Effects of Prenatal and Postnatal Methylmercury Exposure From Fish Consumption on Neurodevelopment Outcomes at 66 Months of Age in the Seychelles Child Development Study

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Context.—Human neurodevelopmental consequences of exposure to methylmercury (MeHg) from eating fish remain a question of public health concern.

Objective.—To study the association between MeHg exposure and the developmental outcomes of children in the Republic of Seychelles at 66 months of age.

Design.—A prospective longitudinal cohort study.

Participants.—A total of 711 of 779 cohort mother-child pairs initially enrolled in the Seychelles Child Development Study in 1989.

Setting.—The Republic of Seychelles, an archipelago in the Indian Ocean where 85% of the population consumes ocean fish daily.

Main Outcome Measures.—Prenatal and postnatal MeHg exposure and 6 age-appropriate neurodevelopmental tests: the McCarthy Scales of Children’s Abilities, the Preschool Language Scale, the Woodcock-Johnson Applied Problems and Letter and Word Recognition Tests of Achievement, the Bender Gestalt test, and the Child Behavior Checklist.

Results.—The mean maternal hair total mercury level was 6.8 ppm and the mean child hair total mercury level at age 66 months was 6.5 ppm. No adverse outcomes at 66 months were associated with either prenatal or postnatal MeHg exposure.

Conclusion.—In the population studied, consumption of a diet high in ocean fish appears to pose no threat to developmental outcomes through 66 months of age.

INORGANIC MERCURY (Hg) discharged into lakes, rivers, and oceans is converted to methylmercury (MeHg) by microorganisms and bioaccumulated up the aquatic food chain.1 Concern about the potential public health threat from MeHg arose in the United States in the early 1970s when elevated concentrations were found in fish in the Great Lakes. Today, recreational fishing is restricted in many states and Food and Drug Administration guidelines regulate interstate commerce of fish because of their MeHg content.
clinical cases of MeHg poisoning. The possibility that prenatal MeHg exposure from maternal consumption of a fish diet may be associated with subtle changes in children’s cognitive and neurological development has been examined in these studies with inconclusive results.\(^\text{13,14}\)

We have followed longitudinally\(^\text{2,3}\) a large inception cohort of mother-child pairs in the Republic of Seychelles, a westernized archipelago in the middle of the Indian Ocean where 85% of the population consumes marine fish daily.\(^\text{14}\) This article presents the results of the neu- rodevelopmental examination of the Seychelles Child Development Study (SCDS) cohort at 66 months, an age at which neuropsychological tests may be given that are sensitive enough to assess potential associations between developmental outcomes and MeHg dietary exposure. Our results also include examination of the role of postnatal exposure from fish consumption.

**METHODS**

**Subjects**

The cohort consisted of 711 mother-child pairs living in the Republic of Seychelles, representing 91% of the 779 pairs originally enrolled in the SCDS main study.\(^\text{11}\) Informed consent was obtained from the caregiver of every participating child. The protocol was approved by human subjects review boards at the University of Rochester, Rochester, NY, and the Ministry of Health, Victoria, Mahé, Republic of Seychelles, before enrollment began. The sample size was sufficient to detect a 5.7-point difference on any test with a mean (SD) of 100 (16) between low (0-3 ppm) and high (>12 ppm) MeHg groups for a 2-sided test (\(a = .05\) at 80% power).

Twenty-eight mother-child pairs were excluded because of medical problems that might seriously affect development.\(^\text{15}\) An additional 16 pairs had insufficient maternal hair available to accurately recapitulate prenatal exposure, and 24 did not return for testing at 66 months.

