + DPA who had low hair mercury 2 ppm or lower had a 67% reduced risk of acute coronary events, compared with men in the lowest fifth DHA + DPA with a hair mercury level above 2 ppm. The authors of that study concluded that their data provided support for the concept that fish oil-derived fatty acids reduce the risk of acute coronary events (Rissanen et al., 2000).

To examine the effects of dietary fats on MeHg-induced acute toxicity, Jin et al. (2007) divided young male Sprague Dawley rats into five different diet groups of 18 animals per group. Semi-purified casein-based diets containing either soy oil, seal oil, docosahexaenoic acid (DHA), fish oil, or lard were provided to the rats for 28 days. On day 29, rats were administered daily doses of 0, 1, or 3 mg MeHg/kg bw by gavage for 14 days. On day 43 of the study, the rats were sacrificed and blood, feces, liver, spleen, thymus, testis, and brain were harvested for examination. Many effects of MeHg were dependent on the particular dietary fat consumed. Data on relative liver, spleen, and testis

weight suggest that lard diet enhanced MeHg toxicity in these organs. For example, rats receiving a daily MeHg dose of 3 mg MeHg/kg bw and a lard diet had significantly increased relative liver and spleen weights compared with vehicle controls. In contrast, rats on fish oil, soy oil, seal oil, or DHA diets all had lesser or absent responses to the same level of MeHg exposure, suggesting a protective effect of these diets. Interestingly, the fish oil diet contained 1.2 mg selenium/kg, but this was not true of the other diets that contained no or near-zero selenium.

According to Jin et al. (2007), data on serum ALT, LDH-L, and AP activities pointed to possible structural damage of these liver enzymes in addition to a decrease in protein synthesis caused by MeHg. Total protein and albumin levels in serum suggested an inhibition of protein synthesis or loss of proteins in rats fed lard and fish oil diets, but inhibition was alleviated in the soy oil or DHA diets. The authors also noted that their results implied the presence of significant modulating effects of dietary fats on MeHg toxicity, which may translate into either more severe or protective clinical outcomes. Based on their findings in this study, Jin et al. (2007) speculated that the outcomes of MeHg intoxication in humans may differ in people with different dietary habits.

The positive effect of fish consumption on infant cognition was investigated by Oken et al. (2005), who specifically wanted to know whether maternal fish consumption during pregnancy harms or benefits the development of the fetal brain. Women were recruited for this study at their initial clinical obstetric appointment, with women considered eligible if their length of gestation was less than 22 weeks at this visit. At their second clinical visit (26-28 weeks of gestation) participants completed a food frequency questionnaire, which quantified the average frequency of consumption of more than 140 specified foods and beverages, including alcohol, during the preceding three-month period. The four questions regarding fish consumption asked about canned tuna (3-4 oz.), shrimp, lobster, scallops, clams (one serving), dark meat fish (*e.g.*, mackerel, salmon, sardines, bluefish, swordfish; 3-5 oz.), and other fish (*e.g.*, cod, haddock, halibut: 3-5 oz.). The six options ranged from "never/less than 1 per month" to "1 or more servings per day." Of the initial recruited cohort, only 135 mothers who agreed to supply a hair

sample had complete information concerning both maternal second trimester diets and 6-month cognitive testing results for their infant. Cognitive testing was performed on the infants at approximately six months of age (range 5.5 to 8.4 months) using the visual recognition memory (VRM) paradigm. All subjects were initially tested for visual acuity, and trained test administrators then presented the infant with two identical photographs of an infant's face. After a number of habituation trials, the infant was presented with the previously seen photo simultaneously with a novel photo of the face of another infant. The test administrator tracked the amount of time that the infant looked at each stimulus using a laptop computer. Each infant had two test trials, with the positions of the two faces alternated. The test reflects the infant's ability to encode a stimulus into memory, to recognize that stimulus, and to look preferentially at a novel stimulus. This test is correlated with later IQ.

Oken et al. (2005) found total maternal fish intake to be moderately associated with hair mercury content. For each additional weekly total fish serving, hair total Hg was found to be 0.07 ppm higher. The mean VRM score (reflecting percent novelty preference) was 59.8 (range 10.8 – 92.5). The VRM score did not differ by maternal and infant characteristics, including maternal use of cigarettes or alcohol during pregnancy. After adjusting for maternal and infant characteristics, it was determined that each additional weekly fish serving was associated with an increase in VRM of 2.8 points (range 0.2 – 5.4). It was also found that following adjustments, an increase of one ppm Hg in maternal hair was associated with a decrease in VRM score by 7.5 points (range 1-2 – 13.7). However, for each additional weekly fish serving consumed by the mother, the VRM score increased by 4 points (range 1.3 – 6.7). Of the 14 mothers in the study with total Hg hair concentrations greater than 1.2 ppm, the VRM scores were 9.3 points (range 0.8 – 19.3) lower than those of infants born to mothers with hair Hg concentrations of 1.2 ppm or less. Unadjusted VRM scores were highest among infants whose mothers had low hair Hg levels and who reported high fish intake levels, whereas those having low fish intake and high hair Hg had the lowest scores. The authors believed that their results support the recommendation that women continue to eat fish during pregnancy, but select varieties with lower mercury concentrations.

As part of the Seychelles Child Development Nutrition Study (SCDNS), Strain et al. (2008) studied the association between early child development and their prior fetal exposure to MeHg through maternal fish consumption. Specifically, they investigated the relationship of maternal LCPUFAs and the child's neurologic development, focusing on the associations of omega-3 and omega-6 LCPUFAs with outcomes of the Bailey Scales of Infant Development II (BSID-II) tests administered at 9 months and 30 months-of-age. The study cohort consisted of 229 mother-infant pairs. The mothers had a mean maternal age of 27.2 years (range 16-43) and reported consuming an average of 9 meals containing fish per week, which was estimated by the study authors to be ~537 g/week. Maternal blood samples were drawn at 28 weeks of gestation and one day after delivery.

The BSID-II, a well-standardized measure of infant cognition and development, was administered by specially trained evaluators (Strain et al. 2008). The BSID-II yields two primary endpoints: a mental developmental index (MDI) and a psychomotor developmental index (PDI). Five models were used in the analysis of the omega-3 and omega-6 LCPUF data. Model 1 adjusted for DHA and AA (the primary LCPUFA associated with neurologic development); model 2 adjusted for the sum of AA plus DHA and eicosapentaenoic acid (EPA) (as a measure of the of the omega-3 LCPUFAs found in fish); model 3 adjusted for omega-3 (sum of DHA, EPA, and alpha-linolenic acid (ALA)) and omega-6 (sum of AA and linoleic acid (LA)); model 4 adjusted for ratios of AA-to-DHA; and model 5 adjusted for the ratio of omega-6 to omega-3 LCPUFAs. Each of the five LCPUFA measurements was given as the geometric mean of the maternal serum values at 28 weeks and at delivery, since the transfer of LCPUFAs from the mother to the fetus occurs primarily during the third trimester. These mean values (in mg/mL) were 0.17 for DHA (range: 0.07-0.32), 0.61 for AA (range: 0.37-1.07), 0.02 for EPA (range: 0.00-0.05), 0.01 for ALS (range: 0.00-0.05), and 7.0 for LA (range: 3.73-9.78). The mean AA/DHA ratio was 3.7 (range 1.75-7.09). The total omega-3 and omega-6 mean values were 0.20 mg/mL (range: 0.08-0.36) and 7.61 mg/mL (range: 4.10-10.77), respectively. The mean omega-6 to omega-3 ratio was 40.17 (range:13.21-90.35). The mean hair MeHg value was 5.7 ppm (range 0.2018.5 ppm).

