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Association between Methylmercury Exposure from Fish Consumption and Child Development at Five and a Half Years of Age in the Seychelles Child Development Study: An Evaluation of Nonlinear Relationships[1]

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Studies to date of the developmental effects of pre- and postnatal methylmercury exposure from fish consumption in the Seychelles Islands, using linear regression models for analysis, have not shown adverse effects on neurodevelopmental test scores. In this study we evaluated whether nonlinear effects of methylmercury exposure were present, using scores on six tests administered to cohort children in the Seychelles Child Development Study at 66 months of age. Prenatal exposure was determined by measuring mercury in a segment of maternal scalp hair representing growth during pregnancy. Postnatal exposure was measured in a segment of the child's hair taken at 66-months of age. Generalized additive models (GAMs), which make no assumptions about the functional form of the relationship between exposure and test score, were used in the analysis. GAMs similar to the original linear regression models were used to reanalyze the six primary developmental endpoints from the 66-month test battery. Small nonlinearities were identified in the relationships between prenatal exposure and the Preschool Language Scale (PLS) Total score and Child Behavior Check List (CBCL) and between postnatal exposure and the McCarthy General Cognitive Index (GCI) test scores. The effects are best described graphically but can be summarized by computing the change in the predicted test score from 0 to either 10 or 15 ppm and then above this point. For the PLS the trend involved a decline of 0.8 points between 0 and 10 ppm followed by an increase (representing improvement) of 1.3 points above 10 ppm. For the CBCL there was an increase of 1 point from 0 to 15 ppm, and then a decline (improvement) of 4 points above 15 ppm. The GCI increased by 1.8 points through 10 ppm and then declined 3.2 points (representing worse performance) above 10 ppm. These results are not entirely consistent. Two of the trends involve what appear to be beneficial effects of prenatal exposure. The one possibly adverse trend involves postnatal exposure. In every case the trend changes direction, so that an effect in one direction is followed by an effect in the opposite direction. Because of the descriptive nature of GAMs it is difficult to provide a precise level of statistical significance for the estimated trends. Certainly above 10 ppm there is less data and trends above this level are estimated less precisely. Overall there was no clear evidence for consistent (across the entire range of exposure levels) adverse effects of exposure on the six developmental outcomes. Further nonlinear modeling of these data may be appropriate, but there is also the risk of fitting complex models without a clear biological rationale.

Key Words: **child development; fish consumption; generalized additive models; methylmercury; nonlinear association.**

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INTRODUCTION

Methylmercury is a known neurotoxin, but it is still unclear if prenatal exposure at the levels achieved by consumption of ocean fish adversely affects child development. Epidemiologic studies have reported both associations (Marsh *et al.*, 1987; Cox *et al.*, 1989; Grandjean *et al.*, 1997; Myers *et al.*, 1995a, b*)* and lack of associations (Marsh *et al.*, 1995a; Davidson *et al.*, 1998) between child development and methylmercury exposure at the levels achieved by regular fish consumption.

The Seychelles Child Development Study (SCDS) is an ongoing longitudinal study of the effects of pre- and postnatal methylmercury (MeHg) exposure from a diet high in fish on child development. The study population is a cohort of over 700 children in the Republic of Seychelles. Prenatal exposure to mercury was measured in a segment of maternal hair corresponding to pregnancy. The children's neurological and developmental status has been evaluated at 6.5, 19, 29, and 66 months of age (Myers *et al.*, 1995b; Davidson *et al.*, 1998; Davidson *et al.*, 1995a) using standard methods of assessment. Postnatal exposure was measured in a scalp hair sample from each child at the time of the 66-month evaluation. This might better be termed concurrent exposure. Since we have used the term "postnatal exposure" in previous work we will continue to use it to avoid confusion. The relationships between pre- and postnatal exposure and child development were evaluated previously using multiple regression analysis. No definite adverse effects of mercury exposure on child development through age 66 months have been identified at the exposure levels represented in this cohort (Davidson *et al.*, 1998, 1995a; Myers *et al.*, 1995c, 1997).

The original multiple regression analysis of exposure and neurodevelopmental test scores at 66 months of age identified apparent beneficial effects, mostly small in magnitude, associated with increasing MeHg exposure in the low exposure range studied (Davidson *et al.*, 1998). Increasing prenatal MeHg exposure was associated with improvements in the Preschool Language Scale total score. Increasing postnatal exposure was associated with improvements in the General Cognitive Index from the McCarthy Scales of Children's Abilities and the Applied Problems subtest from the Woodcock Johnson Tests of Achievement, and a decrease in errors for males on the Bender Gestalt test. One possible explanation for these associations is that mercury exposure is confounded with fish consumption, and mercury level may in fact be a surrogate measure of nutrition. On the other hand, what appears as an increasing linear relationship may in fact be produced by a nonlinear trend that, for example, declines after an initial increase. Nonlinear relationships between environmental toxins and biological parameters have been described previously (Davis and Svendsgaard, 1990), although previous studies of the association between pre- and postnatal mercury exposure and child development have not looked carefully at this question. Nonlinear effects were examined in one previous SCDS analysis of the association between prenatal exposure and age at achieving developmental milestones (Axtell *et al.*, 1998). A possible nonlinear relationship between prenatal exposure and age at first walking independently was identified, but a consistent, dose–response relationship with mercury exposure could not be demonstrated.