Demographic characteristics of the Seychelles and the cohort were reported earlier.\(^\text{16}\) At enrollment, the mothers reported eating an average of approximately 12 marine fish meals per week. Sea mammals are not consumed in the Seychelles. We have previously documented that lead levels in whole blood are less than 0.48 \(\mu\)g/dL (10 \(\mu\)g/dL) in a representative group of Seychellois children and mothers.\(^\text{16}\)

**Procedure Test Battery**

Each child was evaluated at 66 months (±6 months) at a child development center staffed by a team of specially trained Seychellois nurses blinded to MeHg levels and the results of testing during previous visits. Five children were tested between 72 months and 79 months of age. All evaluations were conducted between July 1994 and October 1995. The test battery assessed multiple developmental domains\(^\text{17}\) and was similar to those used to demonstrate adverse developmental effects of exposure to lead\(^\text{18}\) and polychlorinated biphenyls (PCBs)\(^\text{19}\) and to those used in earlier studies to measure MeHg exposure effects.\(^\text{20,21}\) The tests are sufficiently sensitive and accurate to detect neurotoxicity in the presence of a number of confounding factors.\(^\text{21}\)

The test battery included the following 6 primary measures: (1) the General Cognitive Index (GCI) of the McCarthy Scales of Children’s Abilities\(^\text{22}\) to estimate cognitive ability; (2) the Preschool Language Scale\(^\text{23}\) (PLS) total score to measure both expressive and receptive language ability; (3) the Letter and Word Recognition and (4) the Applied Problems subtests of the Woodcock-Johnson (W-J) Tests of Achievement\(^\text{24}\) to measure reading and arithmetic achievement; (5) the Bender Gestalt test\(^\text{25}\) to measure visual-spatial ability; and (6) the total T score from the Child Behavior Checklist (CBCL)\(^\text{26}\) to measure the child’s social and adaptive behavior. The CBCL questionnaire was completed by each child’s primary caregiver. All tests were given in Creole, the language spoken by 98% of Seychellois at home.

Pure tone hearing thresholds were tested using a portable audiometer. Caregiver IQ was determined using the Raven Standard Progressive Matrices, a non-verbal test designed to minimize the effects of culture on measurement of IQ.\(^\text{27}\) When the children were between 42 and 56 months of age, the Home Observation for Measurement of the Environment (HOME) Inventory for Families of Preschool Age Children\(^\text{28}\) was administered during home visits. Following procedures described earlier,\(^\text{17}\) on-site test administration reliability was assessed by an independent scorer; percentage of disagreement ranged from 0% to 8%. Mean intraclass correlations for interrater reliability were 0.96 to 0.97. Reliability of final scoring in Rochester was conducted by rescore a sample of tests; the mean intraclass correlation coefficient was 0.96.

**Mercury in Fish**

Total Hg was analyzed in 5 or more samples of species of fish caught and consumed in the Seychelles, including yellow fin tuna (Thunnus albacares), Indian mackerel (Rastrelliger kanagura), brown spotted grouper (Epinephelus chlorostigma), green jobfish (Aprion virens), bonito (Euthynnus affinis), blugher (Carangoides gymnostethus), and spangled emperor (Lethrinus nebulosus).

**Maternal and Child Mercury Analysis**

The analysis of hair samples and fish homogenates for THg and inorganic Hg was done by cold vapor atomic absorption spectrometry. The analysis technique and quality control procedure are given elsewhere.\(^\text{16}\)

**PCBs in Blood**

Polychlorinated biphenyls are known to be present in some ocean fish and may be associated with developmental delays in children. Levels of PCBs in serum of 49 of the children at 66 months of age were analyzed at the laboratories of the US Centers for Disease Control and Prevention, Atlanta, Ga. The analytic method for measuring the PCBs involved deproteinization of the serum with formic acid, elution through a column containing octadecyl (C18) packing material, elution through a column containing Florisil packing material, concentration of the organic eluents, and analysis by dual capillary column–gas chromatography with electron capture detection.\(^\text{30}\)
Statistical Analysis

The effect of prenatal and postnatal MeHg on each outcome variable was adjusted for covariates, specified as part of the study design, and selected because of their potential to bias the assessment of the association between Hg and outcome.17 Covariates associated with the child included birth weight, birth order, sex, history of breast-feeding, hearing status, and the child’s medical history. Covariates associated with the mother and family included maternal age, maternal smoking during pregnancy, maternal alcohol consumption during pregnancy, maternal medical history, caregiver intelligence, language spoken in the home, Hollingshead socioeconomic status (SES), and HOME score. Two multiple linear regression analyses (with 2-tailed significance tests using a significance level of P<.05) including both prenatal and postnatal THg were performed for each of the 6 primary measures. The first involved all covariates (full model) and the second included only covariates believed to most likely influence child development in the Seychelles (reduced model), including sex, birth weight, child’s medical history, maternal age, HOME score, caregiver IQ, SES, and hearing status. Each full and reduced model was run both with and without THg by sex interaction tests to test the hypothesis that males and females have different THg slopes.