When the infants were evaluated at 9 months, BSID-II PDI (but not MDI) values were found to be positively correlated with LCPUFs in models 3 and 5. In model 3, omega-3 were significantly correlated with PDI scores both with ($p = 0.03$) and without ($p = 0.002$) outliers. No such association was found for omega-6 PUFAs in model 3. When Hg was included in the model, the statistical significance increased ($p = 0.02$ with outliers; $p = 0.001$ without outliers). Model 5 analysis revealed a significant positive correlation ($p = 0.03$) between the omega-6/omega-3 ratio and PDI scores for LCPUFAs including outliers, but not Hg. When outliers and Hg were both excluded from the model, the significance increased to $p = 0.005$. When Hg was included, the *p*-values increased to 0.02 and 0.001 for including and excluding outliers, respectively.

When compared with 30-month test results, LCPUFA levels were not correlated with PDI scores when Hg was not included in the models. (There were no outliers for LCPUFAs with any model.) When Hg was added to the models, the correlations were statistically significant in models 1, 2, 3, and 5. For model 1, the *p*-values were 0.05 (all data) and 0.03 when Hg outliers were excluded. For model 2, the *p*-values were 0.04 (all data) and 0.03 when Hg outliers were excluded. For model 3 and 5, the *p*-values were 0.05 and 0.03, respectively; there were no outliers in those models. Thus, at both 9 and 30-month testing, the associations with the PDI outcomes were stronger when outliers were excluded and weaker when the models did not adjust for MeHg.

In another study showing beneficial effects of LCPUFAs, Jacobson et al. (2008) examined the relation of umbilical cord plasma DHA concentration to infant visual acuity and cognitive and motor development, as well as any effects on growth and development associated with DHA from breast milk. The study population consisted of Inuit mother-infant pairs who participated in the Environmental Contaminants and Child Development Study in Nunavik, a province of Quebec, Canada. Data were collected from cord LCPUFA concentration for 109 mother-infant pairs. In addition, maternal plasma LCPUFA concentrations were available for 91 of the infants, and maternal milk LCPUFA

concentrations were available for 67 of the infants. Infant growth, visual acuity, and cognitive and motor function were assessed at 6 and 11 months post-partum.

Jacobson et al. (2008) found that the mean cord plasma DHA concentration was significantly higher ($p < 0.01$) than the maternal plasma DHA concentration. There was also a strong correlation between maternal and cord plasma phospholipids ($p < 0.001$) and the DHA/AA ratio in maternal and cord plasma ($p < 0.001$). After adjustment for confounders and suppressors, it was also found that higher cord DHA/AA ratios were associated with better visual acuity at 6 months ($p < 0.01$), but not at 11 months. Further, higher cord DHA and DHA/AA ratio were found to be significantly associated with greater novelty preference on the Fagan Novelty Preference test at 6 months ($p < 0.01$ and $p < 0.05$, respectively). In addition, a positive correlation between DHA and scores on the Bayley mental ($p < 0.05$) and psychomotor development ($p < 0.05$) indices was observed at 11 months. The authors concluded that the association between higher cord DHA concentrations with more optimal visual, cognitive, and motor development is consistent with the need for substantial increases in DHA during the third trimester, when a spurt in synaptogenesis in brain and photoreceptor development occurs.

In the Jacobson et al. (2008) study, breast milk intake and maternal DHA body burden were found to be independently related to 11-month weight gain ($p < 0.001$ and $p < 0.05$, respectively). The authors reported that these data suggest that a higher DHA concentration in breast milk contributes to slower 11-month weight gain over and above the reduction in weight gain generally observed in breast-fed infants. It was also found that most of the benefit occurs when the DHA concentration reaches 3% of fatty acids, with little benefit thereafter.

Kaur et al. (2008) investigated the ability of DHA to modulate MeHg-induced neurotoxicity in primary astrocytes and neurons from the cerebella of 7-day-old Naval Medical Research Institute (NMRI) mice. To examine the effects of DHA, cerebellar neurons and astrocytes were incubated in 30 or 90 μM DHA for 24 hours. On the day of the experiment, the cells were incubated with either 10 μM MeHg or HEPES buffer (for

controls) for 50 minutes, the last 20 minutes of which the cells were incubated with either 7 μM CMH_2DCFDA or 40 μM MCB or with the colorimetric reagent MTT (2.4 mM). The content of free thiol levels was determined using the fluorescent indicator MCB. Intracellular ROS accumulation was monitored using either 7 CMH_2DCFDA, which yields a fluorescent adduct upon oxidation, or the MTT. Cell-associated MeHg studies were performed using ^{14}C-labeled MeHg (10 μm). The composition of fatty acids in total lipids was analyzed using a trace gas chromatography ultra gas chromatograph.

Total fatty acids were elevated relative to controls in both astrocytes and neurons bathed in 30 μM and 90 μM DHA ($p < 0.05$), and the increase with 90 μM DHA relative to 30 μM DHA was also significant ($p < 0.05$) in both cerebellar astrocytes and neurons (Kaur et al. 2008). After pretreatment of cerebellar astrocytes with 90 μM DHA, the observed increase in DHA was associated with an increase in EPA and a decrease in AA. Further, in both astrocytes and neurons, DHA-treatment resulted in a significant increase in the DHA/AA ratio, most prominent in the neurons.

Kaur et al. (2008) found that the effects of DHA pretreatment were not always the same for astrocytes and neurons. In both cerebellar astrocytes and neurons, treatment with 30 μM DHA resulted in a significant ($p < 0.05$) decrease in cell-associated MeHg effects compared to the effects seen in the MeHg-treated group not treated with DHA. With 90 μM DHA, however, the decreased cell-associated MeHg only occurred in neuronal cells. In cerebellar neurons, treatment with 90 μM DHA alone resulted the induction of ROS. However, in DHA-treated cerebellar neurons exposed to MeHg, there was a decrease in ROS production ($p < 0.001$) when compared with neurons treated with MeHg alone. In astrocytes, treatment with both DHA and MeHg resulted an ROS level not significantly different from controls. The authors concluded that the reduced response of MeHg (production of MeHg-induced ROS) seen in this study after pretreatment with DHA could be due to the production of less ROS due to reduced availability of intracellular MeHg and may include an increase in the DHA/AA ratio and in GPx activity, which in turn may further influence the downstream transcription factors and signal transduction pathways (Kaur et al. 2008).