The primary goal of this study was to analyze data on the six primary developmental measures from the 66-month test battery to determine whether nonlinear relationships were present between mercury exposure and the developmental outcomes. The multiple regression model that was used in the primary analyses assumes a linear association between exposure and outcome, and fits a straight line to the data to describe this relationship. While this parametric multiple regression analysis has advantages if the underlying trends are linear, there are disadvantages if the assumption of linearity is incorrect. The advantage of the parametric analysis is that exact statistical tests are available for evaluating the strength of the evidence that the slope of the line describing the exposure/outcome relationship differs from zero (no effect). If the true exposure/outcome relationship is nonlinear, however, forcing a straight line fit to the data may lead to biased inference about the nature of the association. In this study, we therefore analyzed the data using models that make no assumptions about the form of the functional relationship between the continuous exposure variables and the outcome variables. Using generalized additive models (GAMs), the response variables were modeled as the sum of nonparametric terms that were smooth functions of the continuous independent variables and parametric terms that were linear functions of the categorical independent variables (Hastie and Tibshirani, 1990).

MATERIALS AND METHODS

Subjects

The cohort consisted of 779 children enrolled at 6.5 months of age. Of these, 711 were available for the 66-month test battery. Detailed descriptions of the cohort and exclusion criteria have been published elsewhere (Davidson *et al.*, 1998; Myers *et al.*, 1995c, 1997; Marsh *et al.*, 1995b; Shamlaye *et al.*, 1995). Informed consent was obtained for each child from their parent or guardian before the child participated in the study.

Exposure Assessment

Prenatal exposure was measured using the mean of the total mercury (THg) concentration in segments of maternal hair representing growth during pregnancy as discussed previously (Cernichiari *et al.*, 1995). THg was used as the measure of exposure because 80% of THg in hair samples collected from fish-eating populations is MeHg (Cernichiari *et al.*, 1995; Phelps *et al.*, 1980). THg in maternal hair correlates well with MeHg levels in maternal hair and blood (WHO, 1990). THg in each hair segment was measured by cold vapor atomic absorption (Cernichiari *et al.*, 1995). Postnatal exposure was measured in a 1-cm segment of the child's hair closest to the scalp at the time of the 66-month evaluation.

Developmental Assessment

A battery of developmental tests was given to each child. The battery was designed to provide a comprehensive assessment of cognitive, perceptual, motor, and language functions and scholastic readiness in typical 5-year old children. The battery yielded a total of six primary endpoints that were used both in our earlier primary analysis and in the present study. The endpoints were as follows: the General Cognitive Index (GCI) of the McCarthy Scales of Children's Abilities (McCarthy, 1972) to estimate cognitive ability; the Preschool Language Scale Total score (Zimmerman *et al.*, 1979) to measure receptive and expressive language; the Letter and Word Recognition and the Applied Problems subtests of the Woodcock–Johnson Tests of Achievement (Woodcock and Johnson, 1989) to document reading and math readiness; the total error score from the Bender Gestalt test (Koppitz, 1963) to estimate visual–spatial ability; and the total T score from the Child Behavior Checklist (CBCL) measuring social and adaptive behavior (Achenbach, 1991). The CBCL questionnaire was completed by each child's primary caregiver. For all tests except the Bender and CBCL, an increase in the score is associated with an improvement in performance on the test. The rationale for including these particular tests in the battery is discussed elsewhere (Davidson *et al.*, 1994, 1995b). Extensive pilot testing (Davidson *et al.*, 1998, 1994, 1995b) indicated that the tests performed as they would with U.S. children. All tests were given in Creole, the language spoken in over 98% of Seychellois homes.

Statistical Analyses

The relationships between pre- and postnatal THg exposure and the six primary developmental measures at the 66-month evaluation, adjusted for covariates, were evaluated using GAMs. Each developmental outcome was modeled as the sum of terms that were linear functions of categorical independent variables and terms that were smoothed functions of the continuous independent variables. This model makes no assumptions about the functional form of the relationship between the dependent variable and THg or other continuous independent variables. It does assume, however, that the effects of the various independent variables are additive. This makes it possible to look at the relationship between the outcome and one independent variable at a time, while controlling for the other independent variables. The error terms for the parametric portion of the model were assumed to be normally distributed, with constant variance.