All models were examined for statistical outliers and influential points.31 Each model was run first with outliers, then without outliers, and the results were compared. All of the results were essentially the same with or without outliers. The regression analyses for all 6 primary measures were also repeated without influential points to determine whether the original results were dependent on such points.31 The final analysis included influential points that were not also outliers. The results without influential points were consistent with the original analysis.

Secondary analyses tested the hypothesis that associations between developmental outcomes and THg exposure might be nonlinear. All regression analyses were repeated, first using the log of the prenatal and postnatal THg values, then classifying THg variables into 5 groups each for prenatal and postnatal exposure.

RESULTS

Mercury in Fish

A total of 350 samples of fish were analyzed. The median THg for each of the 25 species ranged from 0.004 ppm to 0.75 ppm, with most medians in the range of 0.05 to 0.25 ppm. These levels are comparable with fish in the US market. The lowest levels occurred in reef fish. Methylmercury accounted for over 90% of the THg in 34 fish homogenates analyzed by gas chromatography–atomic fluorescent detection. This finding confirms many previous observations.32

PCBs in Blood

Twenty-eight PCB congeners ranging from congener 28 to 206 were measured in each serum sample. All samples had no detectable levels of any PCB congeners. The detection limit for the PCB analysis was 0.2 ng/mL. These results are typical for persons with no known exposure to PCBs.31

Mercury Exposure

The mean (SD) maternal hair level of THg during pregnancy was 6.8 (4.5) ppm (n = 711), and the mean child hair level at 66 months was 6.5 (3.3) ppm (n = 708). The ranges (maternal hair, 0.5-26.7 ppm; child hair; 0.9-25.5 ppm) were sufficient to test for exposure effects using THg analysis. Maternal and child THg concentrations were not highly associated Pearson r = 0.15, n = 708, P<.001) as observed by others.20 The exposure levels found in the Seychelles are typical of populations that depend on fish as a major dietary source of protein and calories.34

Test Performance

Tables 1 and 2 show test score means and SDs for prenatal and postnatal exposure levels. These results are similar to what would be expected from a healthy, well-developing US population. No test indicated a deleterious effect of MeHg exposure. Four of the 6 measures showed better scores in the highest MeHg groups compared with lower groups for both prenatal and postnatal exposure.

Regression Analyses

Primary Analyses.—The models with all covariates (full models) and limited covariates (reduced models) were both significant (ie, each model was able to describe the data) and yielded similar results for every measure. The THg by sex interaction test for prenatal exposure was not significant in any regression model. The THg by sex interaction was significant for postnatal exposure for the Bender Gestalt test; hence, we report the results of the reduced model with both interaction terms included. For all

### Table 1.—Neurodevelopmental Test Means (SDs) by Prenatal Exposure Levels

<table>
<thead>
<tr>
<th>Test</th>
<th>Subgroupings by Prenatal Total Mercury Exposure Level, Mean (SD), ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤3 ppm (Mean, 2.0) (n = 159)</td>
</tr>
<tr>
<td>McCarthy Scales of Children’s Abilities General Cognitive Index</td>
<td>94.0 (12.3)</td>
</tr>
<tr>
<td>Preschool Language Scale total score</td>
<td>69.6 (6.7)</td>
</tr>
<tr>
<td>Bender Gestalt errors</td>
<td>10.2 (3.9)</td>
</tr>
<tr>
<td>Woodcock-Johnson Tests of Achievement Letter and Word Recognition</td>
<td>76.1 (10.8)</td>
</tr>
<tr>
<td>Applied Problems</td>
<td>85.6 (17.2)</td>
</tr>
<tr>
<td>Child Behavior Checklist total T score</td>
<td>60.4 (9.7)</td>
</tr>
</tbody>
</table>

*N = 711.
†The Preschool Language Scale raw scores were used since the test and its norms were based on English.
‡The Koppitz scoring method was used. The score represents the number of indicator errors.
§The Child Behavior Checklist yields a percentile (T) score. The threshold for abnormality is beyond the 75th percentile.