LCPUFA: negative correlation

Wennberg et al. (2007) investigated whether the intake of fish, Hg, or the sum of proportions of the omega-3 fatty acids eicosapentaenoic acid (EPA) acid and docosahexaenoic acid (DHA) influence the risk of hemorrhagic or ischemic stroke. The study population was recruited from a community intervention program on cardiovascular disease (CVD) and diabetes (Vasterbotten Intervention Programme) and the WHO Multinational Monitoring of Trends and Determinants in Cardiovascular Disease (MONICA) study in Northern Sweden. In this study, it was found that fish intake was positively correlated with erythrocyte Mg content ($p < 0.001$). In univariate conditional logistic regression analyses, it was found that fish intake, but not erythrocyte Hg content or EPA + DHA, was associated with the risk of stroke ($p = 0.05$). These analyses also revealed that serum cholesterol ($p = 0.004$), smoking ($p = 0.008$), BMI ($p < 0.001$), self-reported diabetes ($p < 0.001$), hypertension ($p < 0.001$), diastolic blood pressure ($p < 0.001$), and systolic blood pressure ($p < 0.001$) were highly correlated with stroke risk. Multivariate conditional logistic regression analyses similarly revealed an elevated risk of stroke with increasing fish intake ($p = 0.04$ for ischemic stroke risk and for risk of all stroke types). Multivariate analyses also revealed that the effect of fish intake was significantly ($p = 0.03$) modified by sex, as fish intake had no correlation with the risk of stroke in females.

Wennberg et al. (2007) concluded that fish intake appears to adversely affect the risk of stroke in men, but not women. Further increased levels of EPA + DHA did not decrease the risk for stroke, and there was no association between stoke risk and Hg at low levels.

To assess the impact of chronic exposure to PCBs and MeHg, Saint-Amour et al. (2006) selected 110 children, average age of 5.44 years (range: 5.07 to 5.81) for their study. The children were from mother-infant pairs who had participated in the Nunavik Cord Blood Monitoring Program, in which Hg and other persistent pollutants were measured in

umbilical cord blood. Blood and hair samples were collected at the time of testing and analyzed for total Hg, PUFAs, and Se. After testing, adequate electrophysiological data were available for 78 children. While effects on neurobehavioral parameters were reported for MeHg and PCBs, no significant interactions were found between PUFAs and those contaminants.

Selenium: positive correlation

Watanabe et al. (1999b) used ICR mice to test their hypothesis that *in utero* selenium (Se) deficiency and MeHg exposure would affect the neurobehavioral function of the offspring in an additive manner. Initially, female mice were given diets with selenium concentrations considered deficient (< 0.02 mg Se/kg), marginal (~ 0.05 mg Se/kg), or adequate (~ 0.4 mg Se/kg) for four weeks. Ordinary commercial diets normally used in their laboratory contain ~ 0.4 mg Se/Kg. After four weeks on their respective diets, the rats were mated with normal males fed a Se-adequate diet. On gestation day 12, each group of mice was divided into three subgroups that were then injected with *s.c.* doses of MeHg of either 0, 3, or 5 mg/kg bw. The 0 and 3 mg/kg subgroups received additional injections of the same doses on gestational days 13 and 14. On post-natal days (PNDs) 4, 7, and 10, the pups were examined and scored for righting reflex and walking ability. Walking ability was again examined on G3 PND 12. In addition, thermal preference was examined on PNDs 8 and 14 using a thermogradient apparatus, and an open-field test was conducted when the pups were one and four weeks of age.

Watanabe and co-investigators (1999b) found that MeHg, but not Se, significantly affected the development of the righting reflex ($p < 0.05$). This effect of MeHg was interpreted as one of retardation, rather than inhibition, since all test animals performed the test on post-natal day 10. On PND 4, a significant ($p < 0.01$) difference in the development of walking ability was observed between the Se-deficient group and the adequate and marginal Se groups; however, MeHg did not cause the same retardation in walking ability. These differences remained significant when re-examined on PND 12. On PND 8, Se deficiency, but not MeHg, significantly raised the thermal preference of the

pups ($p < 0.01$). On PND 14, both Se-inadequate and MeHg-exposed mice had a significantly increased preference for warmer temperatures ($p < 0.05$). In the latter case, there were no significant differences between groups, presumably because the upper limit on the thermogradient apparatus was 36°C and preferences above that temperature could therefore not be measured. In the open-field test Se deficiency, but not MeHg, reduced the activity of the mice ($p < 0.01$). Watanabe et al. (1999b) concluded that the groups most affected were the group receiving the lowest amount of Se and the group given the highest dose of MeHg. Thus, the neurobehavioral outcome of *in utero* Se deficiency and MeHg exposure converged; that is to say that Se deficiency may result in greater effects of MeHg on neurobehavioral performance.

Newland et al. (2006) investigated the concentrations of mercury and selenium in the brain and blood of 114 Long-Evans rats under exposure conditions designed to model conditions of human exposure under two regimens: chronic and developmental. In the chronic exposure regimen, exposure to MeHg was combined with low- or high-Se in the diet. In this portion of the study, dietary exposure of females to Se began at around 17-18 weeks of age. Dietary selenium levels corresponded to the low and high ends of recommended intakes (0.06 ppm and 0.6 ppm, respectively). After three weeks on the respective diets, MeHg exposure began and was continued for 6 or 18 months. These females became the mothers of the animals used for the second experimental regimen, developmental exposure. MeHg concentrations of either 0, 0.5, or 5 ppm were administered in drinking water.

In the chronic exposure regimen, there was a significant interaction between Hg and Se with respect to Se concentration in the brain ($p = 0.002$) as measured at 6 months (Newland et al. 2006). For all exposure groups except 5 ppm MeHg, the brain Se concentration was about 0.1 ppm. For the 5 ppm MeHg rats, six months of MeHg exposure resulted in a three- four-fold elevation in brain selenium. There was also a main effect of dietary selenium on blood Se ($p < 0.001$), but not blood Hg. A main effect of MeHg intake on Hg content in the brain ($p < 0.001$) and blood ($p < 0.001$) was also seen. However, there was no effect of Se on Hg content of the brain or blood after six months of

exposure. At 18 months, there was a significant interaction ($p = 0.031$) of MeHg and Se, due primarily to the 5 ppm MeHg group. In the high-Se/high-MeHg group, there was a significant effect on brain Se ($p < 0.001$). Also in 5 ppm MeHg animals, those on the low-Se diet had less blood mercury than controls, while the 5 ppm MeHg rats on the high-Se diet had slightly more selenium than controls. There was a significant ($p = 0.043$) interaction between MeHg exposure and Se on blood Hg concentration. The rats exposed to 0.5 ppm MeHg and high-Se had increased blood Hg levels, whereas low-MeHg/ high-Se exposures resulted in lower blood Hg levels.

For the developmental regimen, Newland et al. (2006) reported a main effect of mercury ($p < 0.001$) and selenium ($p = 0.001$*),* but no interaction on brain Se concentration. There was also a main effect of mercury *(*$p < 0.001$) and selenium ($p < 0.001$) on the blood of neonates, but without MeHg/Se interaction of blood Se concentration. The authors concluded that their results indicated that the dosing regimen of 0.5 ppm and 5 ppm spans an important threshold defined by whether there is a molar excess of Hg or Se in the brain. At the 5 ppm exposure level, there was always a large molar excess of Hg over Se in the brain and in the blood. But whether this is also true for the 0.5 ppm group depends on whether exposure begins *in utero* or in adulthood. On one hand, if exposure begins in adulthood, then Se atoms outnumber Hg atoms in whole brain tissue. However, if exposure is during fetal developmental only, Hg and Se are approximately in balance, and the concentrations of Hg and Se in the developing organism are especially labile.