Full and reduced models without interaction terms, specified *a priori*, were fit for each developmental outcome except the Bender error score. Both prenatal and postnatal exposure were included in all models. Separate models were fit for male and female for the Bender, and approximate smooths were also generated from a model with THg by gender interaction terms, because the postnatal exposure by gender interaction term was significant in the original multiple regression analysis (Davidson *et al.*, 1998). All observations without missing values were included in each analysis.

The full model included the following independent variables: maternal and child hair mercury levels, gender, birth weight, birth order, history of breast feeding, the child's medical history, and the child's hearing score. The child's medical history was defined as positive if a diagnosis of intrauterine growth retardation was made at birth or if the child's head circumference was more than 2 standard deviations above or below the Seychellois mean at any age. Covariates associated with the child's environment included a measure of the child's home environment (the Caldwell–Bradley preschool version of the Home Observation for Measurement of the Environment (HOME) (Caldwell and Bradley, 1984)), and the Hollingshead measure of socioeconomic status (SES). Finally, covariates measuring characteristics of the mother included maternal age, maternal medical history, caregiver intelligence (Raven, 1958), and history of maternal cigarette smoking or alcohol use during pregnancy (yes/no).

The reduced model included the subset of these covariates thought to be most important in the Seychelles. The covariates in the reduced model were the child's gender, birth weight, hearing status, and medical history, the mother's age and intelligence category, the HOME score, and the Hollingshead score of SES. For both the full and reduced models, the HOME, Hollingshead, maternal intelligence, and child hearing scores were categorized before they were included in the regression models.

Two types of approximate *F* tests were used to evaluate the significance of the smoothed terms in the model (Hastie and Tibshirani, 1990). An approximate *F* test for nonlinearity was used to determine whether including the nonlinear component of each smooth term in the model resulted in a significantly better fit than assuming a linear relationship. A second type of approximate *F* test was used to estimate the overall significance of the THg smooth in each model by comparing models with and without the smoothed THg term. Although the test statistics calculated from GAM models do not have exact or even asymptotic *F* distributions, Hastie and Tibshirani (1990) report that simulations show them to be useful approximations.

The S-plus software package was used to fit the GAM models (Statistical Sciences, 1993). Smoothing splines with 4 and 2 degrees of freedom (df) were used to compute the smooth term for each continuous predictor variable (Statistical Sciences, 1993; Chambers and Hastie, 1993). More parsimonious models with 2 df for each smoothed term were also estimated and these results were compared with those for the 4-df models. In toxicology, the most common functional form is a monotonic relationship (nondecreasing or nonincreasing, depending on the outcome). From this point of view, a smooth for THg with 2 df may be more interpretable than smooths with higher degrees of freedom, which are more likely to follow local trends in the data and therefore to show some form of oscillation.

For each model, the estimated association between each continuous predictor and the response variable was examined graphically. Each plot describes the contribution of the particular independent variable to the additive predictor for the developmental outcome, and has been centered to have a mean of zero. The points are partial deviance residuals for the independent variable (scaled values of the dependent variable adjusted for all of the independent variables, continuous and categorical, except the one of interest for the plot) (Statistical Sciences, 1993; Chambers and Hastie, 1993). Nonsimultaneous, approximate confidence bands (plus/minus two times the standard error) were included in the smoothed plots for the continuous independent variables. The rug plot (vertical marks) along the bottom of each graph illustrates the distribution of the values of the independent variable in the data set.

TABLE 1

Neurodevelopmental Test Score Means (Standard Deviations) by Pre- and Postnatal Total Mercury Category[a]

	Prenatal total mercury exposure level in ppm (*n*)					Postnatal total mercury exposure level in ppm (*n*)				
Developmental test	0.5–3 (159)	4–6 (206)	7–9 (156)	10–12 (95)	> 12–26.7 (95)	0.8–3 (73)	4–6 (299)	7–9 (213)	10–12 (76)	> 12–25.8 (47)
McCarthy GCI[b]	94.0(12.3)	93.8(13.1)	94.3(13.8)	92.4(11.6)	95.9(12.6)	90.6(14.3)	94.0(12.8)	94.7(12.5)	95.8(14.1)	93.0(10.5)
PLS Total Langauge[b,c]	69.6(6.7)	69.6(6.7)	69.6(6.8)	70.2(6.3)	72.1(6.6)	68.8(6.8)	69.6(6.7)	70.7(6.5)	70.6(7.8)	70.1(4.8)
Bender errors[d]	10.2(3.9)	10.4(3.7)	10.0(4.1)	10.5(3.7)	9.4(3.9)	10.4(4.0)	10.2(3.9)	10.1(3.6)	10.0(4.4)	10.1(4.0)
Woodcock–Johnson[b]										
Letter–Word[b]	76.1(10.8)	77.6(11.1)	76.9(9.9)	76.1(9.9)	77.7(10.9)	74.7(11.0)	77.8(11.3)	76.7(9.5)	76.8(10.9)	75.6(9.0)
Applied problems[b]	85.6(17.2)	87.3(17.7)	87.3(17.5)	87.0(18.0)	90.1(17.9)	85.6(18.1)	86.7(17.6)	88.0(18.2)	90.0(16.0)	85.0(16.3)
CBCL Total T Score[b,e]	60.4(9.7)	59.7(10.5)	59.3(10.2)	59.3(9.8)	59.7(8.8)	47.6(9.4)	60.1(10.0)	60.2(10.7)	59.3(9.3)	59.5(7.6)