### Table 2.—Neurodevelopmental Test Means (SDs) by Postnatal Exposure Level

<table>
<thead>
<tr>
<th>Test</th>
<th>Subgroupings by Postnatal Total Mercury Exposure Level, Mean (SD), ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤3 ppm (Mean, 2.2) (n = 73)</td>
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<tr>
<td>McCarthy Scales of Children’s Abilities General Cognitive Index</td>
<td>90.6 (14.3)</td>
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<tr>
<td>Preschool Language Scale total score</td>
<td>68.8 (6.8)</td>
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<tr>
<td>Bender Gestalt errors‡</td>
<td>10.4 (4.0)</td>
</tr>
<tr>
<td>Woodcock-Johnson Tests of Achievement Letter and Word Recognition</td>
<td>74.7 (11.0)</td>
</tr>
<tr>
<td>Applied Problems</td>
<td>85.6 (18.1)</td>
</tr>
<tr>
<td>Child Behavior Checklist total T score§</td>
<td>57.6 (9.4)</td>
</tr>
</tbody>
</table>

*N = 708.
†The Preschool Language Scale raw scores were used since the test and its norms were based on English.
‡The Koppitz scoring method was used. The score represents the number of indicator errors.
§The Child Behavior Checklist yields a percentile (T) score. The threshold for abnormality is beyond the 75th percentile.

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other analyses, we report the results for reduced models without THg by sex interactions.

The regression coefficients for all variables in the 6 sets of analyses are shown in Table 3. Figure 1 shows partial residual plots (end points adjusted for covariates) for prenatal and postnatal exposure for the McCarthy GCI, the PLS total score, and the W-J Applied Problems test score. For the McCarthy GCI analysis, the model (F [15, 628] = 4.41, P < .001, R² = 0.10) indicated that slopes for both THg exposures did not differ from 0 (P = .59 and .06 for prenatal and postnatal exposure, respectively). For the PLS analysis, the model (F [15, 590] = 6.25, P < .001, R² = 0.14) showed effects of both prenatal and postnatal THg exposure (P = .02 for both), but the effects were very small and in a direction of enhanced performance. The total increase in scores across the entire range of THg exposures was less than 4.5 points. The W-J Applied Problems model (F [15, 625] = 5.38, P < .001, R² = 0.11) indicated that the slope for prenatal exposure did not differ significantly from 0 (P = .41). There was a significant beneficial postnatal exposure effect (P = .05), but no evidence for an adverse effect. The postnatal exposure slope for the W-J Applied Problems test was 0.36 ppm, representing a 9.7-point increase over the full exposure range, or a 10% improvement in performance.

Figure 2 shows partial residual plots for the Bender Gestalt test, separating male from female subjects. The model (F [17, 613] = 4.53, P < .001, R² = 0.11) showed no significant association with prenatal exposure. The interaction of postnatal THg with sex was significant (P = .004). The slightly positive slope for female errors was not significant (P = .14). The regression coefficient of −0.16 ppm for male subjects was significant (P = .009), resulting in a reduction of 4.3 errors over the entire exposure range (a 40% performance improvement given that the average score for the lowest-exposure group was about 10 errors).

Although the models for the W-J Letter and Word Recognition achievement score and the CBCL yielded significant overall F statistics, none of these was significantly associated with either prenatal or postnatal exposure.

The data for covariate effects shown in Table 3 indicate that test scores were frequently influenced by sex (female subjects scored higher than male subjects), and were directly related to SES, quality of home stimulation, and caregiver IQ, as would be expected in westernized cultures. These data also indicate that performance by Seychellois children on these tests was similar to what would be expected of US children. The slopes provided for the difference between each level of the covariate and the reference (highest) level. Indications of statistical significance are provided for these individual effects as well as for overall effects.
that the effects are not statistically significant.

Secondary Analysis.—Log transformations of the 2 THg variables did not alter the direction of any effects. In the categorical analyses, test scores for children with an MeHg exposure level greater than 12 ppm were not significantly different than for children with exposure levels of 3 ppm or less.