Selenium: negative correlation

Reed et al. (2006) investigated the interactions between low-level MeHg exposure and selenium during development on discrimination reversal later in adulthood using 44 female Long-Evans rats F_1 generation). The animals (6-8 per group) were exposed *in utero* through maternal ingestion of drinking water containing 0, 0.5, or 5 ppm mercury (equivalent to 0, 40, and 400 μg/kg/day, respectively) as MeHg chloride and a diet containing ~ 0.06 or 0.6 ppm selenium. MeHg exposure was discontinued after birth, but

the selenium regimen was continued throughout life. After undergoing training (autoshaping of lever-pressing and two-lever training), original discrimination and spatial discrimination reversal tests were conducted.

One-way ANOVA showed that low-selenium adult rats required more sessions to complete the first reversal ($p = 0.046$) and made more omissions ($p = 0.017$) than high-selenium animals, but these effects were not seen on subsequent reversals. Low-selenium rats also had longer rear-lever latencies and longer choice latencies during original discrimination than did rats in the high-selenium group ($p = 0.058$ and $p = 0.06$, respectively), but these effects fell just short of having statistical significance. These differences between selenium intake groups were independent of MeHg intake, and the number of sessions necessary to satisfy the test criterion was related to omissions, but not errors.

Adult rats gestationally exposed to MeHg, regardless of selenium exposure, exhibited response patterns similar to control rats during the original discrimination, but made significantly more errors than controls ($p < 0.05$) on the subsequent first and third reversals, away from the a response previously rewarded during original discrimination (Reed et al. 2006). The responses in the high-MeHg group were also significantly different from responses of the low-MeHg group ($p < 0.05$). Those authors concluded that exposure to selenium at the dosages used in their study had no impact on the effects of MeHg exposure.

Saint-Amour et al. (2006) assessed the impact of chronic exposure to PCBs and MeHg in 110 children, average age of 5.44 years (range: 5.07 to 5.81). The children were from mother-infant pairs who had participated in the Nunavik Cord Blood Monitoring Program, in which Hg and other persistent pollutants were measured in umbilical cord blood. Blood and hair samples were collected at the time of testing and analyzed for total Hg, PUFAs, and Se. After testing, adequate electrophysiological data were available for 78 children. Blood concentrations of Se at the time of testing were associated with longer N75 component VEP latencies at the 95% ($p \leq 0.001$) and 30% ($p \leq 0.01$) contrasts, in

addition to longer P100 latency at 95% and 30% contrasts ($p \leq 0.01$). Thus, any effect of Se was not protective of VEP latency.

Selenium: another perspective

Ralston et al. (2008) investigated the interactions between dietary MeHg and Se in order to examine the role of dietary Se in counteracting MeHg toxicity. Sixty weanling male Long-Evans Rats were fed diets containing Se concentrations of 0.1, 1.0, or 10.0 μmol Se/kg diet (equivalent to ~0.01, 0.08, or 0.8 ppm Se, respectively). Mercury levels in the diets were 0.5 μmol/kg and 50 μmol/kg (equivalent to ~0.10 and 10 ppm Hg). The rats were divided equally into six weight-matched groups to accommodate all possible combinations of Se and MeHg dietary concentrations. Animals received the respective diets *ad libitum* for 18 weeks. At that time, the animals were sacrificed and samples of blood, liver, kidney, and brain were harvested and submitted for analysis. The authors reported that the toxicity of MeHg was not predictable by tissue Hg, but instead was related to the presence of both Se and MeHg. MeHg toxicity was found to be related inversely to tissue Se ($p < 0.001$) and directly related to the ratio of Hg-to-Se ($p < 0.001$). They also noted that the formation of MeHg-selenocysteine complexes appeared likely to impair Se bioavailability, thereby interfering with the synthesis of anti-oxidant Se-dependent enzymes. Ralston et al. (2008) concluded that their study provided evidence that simply measuring MeHg exposure is not sufficient to provide accurate, precise information regarding the potential risks of MeHg, unless Se intakes are also factored into the evaluation. They further concluded that the ratio of Hg-to-Se in the blood appears to provide more interpretable and physiologically meaningful information concerning the risk from MeHg exposure, than does blood Hg alone.

3. CHEMICAL AND PHYSICAL INFORMATION

No updated data.

4. PRODUCTION, IMPORT, USE, AND DISPOSAL

No updated data.

5. POTENTIAL FOR HUMAN EXPOSURE

5.5 GENERAL POPULATION AND OCCUPATIONAL EXPOSURE

In the Third National Report on Human Exposure to Environmental Chemicals (CDC 2005), the Centers for Disease Control and Prevention (CDC) reported the results of the National Health and Nutrition Examination Survey (NHANES), 1999-2002. In that report, mercury blood levels were -obtained and reported for children (male and female) ages 1-5 years of age and women considered to be of reproductive age (ages 16-49 years). The results were tabulated as follows.

Table 3. Mercury blood levels

Geometric mean and selected percentiles of blood concentrations (in ug/L) for males and females aged 1 to 5 years and females aged 16 to 49 years in the U.S. population, National Health and Nutrition Examination Survey, 1999-2002.

	Survey years	Geometric mean (95% conf. interval)	Selected percentiles (95% confidence interval) 50th	75th	90th	95th	Sample size
Age Group							
1-5 years (females and males)	99-00	**.343** (.297-.395)	**.300** (.200-.300)	**.500** (.500-.600)	**1.40** (1.00-2.30)	**2.30** (1.20-3.50)	705
	01-02	**.318** (.268-.377)	**.300** (.200-.300)	**.700** (.500-.800)	**1.20** (.900-1.60)	**1.90** (1.40-2.90)	872
Females	99-00	**.377** (.299-.475)	**.200** (.200-.300)	**.800** (.500-1.10)	**1.60** (1.00-2.80)	**2.70** (1.30-5.50)	318
	01-02	**.329** (.265-.407)	**.300** (.200-.300)	**.700** (.500-.800)	**1.30** (1.00-2.10)	**2.60** (1.30-4.90)	432
Males	99-00	**.317** (.269-.374)	**.200** (.200-.300)	**.500** (.500-.600)	**1.10** (.800-1.60)	**2.10** (1.10-3.50)	387
	01-02	**.307** (.256-.369)	**.300** (.200-.300)	**.600** (.400-.700)	**1.30** (.900-1.70)	**1.70** (1.40-2.00)	440
16-49 years (females only)	99-00	**1.02** (.825-1.27)	**.900** (.800-1.20)	**2.00** (1.50-3.00)	**4.90** (3.70-6.30)	**7.10** (5.30-11.3)	1709
	01-02	**.833** (.738-.940)	**.700** (.700-.800)	**1.70** (1.40-1.90)	**3.00** (2.70-3.50)	**4.60** (3.70-5.90)	1928
Race/ethnicity (females, 16-49 years)							
Mexican Americans	99-00	**.820** (.664-1.01)	**.900** (.700-1.00)	**1.40** (1.20-2.00)	**2.60** (2.00-3.60)	**4.00** (2.70-5.50)	579
	01-02	**.667** (.541-.824)	**.700** (.500-.800)	**1.10** (1.00-1.40)	**2.10** (1.70-3.00)	**3.50** (2.30-4.40)	527
Non-hispanic blacks	99-00	**1.35** (1.06-1.73)	**1.30** (1.10-1.70)	**2.60** (1.80-3.40)	**4.80** (3.30-6.60)	**5.90** (4.20-11.7)	370
	01-02	**1.06** (.871-1.29)	**1.10** (.800-1.20)	**1.80** (1.50-2.20)	**3.20** (2.20-3.90)	**4.10** (3.30-6.00)	436
Non-hispanic whites	99-00	**.944** (.726-1.23)	**.900** (.700-1.10)	**1.90** (1.30-3.30)	**5.00** (3.00-6.90)	**6.90** (4.50-12.0)	588
	01-02	**.800** (.697-.919)	**.800** (.700-.800)	**1.50** (1.30-2.00)	**3.00** (2.20-3.70)	**4.60** (3.30-6.80)	806

