Prenatal methylmercury exposure from ocean fish consumption in the Seychelles child development study

Gary J Myers, Philip W Davidson, Christopher Cox, Conrad F Shamlaye, Donna Palumbo, Elsa Cernichiari, Jean Sloane-Reeves, Gregory E Wilding, James Kost, Li-Shan Huang, Thomas W Clarkson

Summary

Introduction Exposure to methylmercury (MeHg) before birth can adversely affect children's neurodevelopment. The most common form of prenatal exposure is maternal fish consumption, but whether such exposure harms the fetus is unknown. We aimed to identify adverse neurodevelopmental effects in a fish-consuming population.

Methods We investigated 779 mother-infant pairs residing in the Republic of Seychelles. Mothers reported consuming fish on average 12 meals per week. Fish in Seychelles contain much the same concentrations of MeHg as commercial ocean fish elsewhere. Prenatal MeHg exposure was determined from maternal hair growing during pregnancy. We assessed neurocognitive, language, memory, motor, perceptual-motor, and behavioural functions in children at age 9 years. The association between prenatal MeHg exposure and the primary endpoints was investigated with multiple linear regression with adjustment for covariates that affect child development.

Findings Mean prenatal MeHg exposure was 6·9 parts per million (SD 4·5ppm). Only two endpoints were associated with prenatal MeHg exposure. Increased exposure was associated with decreased performance in the grooved pegboard using the non-dominant hand in males and improved scores in the hyperactivity index of the Conner's teacher rating scale. Covariates affecting child development were appropriately associated with endpoints.

Interpretation These data do not support the hypothesis that there is a neurodevelopmental risk from prenatal MeHg exposure resulting solely from ocean fish consumption.

Lancet 2003; **361:** 1686–92
See Commentary page 1667

Department of Neurology (Prof G J Myers MD, D Palumbo PhD), **Department of Pediatrics** (Prof G J Myers, Prof P W Davidson PhD, J Sloane-Reeves MS), **and Division of Epidemiology, Statistics, and Prevention** (C Cox PhD), **National Institute for Child Health and Development, National Institutes of Health, Department of Health and Human Services, Bethesda, USA; Departments of Biostatistics and Computational Biology** (C Cox, G Wilding PhD, J Kost PhD, L-S Huang PhD) **and Environmental Medicine** (E Cernichiari MS, Prof T W Clarkson PhD), **University of Rochester School of Medicine and Dentistry; Ministry of Health, Republic of Seychelles** (C F Shamlaye MBChB)

Correspondence to: Dr G J Myers, University of Rochester Medical Center, 601 Elmwood Avenue, Box 631, Rochester, NY 14620, USA (e-mail: gary_myers@urmc.rochester.edu)

Introduction

Methylmercury (MeHg) is highly and selectively toxic to the CNS. The prenatal period is believed to be the most susceptible stage of life.[1] MeHg inhibits processes fundamental to brain development such as neuronal cell division and migration.[2] Although outbreaks of MeHg poisoning have occurred, human exposure is almost always from fish and other seafood. Inorganic mercury (Hg) occurring naturally or from pollution can be converted to MeHg by micoorganisms and is bioaccumulated up the food chain.[3] Ingested MeHg is almost totally absorbed and readily crosses the placenta and blood-brain barriers. Pregnant women who consume fish expose the fetus to MeHg.

Studies[4] in Iraq raised concern that prenatal MeHg exposure at the concentrations achieved by maternal consumption of ocean fish might adversely affect a child's neurodevelopment. However, the poisoning in Iraq resulted from seed grain treated with MeHg in a subsistence desert community and its relevance to fish consumers was unclear. If consumption of fish is associated with adverse neurodevelopmental consequences from MeHg exposure, public-health measures could reduce the risk. However, fish is an important source of protein worldwide and also has health benefits for adults. Consequently, restricting its consumption might adversely affect health.[5–7]

The Seychelles Child Development Study was specifically designed to test the validity of this hypothesis in a well-nourished population exposed to MeHg only from high consumption of unpolluted ocean fish. In Seychelles, women of childbearing age consume fish containing similar concentrations of MeHg to those in the USA (average about 0·3 μg/g), but the fish consumption rate is much higher (average 12 meals per week in our cohort compared with one or fewer in the USA). Consequently, concentrations of mercury in hair and other indicator media are many times higher than those in the US population and any effects should be detectable earlier. The main cohort was established in 1989 and previous assessments when the children were 6, 19, 29, and 66 months of age have been described in detail elsewhere.[8–11] Up to now, we have detected no adverse effects. We now report the results of our investigation at 9 years of age.