COMMENT

Results from this study are relevant for the United States and other countries with similar dietary intake of fish. The major source of MeHg in the Seychelles is ocean fish and the average fish levels are similar to those on the US market. Seychellois MeHg levels are 10 to 20 times higher than in the United States because the Seychellois consume more fish, not because they eat a few fish with abnormally high MeHg levels. Thus, any potential adverse effects of MeHg in fish should be detected in the Seychelles long before such effects would be seen in the United States. Our findings confirm our earlier report in which no adverse developmental effects were found in toddlers following prenatal MeHg exposure.12 Our data extend preliminary results from the SCDS Pilot Study,9 involving a less statistically powerful, less well-controlled developmental evaluation of 217 Seychellois children at 66 months using most of the same measures but lacking many of the covariates used here.

We applied multiple regression analysis to our data as has been done in other studies on the effects of mercury, lead, or PCBs on child development. Although our models showed no negative associations between MeHg and outcome scores, our test procedures did detect other factors known to be associated with child development. The HOME test indicated that the quality of the home environment had a substantial impact on child performance, significantly affecting the results of all tests. The SES of the family, the caregiver’s IQ, and the child’s sex were all found to have an influence on performance scores, in keeping with the literature on child development. These results increase confidence in the sensitivity of our tests to child development functions. Postnatal exposure to MeHg at 66 months of age was associated with a small but statistically significant increase on several developmental outcomes. Our hypothesis did not predict positive effects, since there are no reasons to suppose that such effects are associated with exposure to MeHg. However, MeHg levels in hair are known to correlate closely with fish intake, and other factors or agents associated with fish, such as omega-3 fatty acids, may have beneficial effects. A large cohort study under way in the Faroe Islands found enhancement of developmental milestones in suckling infants exposed to MeHg in breast milk. They suggested that MeHg levels in the infant were a surrogate for the length of breast-feeding, which is reported to have a positive association with developmental outcomes.

In contrast with the conclusions from this and our earlier studies of the main Seychellois cohort,12,14 the Faroe Islands study found evidence of cognitive deficits associated with prenatal exposure to THg when children were tested at 7
Important differences between the two populations may explain the divergent outcomes (eg, nutritional practices, housing, and lifestyle). However, the major difference between our study and the Faroe Islands study is the source of exposure. Ocean fish are the source of MeHg in the Seychelles, whereas pilot whales are the predominant source in the Faroe Islands. The average MeHg level in the meat of pilot whales sampled in the Faroe Islands was 1.6 ppm, approximately 10 times higher than the average level in fish consumed in the Seychelles. Approximately the same level of inorganic Hg is also present in whale meat. In addition, whale blubber is also consumed by the Faroese and is heavily contaminated with fat-soluble pollutants. The average PCB concentration in pilot whale blubber from Faroese waters is elevated (about 30 ppm). In general, fatty tissues of marine mammals in the North Atlantic also contain elevated levels of persistent organochlorine compounds including dibenzofurans and dioxins, DDT and its metabolites, and other pesticides. It is difficult to determine the relative toxicological impact of individual compounds. Some of these contaminants are believed to affect child development. The Faroese study may be relevant to populations consuming large, perhaps episodic, amounts of marine mammals, but its relevance to people consuming ocean fish remains to be established.

A Swedish expert group conducted the first extensive evaluation of human health risks from MeHg in fish in 1971. They concluded the lowest toxic level in hair was 50 ppm in adults. The World Health Organization (WHO) expert group subsequently reaffirmed the Swedish conclusion and applied a safety factor of 10 to cover risks to the most sensitive subgroup of the population, assumed to be those who are prenatally exposed. Thus, 5 ppm in hair was adopted as the international standard for the upper tolerable level of Hg in hair. Subsequent epidemiological studies of human populations prenatally exposed to MeHg from fish have given strong support to the WHO guideline. Our results add further support to the validity of this long-standing guideline.

In summary, the results of extensive performance tests conducted with cohorts of children at 66 months of age strongly support our findings reported at younger ages. The development of these children is proceeding well without any detectable adverse influence of MeHg. Our results support Egeland and Middaugh’s observation that it would be inadvisable to forgo the health benefits of fish consumption to protect against a small risk of adverse effect at the levels of MeHg found in ocean fish on the US market.

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References


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