In 2009, the U.S. Food and Drug Administration (FDA) corrected the NHANES values from 1999-2004 for inorganic mercury content by subtracting the inorganic mercury from the total blood level to show the level of organic mercury in the blood (FDA 2009) as follows.

Table 4. MeHg levels in blood.

Population percentiles from NHANES 1999-2004					
Percentile	**Children 2-5**	**Men 16-45**	**Men 46+**	**Women 16-45**	**Women 46+**
Mean	1.10	1.01	1.14	1.32	1.32
1st	0.1	0.14	0.14	0.2	0.14
25th	0.3	0.3	0.3	0.4	0.3
50th	0.6	0.6	0.6	0.8	0.7
75th	1.2	1.2	1.3	1.6	1.5
90th	2.4	2.2	2.8	3.4	2.9
95th	4.1	3.4	4.2	5.5	4.5
99th	8.8	7.2	7.8	12.0	9.5
99.5th	12.8	8.5	10.3	14.0	12.6
99.9th	15.1	13.7	11.1	22.7	24.6
All values are in blood (ppb or µg/L). The values have been corrected for inorganic mercury content, meaning that inorganic mercury has been subtracted from total					

blood mercury in order to show the level of organic mercury in the blood. Source, FDA 2009.

Dietary Sources of Mercury. Gerstenberger et al. (2010) examined the mercury content of 302 cans of tuna purchased from a Las Vegas grocery store. The study was divided into two parts. In the initial (pilot) study, 155 cans of tuna from three national brands (identified only as Brands 1, 2, and 3) were analyzed for mercury content. To ensure that the mercury concentrations were typical, multiple lot numbers of tuna were included during the purchase period of November 2005 through February 2006. Four variables were examined in the pilot study; namely, brand of tuna, type (chunk white *vs.* chunk light), seasonal variation, and packaging medium (oil *vs.* water). Analyses of the mercury content of each can revealed significant differences among brands and the type of tuna. A follow-up study was then conducted in May of 2006. In this follow-up, all purchases were made at the same store used in the pilot study, but all 147 cans purchased were of the same brand that had the highest mercury concentrations in the pilot study. Also, this second study included the purchase of three types of tuna (solid white, chunk white, and chunk light) of this single brand. Of 54 cans of Brand 1 analyzed during the pilot study, none had mercury concentrations exceeding the 1 ppm standard set by the U.S. Food and Drug Administration (FDA) for fish sold in interstate commerce. Of 46 cans of Brand 2, only 2 of the cans (chunk white) had mercury levels exceeding the FDA standard. However, 14 cans of Brand 3 chunk white (and no solid white or chunk light) tuna exceeded the FDA limit of 1 ppm. Of the 147 cans sampled during the follow-up portion of the study, mean mercury concentrations were 0.576 (S.D. 0.178), 0.619 (S.D. 0.212), and 0.137 (S.D. 0.063) ppm for solid white, chunk white, and chunk light tuna, respectively. The highest concentrations in any can of each type were 0.988, 1.159, and 0.310 ppm for solid white, chunk white, and chunk light, respectively (Gerstenberger et al. 2010).

Morrissey et al. (2004) examined 91 albacore tuna caught along the western coast of the U.S. during the 2003 commercial fishing season for total Hg content in muscle tissue.

The average muscle tissue Hg (combined MeHg and inorganic Hg) was 0.14 (+/- 0.05) ppm (range: 0.027 to 0.26). The authors also found a positive correlation between total Hg and the length and weight of the fish. Morrissey et al. (2004) also noted that the mercury levels recorded are below both the U.S. FDA and Canadian standards of 1 ppm and 0.5 ppm, respectively, for permissible Hg levels in fish.

Atlantic bluefish (*Pomatomus saltatrix*) are widely prized as fighting sportfish and for their taste, as most of the meat is white meat. But since they are predatory fish, feeding on squid, small shrimp and other smaller fish (especially menhaden and silversides), they accumulate mercury in their tissues as they grow in size and age. Burger (2009) examined 206 bluefish caught in New Jersey waters during the period 2005-2008. Mercury content in fish tissue was found to be highly correlated with body length ($p < 0.0001$) and weight ($p < 0.0001$). Fish less than 25 cm in fork length had lower Hg levels than those between 26 and 50 cm; but Hg levels increased quickly with increasing size thereafter.

Storelli et al. (2005) collected fish from two species, hake (*Merluccius mercluccius*) and striped mullet (*Mullus barbatus*) by trawling the waters of the Ionian Sea and the Adriatic Sea. Muscle tissue from these fish was removed and examined for both MeHg and total Hg using gas chromatography and a ^{63}Ni electron capture detector. Average MeHg levels in the hake were 0.09 +/- 0.07 µg/g (ppm) and 0.16 +/- 0.10 µg/g for the Ionian and Adriatic Seas, respectively. Comparable total Hg levels in hake were 0.09 +/- 0.08 ppm and 0.18 +/- 0.12 ppm for Ionian and Adriatic fish, respectively. The percent of total Hg represented by MeHg was 98.3 % (range: 73-100%) for Ionian hake and 90.8% (range: 60-100%) for Adriatic hake.

In striped mullet, Storelli et al. (2005) found average MeHg concentrations of 0.40 +/- 0.42 ppm for Ionian mullet and 0.44 +/- 0.53 ppm for Adriatic mullet. Average total Hg levels were 0.40 +/- 0.42 for Ionian fish and 0.49 +/- 0.54 ppm for fish caught in the Adriatic Sea. For the mullet, the percent of total Hg represented by MeHg was 98.9 % (range: 92-100%) for Ionian mullet and 79.8% (range: 68-100%) for Adriatic mullet.