Methods

Participants

In 1989–90 we enrolled 779 mother-child pairs (about 50% of live births during that period), when the children were 6 months old. We excluded mothers and children with disorders highly associated with adverse neurodevelopment such as traumatic brain injury, meningitis, epilepsy, and severe neonatal illnesses. Although these disorders have been associated with overt and subtle neurodevelopmental problems, no data exist to suggest they are associated with MeHg exposure.[9] The 44 exclusions through 66 months of age have been reported.[9–11] We subsequently excluded 18 children for

Covariate	Definition
Sex	Male or female
Examiner	1, 2, or 3
Family resource scale	Continuous
Family status code	2, 1, or no biological parents in home
HELPS*	Continuous
Child's age at testing	Continuous
Child's medical history	Positive if diagnosed intrauterine growth retardation at birth or head circumference greater than 2 SD from normal
Maternal age	Continuous
HOME score†	≤31, >31 to 35, or >35
K-Bit‡	Low (<16), normal (16–28), or high (>28)
Hollingshead socioeconomic status	Unskilled (≤19), semiskilled (>19 to 29), skilled (>29 to 39), or minor/major business/profession (>39)
Hearing (best ear)	Normal (≤25 dB), borderline (>25 to 35 dB), or abnormal (> 35 dB)
Child's mercury concentration	Continuous

*Henderson early learning process scale. †HOME=home observation for measurement of the environment. ‡K-Bit=Kaufman brief intelligence test to determine caregiver intelligence.

Table 1: **Covariate definitions**

closed head trauma and meningitis. 717 (92%) of the 779 children were still eligible at 9 years of age. Of those eligible, 74 (10%) were not tested, leaving a total of 643 children (83% of the original cohort and 90% of those still eligible). The reasons children were not tested included residing abroad, refusal, and inability to locate them. The research protocols were reviewed and approved by the Institutional Review Boards of the University of Rochester and the Republic of Seychelles.

Procedures

Prenatal exposure to MeHg was determined by measuring total Hg in maternal hair growing during pregnancy, the method most indicative of prenatal exposure.[3,12] We obtained maternal hair at delivery and enrolment and measured the Hg concentrations in the sample that best recapitulated exposure. We assumed a growth rate of 1·1 cm per month and a delay of 20 days between current blood concentrations and appearance of the Hg in the first centimetre of scalp hair. Concentrations of total Hg in the hair of fish-eating populations correlate highly with maternal blood concentrations and are a better exposure index than concentrations of organic Hg.[13] Organic Hg can be partly transformed to inorganic Hg so that the concentration of total Hg more accurately represents the MeHg entering the hair follicle from the blood stream.[13] Concentrations of total Hg in maternal hair at delivery correlated highly with concentrations of Hg in brain samples taken at autopsy from Seychellois infants who died of natural causes.[14] We measured total and inorganic Hg with cold vapour atomic absorption spectroscopy with quality control procedures.[12] Concentrations of Hg are expressed in μg/g, where 1 μg/g=1 part per million (ppm) in hair.

We assessed neurocognitive, language, memory, motor, perceptual-motor, and behavioural functions. Our tests included overall and domain-specific items, covering neurodevelopmental domains associated with prenatal MeHg exposure and included most of the specific tests used in previous studies. Individual tests measured intelligence (the Wechsler intelligence scale for children III [WISC III] full-scale IQ); learning and achievement (the Woodcock-Johnson test of achievement, letter-word recognition, and applied problems subtests and the California verbal learning test); memory (the visual memory subtest of the wide-range assessment of memory and learning); motor functions (finger tapping, trailmaking, grooved pegboard, and most of the Bruininks-Oseretsky test of motor proficiency); language (Boston naming test); visual-motor integration (the Beery-Buktenica developmental test of visual motor integration and a test of haptic matching[15]); and sustained attention (Connor's continuous performance test). We assessed behaviour with the Connor's teacher rating scale and the parent-child behaviour checklist.