[a] $n = 711$ for prenatal THg results and $n = 708$ for postnatal total mercury results (child hair samples were not available for three children).

[b] McCarthy GCI, McCarthy Scales of Children's Abilities General Cognitive Index; PLS, Pre-School Language Scale; Woodcock–Johnson, Woodcock–Johnson Achievement Test; Letter–Word, Letter and Word Recognition; CBCL, Child Behavior Checklist.

[c] The PLS raw scores were used since the test and its norms were based on English.

[d] Koppitz scoring method was used. Score represents number of indicator errors.

[e] The CBCL yields a percentile (*T*) score. The threshold for abnormality is beyond the 75th percentile.

RESULTS

For descriptive purposes, the exposure variables were categorized into five groups each. The unadjusted means and standard deviations of the test scores in each exposure category are presented in Table 1 in order to give a general overview of the data. For each of the six tests, the mean score and the corresponding standard deviation are essentially unchanged across each of the mercury levels for both pre- and postnatal exposure. The stability of the standard deviations for each exposure category indicates that the scatter of the data is not changing with mercury levels. With the possible exception of the CBCL score in the lowest postnatal category, having a value of 47.6 as compared to 59–60 in the other four exposure categories, the unadjusted mean scores in Table 1 indicate the absence of any effect of THg. When the association between each test score and pre- and postnatal exposure was analyzed using

TABLE 2

Approximate *P* Values for Mercury Variables and for Nonlinearity for Continuous Variables from GAM Analyses of 66-Month Data for Full and Reduced Models without Interaction

Developmental test	Predictor variable	Full model[a] (df = 4)		Reduced model[b] (df = 4)		Full model (df = 2)		Reduced model (df = 2)	
		Nonlinearity	Overall	Nonlinearity	Overall	Nonlinearity	Overall	Nonlinearity	Overall
McCarthy GCI	*s*(prenatal THg)	0.54	0.72	0.44	0.61	0.34	0.64	0.27	0.53
	s(postnatal THg)	0.038	0.059	0.066	0.068	0.028	0.083	0.043	0.068
	s(birth weight)	0.17		0.16		0.18		0.15	
	s(maternal age)	0.20		0.097		0.31		0.19	
	s(birth order)	0.17				0.050			
PLS Total Language	*s*(prenatal THg)	0.10	0.023	0.075	0.018	0.061	0.011	0.046	0.010
	s(postnatal THg)	0.27	0.19	0.26	0.11	0.15	0.13	0.17	0.074
	s(birth weight)	0.057		0.11		0.085		0.13	
	s(maternal age)	0.38		0.21		0.43		0.29	
	s(birth order)	0.87				0.57			
Woodcock–Johnson Letter–Word	*s*(prenatal THg)	0.50	0.60	0.32	0.47	0.47	0.65	0.33	0.62
	s(postnatal THg)	0.10	0.44	0.11	0.45	0.17	0.73	0.17	0.71
	s(birth weight)	0.045		0.097		0.047		0.085	
	s(maternal age)	0.10		0.075		0.20		0.16	
	s(birth order)	0.10				0.023			
Woodcock–Johnson Applied Problems	*s*(prenatal THg)	0.57	0.54	0.63	0.68	0.59	0.48	0.59	0.65
	s(postnatal THg)	0.21	0.14	0.24	0.087	0.29	0.24	0.30	0.13
	s(birth weight)	0.031		0.021		0.10		0.080	
	s(maternal age)	0.58		0.57		0.47		0.46	
	s(birth order)	0.12				0.12			
CBCL Total T Score	*s*(prenatal THg)	0.051	0.076	0.036	0.057	0.13	0.21	0.10	0.19
	s(postnatal THg)	0.12	0.36	0.22	0.56	0.10	0.51	0.15	0.66
	s(birth weight)	0.65		0.63		0.43		0.45	
	s(maternal age)	0.17		0.16		0.35		0.29	
	s(birth order)	0.11				0.19			
Bender error with interaction	*s*(prenatal THg-males)	0.74	0.82	0.83	0.85	0.58	0.70	0.65	0.71
	s(prenatal THg- females)	0.32	0.18	0.25	0.15	0.19	0.10	0.14	0.083
	s(postnatal THg-males)	0.13	0.034	0.22	0.034	0.094	0.016	0.16	0.015
	s(postnatal THg-females)	0.80	0.62	0.79	0.68	0.52	0.30	0.56	0.40
	s(birth weight)	0.094		0.12		0.11		0.14	
	s(maternal age)	0.24		0.20		0.30		0.20	
	s(birth order)	0.28				0.23			

[a] The full model included the independent variables in the reduced model (see below) plus the following: birth order, history of breast feeding, whether the mother smoked or consumed alcohol regularly during the pregnancy, and maternal medical history.