In an examination of the Hg levels in albacore tuna (*Thunnus alalunga*), Storelli and Marcotrigiano (2004) measured the total Hg levels in 137 fish collected by trawling in the Adriatic Sea. The mean Hg levels in those tuna were 1.56 ppm (range: 0.88 – 2.34) in edible flesh and 2.41 ppm (0.95 – 4.30) in livers. Hg concentrations above 1 ppm were found in 71.4% of the edible flesh and 85.7% of the livers of all albacore sampled. In another study (Storelli et al. 2003), total Hg concentrations in Adriatic frostfish (*Lepidopus caudatus*), reported to be good eating, exceeded 1 ppm in 15% of the 300 fish sampled. For the 15 species sampled, the measured MeHg levels varied between 70% and 100% of the total Hg present. In the frost fish, mean total Hg levels were reported to be 0.76 ppm (range: 0.19 – 1.77 ppm), 83% (+/- 22) of which was MeHg.

To obtain recent data on MeHg levels in some fish commercially sold in Canada and to compare the relative amounts of MeHg and total Hg in the species of concern, Forsyth et al. (2004) obtained samples from four specialty seafood outlets in each of the following cities: Vancouver, Toronto, and Halifax. Fresh or frozen shark, marlin, and swordfish were collected in samples of 450 g or more edible portion. Fresh, frozen, or canned tuna were also collected from the same 12 stores. The highest average MeHg (1.060 ppm) and total Hg (1.822 ppm) were found in swordfish, followed by shark (0.849 MeHg; 1.360 ppm total Hg), fresh/frozen tuna (0.662 ppm MeHg; 0.929 ppm total Hg)), and marlin (0.489 ppm MeHg; 0.842 ppm total Hg). However, no statistical significance was found, reportedly due to the large range in results. The difference between canned tuna samples (mean value of 0.098 ppm) and fresh/frozen shark, swordfish, marlin and tuna was significant ($p < 0.05$). Among the four categories of canned tuna (albacore, skipjack, yellowfin, and unidentified), the highest MeHg level was found in canned albacore (mean value of 0.166 ppm). The MeHg concentrations in the other three types of canned tuna were 0.057 ppm for yellowfin, 0.047 for skipjack, and 0.025 for unidentified. The highest reported levels for any single predatory fish were 2.35 ppm MeHg and 3.85 ppm total Hg found in one swordfish.

The wahoo, a sportfish similar in appearance to Spanish mackerel and some tuna, is widely prized for white meat and the fight it puts up when hooked on a fishing line. It is an important recreational and commercial species in Florida waters (FMRI 2003). In January of 2003, the Florida DOH issued a health advisory recommending limited consumption of wahoo from the Florida Keys and South Bay (FDOH 2003). Sixty-one wahoo from the Atlantic and Gulf coasts (six different locations) were collected and analyzed for total Hg. For those six locations mean total Hg levels ranged from 0.16 ppm to 0.68 ppm. The lowest single Hg level reported was 0.06 ppm, and the highest was 1.40 ppm. In all, 18% of the wahoo collected had total Hg levels ≥ 0.5 ppm. Analysis of wahoo from one area adjacent to the Indian River Lagoon showed a significant positive correlation between total Hg and the length of the fish.

Ruppel et al. (2008) reported data collected during the third year of the New Jersey Department of Environmental Protection (NJDEP) monitoring program that is investigating the contamination of fish caught in that state. Of 434 fish and crab samples collected, Hg was detected in all. The largest level detected was 1.413 ppm, found in a largemouth bass. The authors reported that although most Hg levels were below 0.5 ppm, levels above 0.5 ppm were found in individual fish samples from a number of lakes. The majority of the higher Hg concentrations were found in freshwater fish in the top trophic level, including largemouth bass, chain pickerel, hybrid striper, and walleye. Only one of the marine/estuarine samples, a white perch, had an "elevated" mercury concentration.

Some of the higher total Hg levels listed in the extensive FMRI (2003) report are provided in Table 5. It should be noted that this table contains only some of the fish identified in areas having the highest level of MeHg in the fish caught. When different fish-catch locations had different reported total Hg levels in the same species, only the highest is typically reported in this table. Most aquatic species had considerably lower total Hg levels. For further information, see FMRI TR-9 (FMRI 2003).

Perugini et al. (2009) reported the results of sampling analysis of 82 selected edible fish, crustaceans, and cephalopod mollusks collected from the Central Adriatic Sea in 2004. Samples were analyzed for total Hg only. The highest man total Hg concentration was found in Norway lobster (0.97 ppm; range 0.29-3.27; $n = 13$). European Hake ($n = 14$) had a mean Hg concentration of 0.59 ppm (range: 0.04 – 1.99), followed by red mullet ($n = 14$) with a mean concentration of 0.48 ppm (range: 0.05 – 1.07), blue whiting ($n = 14$) with a mean total Hg level of 0.38 ppm (range: 0.06 – 1.42), Atlantic mackerel ($n = 13$) with a meal Hg level of 0.36 ppm (range: 0.03 – 1.17), and European squid ($n = 14$) with a mean total Hg level of 0.25 ppm (range: 0.02 – 0.62). The total Hg levels in the lobster were significantly higher than those found in all other species ($p < 0.01$). Perugini et al. (2009) noted that over 25% of the 82 organisms examined exceed 0.5 mg/kg (equivalent to 0.5 ppm).

Mean Hg concentrations reported for tuna sold or caught in the U.S. are provided in Table 6.

Table 5. Fish from FMRI (2003) that have high total Hg values.

		total Hg in ppm			
Common name	***Genus/species***	***mean***	***median***	***range***	***location/area***
blue marlin (*n*=8)	*Makair nigricans*	3.08	2.75	0.98 – 6.80	Florida Keys
great barracuda (*n* = 62)	*Sphyraena barracuda*	0.87	0.72	0.08 – 3.10	Florida Keys
black grouper (*n* = 8)	*Mycteroperca microlepis*	1.16	1.15	0.83 – 1.60	Florida Keys
Goliath grouper (*n*=13)	*Epinephelus itajara*	1.15	1.10	0.09 – 3.30	Tampa Bay & coastal waters
snowy grouper (*n* = 22)	*Epinephelus niveatus*	0.95	0.88	0.26 – 1.90	Indian River Lagoon & coastal waters
red snapper (*n* = 1)	*Lutjanus campechanus*	2.80	2.80	N/A	Indian River Lagoon & coastal waters
blackfin tuna (*n* = 22)	*Thunnus atlanticus*	1.16	1.20	0.16 – 2.00	Indian River Lagoon & coastal waters
little tunny (*n* = 2)	*Euthynnus alletteratus*	2.15	2.15	1.50 – 2.80	Indian River Lagoon & coastal waters
king mackerel (*n* = 19)	*Scomberomorus cavalla*	2.08	1.80	1.20 – 3.80	Sarasota Bay & coastal waters
Amberjack (*n* = 1)	*Seriola dumerili*	0.91	0.91	N/A	Apalichicola & coastal waters
Bluefish (*n* = 25)	*Pomatomus salatrix*	0.87	0.68	0.28 – 2.00	Charlotte Harbor
gafftopsail catfish (*n* = 4)	*Bagre marinus*	0.96	0.98	0.88 – 1.00	Choctawatchee Bay & coastal waters
gafftopsail catfish (*n* = 59)	*Bagre marinus*	0.60	0.54	0.02 - 1.80	Tampa Bay & coastal caters

Table 6. Mercury (mean) concentrations (ppm) in tuna sold/caught in U.S.

Tuna Type/How Sold	*ppm Hg*	*Reference/Source*
canned solid white (albacore)	0.576	Gerstenberger et al., 2010
canned chunk white (albacore)	0.619	Gerstenberger et al., 2010
canned chunk light	0.063 0.118	Gerstenberger et al., 2010 FDA, 2006
canned albacore (not otherwise specified)	0.353	FDA, 2006
fresh/frozen (all)	0.383 0.384	FDA, 2006 Groth 2010
fresh/frozen (albacore)	0.357	FDA, 2006
fresh/frozen (bigeye)	0.639	FDA, 2006
fresh/frozen (skipjack)	0.205	FDA, 2006
fresh/frozen (yellowfin)	0.325	FDA, 2006
fresh/frozen (species unknown)	0.414	FDA, 2006
type(s) not specified	0.6	Burger et al., 2005

Consumption Patterns of Dietary Sources of Mercury.

Fish consumption patterns vary widely, dependent on such factors as proximity to large bodies of water, culture, gender, and socio-economic status. Good pre-pregnancy nutritional status and prudent seafood consumption practices are crucial to the development of the fetal nervous system. Thus, all women of child-bearing years should be knowledgeable of the sources of Hg exposure and the potential impact it could have on fetal well-being. A study of women at a Special Supplemental Nutrition Program for Women, Infants, and Children (WIC) clinic found that 11% of the women from this East Harlem, New York clinic who were fish consumers ate sport fish, despite an advisory recommending that women of child-bearing years not eat any fish from local waters (Bienenfeld et al. 2003). Further, only 55% of those fish consumers was even aware of that advisory.

Silver et al. (2007) investigated the fish consumption habits of low-income women in the Sacramento-San Joaquin Delta area of California, as well as their awareness of existing fish advisories. Due to the presence of MeHg resulting from Hg runoff from abandoned gold mines, the State of California has issued a health advisory recommending consumption limits of 6 oz./month (or 5.7 g/day) for certain fish caught in the Delta and in San Francisco Bay. This advisory was to be followed by consumers in conjunction with a joint U.S. Environmental Protection Agency (EPA) – U.S. Food and Drug Administration (FDA) advisory for commercial and sport fish. This federal advisory recommended that women of child-bearing age, pregnant and breast-feeding women, and children completely avoid fish containing high mercury levels of mercury (specifically shark, swordfish, king mackerel, and tilefish). The joint EPA-FDA advisory also recommends limited consumption of other commercial fish (*i.e.*, 12 oz./week, or 48.6 g/day for most fish; 6 oz/week, or 24.3 g/day for sport-caught fish). In this study, it was found that 29% of the women exceeded federal fish consumption advisory limits.

Among the participants enrolled at a Special Supplemental Nutrition Program for Women, Infants, and Children (WIC) clinic, Silver et al. (2007) found widespread fish

consumption and consumption differences among ethnic groups. Purchasers of commercial fish accounted for 99% of the study population, and 32% consumed sport fish. The overall fish consumption rate among consumers was 27.9 g/day, with a commercial fish consumption rate of 26.3 g/day and a sport fish consumption rate of 10.5 g/day. Overall fish consumption rates were higher in African-American (41.2 g/day) and Asian (35.6 g/day) women, with Cambodian and Hmong women having the highest rates among Asians. Pregnant women were found to consume less fish overall than other women ($p < 0.0001$). Women aware of fish advisory limits also had lower fish consumption rates ($p < 0.02$).

The seafood consumption rates of first-and second-generation Asian American and Pacific Islanders were studied by Sechena et al. (2003) using community-based participatory research (CBPR). A total of 202 Cambodian, Chinese, Filipino, Hmong, Japanese, Korean Laotian, Mien, Samoan, and Vietnamese adults, all of whom were fish eaters living in King County, WA in 1997 participated in the survey. Shellfish comprised 45.9% and finfish 43.3% of all fish consumed. The average seafood consumption rate for the overall study population was 117.2 g/day (median of 89 g/day). As with the aforementioned Silver et al. (2007) study, the consumption rate varied among ethnic groups. The Vietnamese had the highest consumption rate (mean 161.1 g/day, median 148 g/day), followed by the Japanese (~ 115 g/day). Using a measured mean body weight of 62 kg, Sechena et al. (2003) estimated the weight-relative intake to be 2.63 g/kg/day and 2.18 g/kg/day for the Vietnamese and Japanese, respectively. At the other end of the intake scale, the Mien women (0.58g/kg/day) and the Hmong women (0.59 g/kg/day) had the lowest intakes (both ~ 36 g/day). These values may be contrasted with the U.S. EPA (2000) seafood consumption rate of 1.8 g/day for U.S. women and 17.5 g/day for general and recreational fish consumers. A daily intake of 17.5 g/day would equate to 0.29 g/kg/day for a 60 kg individual and 0.32 g/kg/day for a 70 kg individual. Sechena et al. (2003) point out that 0.25 g/day is below the 10^{th} percentile of all participants in their study.

The most commonly consumed fish and shellfish in the Sechena et al. (2003) study were shrimp (98% of the respondents), crab (96%). salmon (93%), tuna (86%), and squid (82%). Among bivalve mollusks, the highest-consumed species were Manila/littleneck clams (72%), oysters (71%), mussels (62%), and scallops (57%). There was also a difference in the consumption of non-fillet parts not ordinarily consumed by U.S. populations. Fillet was eaten with the skin 55% of the time, and overall, the head, bones, eggs, and/or other organs were consumed 20% of the time. Crabs were eaten whole, muscle and organs, 43% of the time. With the exception of some tuna, the fish consumed in this study are generally considered to have lower mercury concentrations.

Holloman and Newman (2010) used community-based participatory research (CBPR) methods to assess the seafood consumption patterns of low income African-American women living along the lower James River in Virginia, USA. Ninety-five surveys were administered among ten different sites located throughout the Southeast community of Newport News, Virginia. The sites were randomly selected from a list of locations suggested by the Community Advisory Council, a partnership of ten African-American women representative of the study population. Although the Southeast community is not considered a fishing population, 83% of the women responding to the survey reported having consumed seafood within the last seven days. The mean seafood consumption rate for the woman surveyed was 147.8 g/day (range 117.6-185.8 g). Based on the amount consumed, the three highest consumption items were shrimp, croaker, and blue crab, followed by whiting, snow crab legs, and tuna. Based on just the frequency of consumption, the top three were whiting, shrimp, and tuna, followed by snow crab legs, blue crab, and croaker. Thus, although the women of the Southeast community do not fish for subsistence, they still consume seafood as a major source of dietary protein, and their seafood consumption rate is consistent with populations considered to be subsistence fishers: native Alaskans (109 g/day, Nobmann et al. 1992); Asian and Pacific Islanders in King County, WA (117.2 g/day, Sechena et al. 1999); Suquamish Indian Tribe (213.9 g/day, Duncan 2000). However, most of the fish commonly consumed are low in Hg and suggest a low risk of dietary MeHg exposure, with the possible exception of some forms of canned tuna.