[b] The reduced model included pre- and postnatal total mercury exposure, birth weight, birth order, maternal age, child's gender, child's medical history, HOME score category, Hollingshead score category, maternal intelligence category, and hearing score category.

multiple linear regression with adjustment for covariates, no adverse effect of THg could be detected (Davidson *et al.*, 1998), The analyses in this article seek to determine whether semiparametric nonlinear regression analysis, with appropriate adjustment for covariates, might detect an adverse influence of THg on the test scores.

Prenatal exposure. Among the models with 4-df smooths, there was some evidence for a nonlinear relationship between prenatal exposure to THg and PLS Total Language (P for nonlinearity = 0.10 and 0.075 for the full and reduced models, respectively) (Table 2). The nonlinear portion of this relationship became more significant when 2 df were allocated for the smoothed terms (P for nonlinearity = 0.061 for the full and 0.046 for the reduced model). The curves from the 4- and 2-df reduced models are illustrated in Fig. 1. As expected, the 4-df smooth shows more oscillation than the 2-df smooth, which is approximately linear and exhibits a modest upward trend. For the 2-df model, the PLS Total Language score declines by 0.8 as prenatal exposure increases from 1 to 10 ppm, and then increases by 1.3 as the exposure level increases above 10 ppm (Table 3). Prenatal exposure was a significant predictor of PLS Total Language (overall $P = 0.023$ and 0.018 for the full and reduced models with 4-df smooths; $P = 0.011$ and 0.010 for the full and reduced models with 2-df smooths) (Table 2). This is consistent with the result from the original multiple regression analysis where there was a significant but small positive coefficient on prenatal THg in the reduced model for PLS Total Language (coefficient = 0.13; $P = 0.02$) (Davidson *et al.*, 1998).

The nonlinear component of the estimated relationship between prenatal THg and the CBCL Total T score was significant (P for nonlinearity = 0.051 and 0.036 for the full and reduced models with 4 df for smoothed terms) (Table 2). When 2 df were allocated for the smooth terms, the nonlinear relationship between prenatal exposure and the CBCL became less significant (P for nonlinearity = 0.13 for the full and 0.10 for the reduced model). The fitted function from the 4-df model shows a 1-point increase in the CBCL as prenatal THg increases from one to 15 ppm, and then a decline (representing an improvement in score) of four points as prenatal exposure increases from 15 to 20 ppm (Fig. 2; Table 3). The pattern for the 2-df smooth is similar; most of the decrease occurs above 15 ppm, where the sparsity of the data makes the estimate less precise. In the original multiple regression analysis, there was a nonsignificant negative coefficient on prenatal THg in the model for CBCL (coefficient = − 0.11; $P = 0.22$) (Davidson *et al.*, 1998).

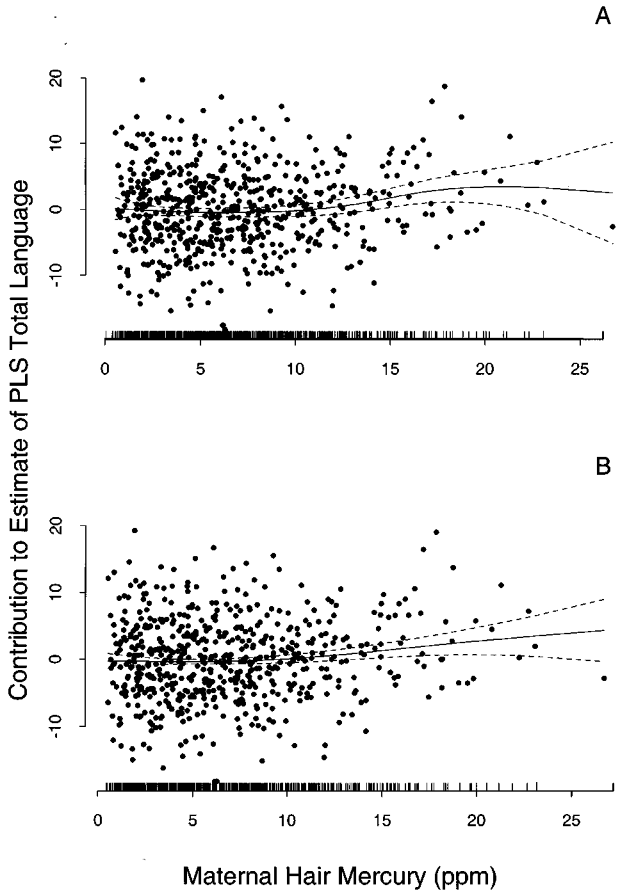

FIG. 1. Results from the reduced model with 4 (A) and 2 (B) df for smoothed terms for PLS Total Language. Estimated relationship (fitted smooth) between maternal hair THg level and the PLS Total Language score, adjusted for covariates. The dashed lines in this plot and those that follow are twice the point-wise standard error curves and the points are partial residuals for the THg variables, calculated by adding the estimated smoothed THg term back to the scaled residuals.