To assess the effectiveness of fish advisories on fish consumption practices in the panhandle of Florida, Karouna-Renier et al. (2008) enrolled both pregnant and non-pregnant women (ages 16-49) who had resided in Santa Rosa and Escambia Counties of Florida for at least one year. To assess MeHg exposure, ~ 100 strands of hair were cut from the occipital portion of the scalp of 601 women and analyzed for Hg content. At the time of hair sampling, participants filled-out a questionnaire inquiring of the participant's use of hair dye or relaxer and their fish consumption practices. The questions related to number of fish meals (0.1, 2, 3, or ≥ 4) consumed during the previous 30, 60, or 90 days and to the participant's awareness of Florida's fish consumption advisories. The ethnic make-up of the study cohort consisted of women, who classified themselves as either white (74.5%), black (10.8%), Hispanic (6.1%), Asian (4.6%), other (2%), or chose not to provide race data (1.9%). A total of 83 women (13.9%) reported being pregnant. Hair mercury levels were 0.28 ppm (range: 0.02 – 4.12) for white women, 0.11 ppm (range: 0.02 - 2.67) for black women, 0.26 ppm (range: 0.02 – 2.30) for Hispanic women, 0.28 ppm (range: 0.02 - 22.14) for Asian women, 0.61 ppm (range: 0.05 – 10.65) for women who classified themselves as "other," and 0.40 ppm (range: 0.10 – 1.26) for women electing not to report race. When comparing pregnant to non-pregnant women, the 83 pregnant women had a mean hair Hg concentration of 0.20 ppm (range: 0.02 – 10.65), while the 515 non-pregnant females had a mean hair Hg concentration of 0.27 ppm (range: 0.02 – 22.14). Hair Hg levels were significantly higher ($p < 0.05$) in women who had consumed fish within the 30 days immediately prior to hair sampling. Of the 95 women (15.8% of the study population) who had hair Hg levels above 1 ppm, 60 (or 62.5%) ate four or more fish meals and 75 (78%) ate three or more fish meals during that 30-day period.

Karouna-Renier reported that only 31% of the participants reported knowledge of existing fish consumption advisories, and hair Hg levels were also significantly higher ($p < 0.05$) in that particular population. Hispanic women had the highest awareness and black women were the least likely to know about the advisory. Further, a smaller percentage of pregnant women knew about the advisory than non-pregnant women.

Karouna-Renier et al, (2008) concluded that public health interventions such as education and fish advisories have not reached the majority of women in the counties surrounding Pensacola, FL, where fish consumption is high and women are at risk from consumption of fish with high levels of MeHg.

The Harvard Center for Risk Analysis convened an expert panel to evaluate the potential public health impact of fish advisories on fish consumption patterns (Cohen et al. 2005). The advisories considered by the panel included those aimed at reducing fish consumption among pregnant women, with consideration given to the possibility that the advisories influence fish consumption in a manner not intended by the advisory. Examples of non-intended effects include the changing of fish consumption patterns among individuals not belonging to the group targeted by the advisory. Cohen et al. (2005) quantified the impact of changes in fish consumption on MeHg exposure, omega-3 PUFA intake, and the average number of fish servings consumed per week. They concluded that following advisories by substituting fish with low MeHg concentrations for fish with high MeHg concentrations among women of childbearing age yields substantial developmental benefits and few negative impacts. But if women instead decrease or stop their consumption of fish, resultant risks substantially reduce those benefits. In addition, if the advisories result in non-targeted adults also reducing fish consumption, the net impact on public health is negative. Cohen et al. cautioned that while high compliance with advisories recommending changes in fish consumption patterns among the target population can improve public health, unintended shifts in consumption by the overall population can lead to public health losses.

Hair and Blood levels in Humans. Walker et al. (2006) enrolled 523 volunteer women from the Northwest Territories and Nunavut in Canada to participate in a study to examine maternal blood and umbilical cord blood for mercury. The volunteers consisted of Inuits, Dene/Metis, Caucasians (n=134), and other non-aboriginal females from five regions of Arctic Canada. From these, 386 maternal blood samples, 407 cord blood samples, and 351 paired cord-maternal blood samples were collected. Total mean (arithmetic) mercury levels (μg/L) in maternal blood were 0.87 (SD 0.91) for Caucasians,

1.72 (SD 1.16) for Dene/Metis, 5.41 (SD 5.38) for Inuits, and 1.75 (SD 1.20) for other women. [Corresponding geometric mean maternal blood Hg levels were 0.87 (SD 1.95), 1.35 (SD 1.60), 3.51 (SD 8.30), and 1.30 (SD 2.14), respectively.] For cord blood total Hg, arithmetic mean values were 1.77 (SD 1.48) for Caucasians, 2.19 (SD 1.78) for Dene/Metis, 10.96 (SD 11.16) for Inuits, and 2.01 (SD 4.29) for other women. [Corresponding geometric mean cord blood Hg levels were 1.22 (SD 2.80), 1.62 (SD 2.29), 6.96 (SD 15.64), and 2.01 (SD 4.29), respectively.] In all cases, the mean values of the Inuits were significantly higher ($p<0.0001$) than corresponding values of the other groups. Three percent of the participating Inuit women had maternal blood MeHg levels in Health Canada's range of concern (20-99 μg/L), and 56% of the Inuit cord blood samples exceeded the blood levels corresponding to U.S. EPA's RfD for MeHg (Walker et al., 2006).

CDC (2009) reported the results of National Health and Nutrition Examination Survey (NHANES) for the years 2003 and 2004. Total blood mercury levels, but not MeHg blood levels, were reported for the two survey years. Since 90-95% of total blood mercury is generally considered to be MeHg, total blood mercury is considered to be a fairly reliable indicator of blood MeHg.

CDC (2009) reported that over the NHANES 1999-2006 survey periods, the geometric mean levels of total blood mercury did not (significantly) change for females aged 16-49 years. Geometric mean values for total blood mercury in women 16-49 years of age were 1.02 μg/L for the 1999-2000 sampling period and 0.833 μg/L for the 2001-2002 sampling period. The corresponding 50^{th} and 95^{th} percentile values were 0.900 μg/L and 7.10 μg/L, respectively for 1999-2000 and 0.700 μg/L and 4.60 μg/L, respectively for 2001-2002. For the 2003-2004 sampling period, the geometric mean total blood mercury value was 0.979 for women 20 years of age and older. The corresponding 50^{th} and 95^{th} percentile values were 1.00 and 5.40, respectively. Total blood mercury values were not reported for the 2005-2006 sampling period.

CDC (2009) did report that, although the geometric mean total blood levels did not change from 1999-2006, non-Hispanic black females had higher levels than non-Hispanic white or Mexican American females. A full report on the NHANES testing data can be found in CDC (2009).

6. ANALYTICAL METHODS

The USGS Mercury Research Laboratory (MRL) has developed an additional method of MeHg analysis (USGS, 2010). The USGS National Water Information System (NWIS) code for this method is P50285, MRL code FMHG. This procedure utilizes gas chromatography with cold vapor atomic fluorescence using filtered and unfiltered water. MeHg filtration methods include: (1) manual MeHg - no isotope dilution; (2) manual MeHg by ICPMS isotope dilution; and (3) Brooks-Rand "MERX" by ICPMS isotope dilution. The detection limit for MeHg using this method is 0.04 ng/L.

7. REGULATIONS AND ADVISORIES

No updated data.

8. REFERENCES

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