Prenatal exposure was marginally significant in the full and reduced models for the CBCL Total T score when 4 df were allocated for the smooths (overall $P = 0.076$ for the full and 0.057 for the reduced model), but not in the 2-df models ($P = 0.21$ and 0.19 for the full and reduced models, respectively). As in the original multiple regression analysis (Davidson *et al.*, 1998) prenatal exposure was not significant in any of the models for the other four endpoints (Table 2).

Postnatal exposure. There was evidence for a nonlinear relationship between postnatal exposure and the McCarthy GCI in the full and reduced models with 4- and 2-df smooths (P for nonlinearity = 0.038

TABLE 3

Change in Developmental Test Score Associated with an Increase in Maternal or Child Hair THg Level as Estimated from the Reduced Models without Total Mercury by Gender Interaction with Four and Two Degrees of Freedom for the Nonparametric Smoothed Terms

Increase in hair total mercury level (ppm)		Change in PLS total language associated with prenatal exposure		Change in CBCL total scale associated with prenatal exposure		Change in McCarthy GCI associated with postnatal exposure	
From	To	df[a] = 2	df = 4	df = 2	df = 4	df = 2	df = 4
1	3	− 0.3	− 0.6	+ 0.1	− 0.3	+ 0.8	+ 2.3
3	5	− 0.3	− 0.5	+ 0.1	+ 0.1	+ 0.7	+ 1.4
5	10	− 0.2	− 0.4	+ 0.3	+ 0.4	+ 0.3	+ 0.2
10	15	+ 0.6	+ 1.3	− 0.2	+ 0.8	− 1.4	− 3.0
15	20	+ 0.7	+ 1.0	− 1.3	− 4.0	− 1.8	− 2.5

[a] df, degress of freedom for each smoothed term in the model.

and 0.066 for the full and reduced models with 4 df; $P = 0.028$, and 0.043 for the full and reduced models with 2 df). There was no evidence for a nonlinear relationship with postnatal exposure for any of the other five endpoints (Table 2).

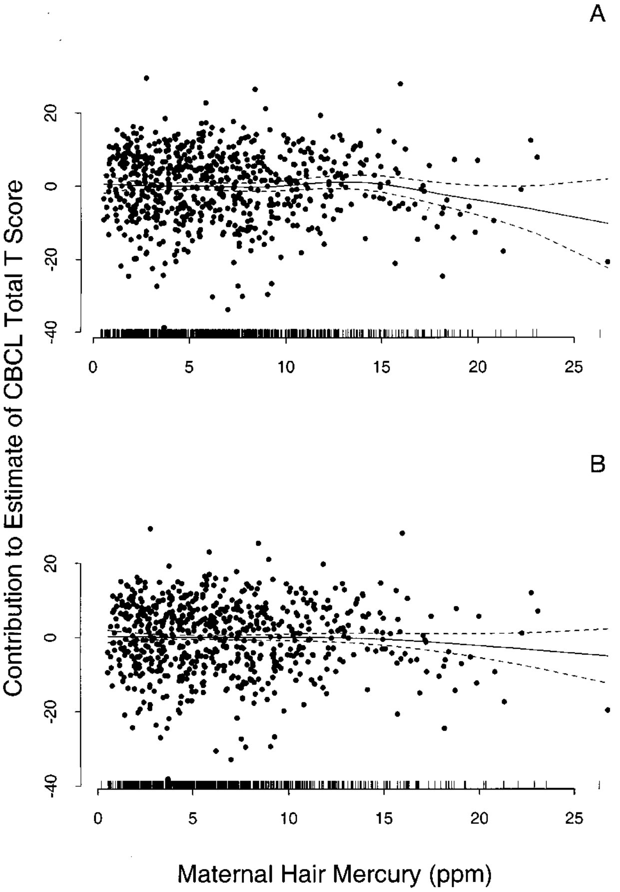

FIG. 2. Results from the reduced model with four (A) and two (B) degree of freedom the CBCL Total T score showing the fitted smooths, twice the point-wise standard error curves, and partial residuals for maternal hair THg.

The estimated smooth for postnatal exposure approached significance in the model for the GCI (overall $P = 0.059$ for the full and 0.068 for the reduced model with 4 df for smoothed terms). The results from the 2-df models were similar (overall $P = 0.083$ and 0.068 for the full and reduced 2-df models, respectively). The estimated curve from the 4-df reduced model shows an increase of 3.9 points in the GCI as postnatal THg increases from one to 10 ppm. After the 10 ppm exposure level there is a decline of 5.5 points as THg increases from 10 to 20 ppm, followed by a slight increase as exposure increases above 20 ppm (Fig. 3; Table 3). This oscillation is smoothed out in the curve from the 2-df model, where the curve increases by 1.8 points up through 10 ppm and then declines 3.2 points as postnatal exposure increases from 10 to 20 ppm (Fig. 3; Table 3). The downward trend was estimated with a greater degree of uncertainty as evidenced by the gradually increasing width of the confidence bands. The graphs of the curves in Fig. 3 do not provide very strong evidence for a consistent downward trend above 10 ppm. There was a small positive coefficient (+ 0.26) on postnatal THg level in the multiple regression analysis ($P = 0.06$) (Davidson *et al.*, 1998).

Covariates. Among the 4-df models, birth weight had a significant nonlinear component in the full model for the Woodcock–Johnson Letter–Word subscale (P for nonlinearity = 0.045) and in the full and reduced models for the Woodcock–Johnson Applied Problems subscale ($P = 0.031$ for the full and 0.021 for the reduced model). The nonlinear component of birth weight approached significance in the full

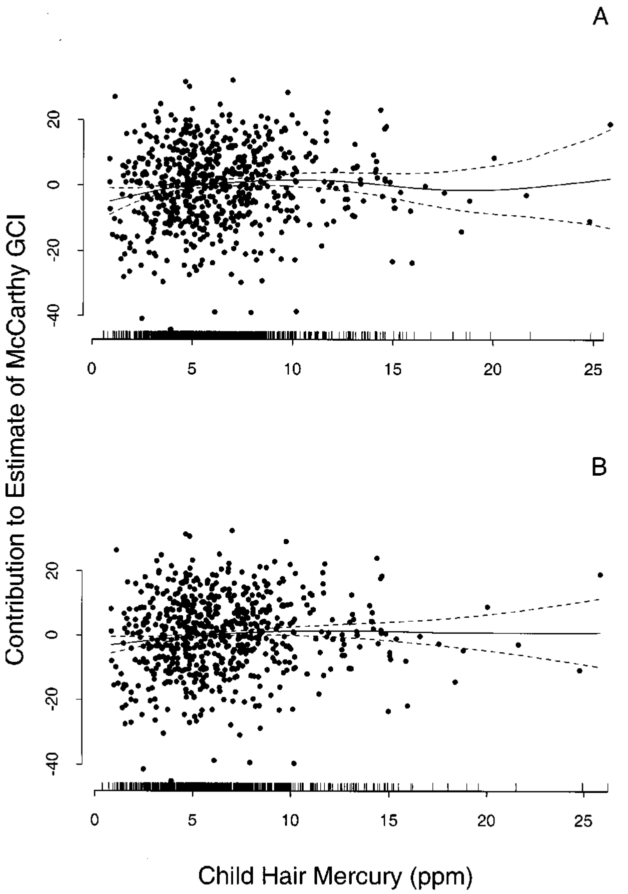

FIG. 3. Results from the reduced model with four (A) and two (B) degrees of freedom for the McCarthy GCI showing the fitted smooths, twice the point-wise standard error curves, and partial residuals for child hair THg.

model for PLS Total Language ($P = 0.057$), but not in the models for the GCI and CBCL (Table 2). When the full and reduced models were estimated with 2-df smooths, the nonlinear trend with birth weight persisted only in the full model for the Woodcock–Johnson Letter–Word subscale (P for nonlinearity $= 0.047$) (Table 2; Fig. 4).

There were no indications that the relationship between the other continuous covariates and any of the outcomes were nonlinear (Table 2). The graphical evaluations of the associations between the categorical covariates and the six outcomes showed increases in HOME score, Hollingshead SES score, and maternal intelligence to be associated with higher (lower for the Bender and CBCL) scores on all tests. Female gender was associated with slightly higher scores on the GCI, PLS, and Woodcock–Johnson subscales. These results are consistent with those from the original regression analysis (Davidson *et al.*, 1998).

DISCUSSION

We explored nonlinear relationships between methylmercury exposure from fish consumption and child development in order to evaluate whether the results from the original multiple linear regression analyses of these data were correct. We found evidence for nonlinear relationships with three of the six primary endpoints. Two of these suggested nonlinear associations with prenatal exposure, and one a nonlinear relationship associated with postnatal exposure, measured concurrently. These relationships can be seen most clearly in the plots of the smoothed curves (Figs. 1–3). The fitted function representing the effect of postnatal exposure on the GCI suggested increasing test scores with increasing postnatal exposure levels in the 0–10 ppm range, followed by declining scores in the 10–20 ppm range. However, the 4-df curve oscillates in an implausible way, and the decline in the 2-df curve in the region

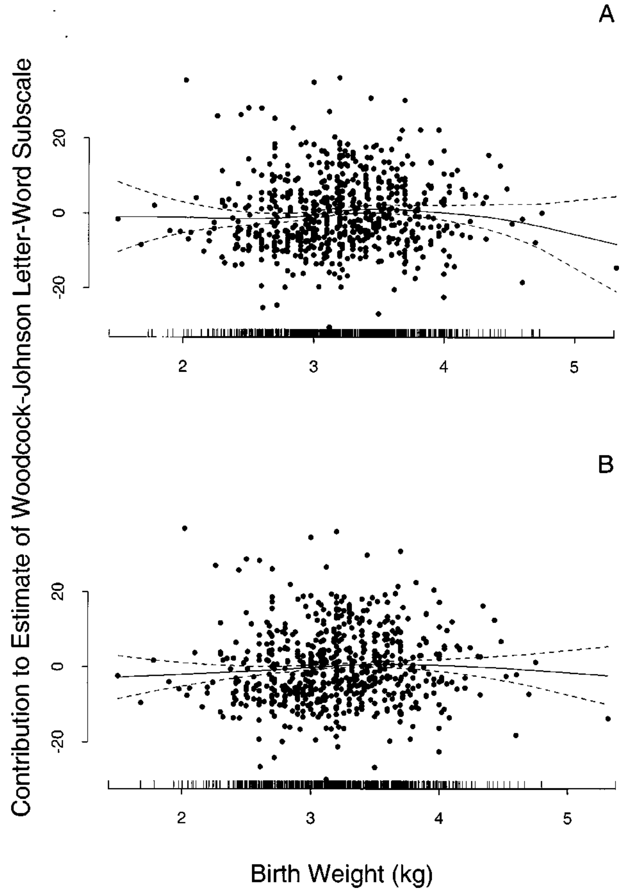

FIG. 4. Results from the full model with four (A) and two (B) degrees of freedom for the Woodcock–Johnson Letter–Word subscale showing the fitted smooths, twice the point-wise standard error curves, and partial residuals for birth weight.

beyond 10 ppm is estimated with less precision because of the smaller amount of data in this range. We also identified nonlinear relationships for the PLS Total Language and CBCL scores, but again, they were small in magnitude and did not change the conclusions of the original analysis. The departures from linearity in the pre- and postnatal MeHg curves for the three outcomes where nonlinearity was present were small, particularly in the 2-df models. In general, the models that allocated 4-df for smoothed terms were more likely to produce MeHg smooths that oscillated, while the 2-df models provided more plausible estimates of the relationship between the exposure variables and the developmental outcomes. Overall, our results indicate that linear models were reasonable for evaluating the effects of MeHg exposure.

Graphical evaluations of the associations between the categorical covariates and the six primary outcomes yielded results that were consistent with the original analysis. As would be expected, the HOME score, Hollingshead SES, and maternal intelligence were positively associated with improvements in test scores for all outcomes. Female gender was also associated with higher test scores on the GCI, PLS, and Woodcock–Johnson subscales, although the differences between the genders were small.

An important issue for the assessment of nonlinear trends in data from studies of the toxic effects of an environmental exposure such as methylmercury is the assumption that the true dose–effect relationship is monotone. This is the property that the curve never changes direction, that it moves consistently either up or down, depending on the particular test, with increasing exposure. A monotone function may be flat (or nearly so) over part of the range of exposure levels, but it never reverses direction; an increase in exposure from any point always has the same effect, although the magnitude may differ from one part of the curve to another. This is commonly the case in toxicology, and our original expectation was that any dose–effect relationships between methylmercury exposure and developmental test scores would be monotone. This assumption provides support for the use of conventional linear regression models for the analysis of data. We did not, however, employ a monotone smoother in our GAM models, since we wanted to let the data make this determination. This was especially important since the original analyses of these same endpoints had demonstrated apparent beneficial effects, which we hypothesized might be due to the association between exposure and the nutritional benefits of fish consumption. Of course if such beneficial effects exist, then they must eventually be overwhelmed by the toxic effects of methylmercury. This would lead to exactly the kind of non-monotone relationship we observed in the relationship between postnatal exposure and the McCarthy GCI. At this point it is tempting to employ further nonlinear modeling to better define such "U-shaped" relationships in our data. Such functional relationships have been observed previously in toxicology (see Davis and Svendsgaard, 1990, for a review). However, to really study the question of possible nutritional effects requires assessment of nutrition, which is beyond the scope of the present study.

APPENDIX 1

Seychelles Child Development Study Group

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