A team of three Seychellois child health and development professionals (a senior nurse, a child psychologist, and a special educator) assessed the children.[16] They received extensive training in child development and psychometric assessment procedures at the University of Rochester before the assessments. All personnel working in Seychelles were unaware of the MeHg exposure from the start of the study and no individual MeHg concentrations have been shared with families, clinical investigators, or anyone in Seychelles.

We investigated test reliability among testers and between each tester and a psychologist (PWD or DP). Pair-wise intertester reliability was assessed once a week by having a child's performance during testing scored by two team members simultaneously. We investigated gold-standard reliability by on-site simultaneous scoring of about 5% of test sessions by one of the psychologists.

The cohort was initially tested between February, 1997, and November, 1998. Every child was seen twice about 1 month apart. The sessions lasted about 3 h. During the first session the caregiver completed a demographic questionnaire and the parental child behaviour checklist while the child was given the WISC III and had audiometry and tympanometry. All remaining tests were administered individually during the second session. Testing took place in a specially established child development centre, mostly in the morning, and the tests were given in the same sequence in each session.

	Normal (mean [SD] or normal range)	Overall data (mean [SD])	Data by MeHg in maternal hair (mean [SE])					Regression coefficient (SE)*	p	95% CI for a 10 μg/g change
			≤3 (n=135)	>3 to 6 (n=190)	>6 to 9 (n=143)	>9 to 12 (n=87)	>12 (n=88)			
WISC III full scale IQ	100 (15)	81·6 (11·6)	79·4 (1·0)	81·1 (0·9)	80·3 (1·0)	80·8 (1·2)	81·7 (1·2)	–0·13 (0·10)	0·20	–3·3 to 0·7
CVLT										
Short delay recall	0 (1)	–0·02 (1·04)	–0·1 (0·1)	–0·6 (0·1)	0·0 (0·1)	0·0 (0·1)	0·1 (0·1)	0·013 (0·010)	0·19	–0·1 to 0·3
Long delay recall	0 (1)	–0·1 (1·03)	–0·2 (0·1)	–0·2 (0·1)	0·0 (0·1)	0·0 (0·1)	0·0 (0·1)	0·011 (0·010)	0·28	–0·1 to 0·3
BNT total score	43 (5)	26·5 (4·8)	26·3 (0·4)	26·6 (0·4)	25·9 (0·4)	27·3 (0·5)	26·7 (0·5)	–0·012 (0·046)	0·79	–1·0 to 0·8
W-J test										
LW recognition	90–110	131·7 (40·3)	130·4 (3·6)	126·3 (3·1)	135·3 (3·4)	131·3 (4·6)	140·1 (3·8)	0·19 (0·39)	0·62	–5·8 to 9·6
Applied problems	90–110	95·4 (15·5)	94·8 (1·4)	95·1 (1·2)	94·1 (1·3)	97·2 (1·8)	97·2 (1·7)	–0·057 (0·15)	0·71	–3·5 to 2·4

Higher scores in all tests indicate better performance. WISC III=Wechsler intelligence scale for children version III. CVLT=California verbal leaming test. BNT=Boston naming test. W-J=Woodcock-Johnson test of achievement. LW Recognition=letter word recognition. *Data from prenatal mercury term in the multiple regression models. †95% CI, on the scale of the original measurement.

Table 2: **Results of neurodevelopment tests for cognition and achievement by prenatal exposure**

	Normal (mean [SD] or normal range)	Overall data (mean [SD])	Data by MeHg in maternal hair (mean [SE])					Regression coefficient (SE)*	p	CI for a 10 μg/g change†
			≤3 (n=135)	>3 to 6 (n=190)	>6 to 9 (n=143)	>9 to 12 (n=87)	>12 (n=88)			
VMI	100 (15)	96·0 (11·7)	95·1 (1·2)	95·8 (0·8)	96·7 (0·9)	95·7 (1·2)	96·6 (1·4)	–0·010 (0·12)	0·93	–2·4 to 2·2
Bruninks-Oseretsky‡	..	44·6 (6·1)	44 (0·6)	44·3 (0·5)	45 (0·5)	45 (0·6)	45·3 (0·6)	0·093 (0·056)	0·10	–0·2 to 2·0
Haptic discrimination test (total correct out of 10)‡	..	4·1 (1·8)	4·2 (0·2)	4·1 (0·1)	4 (0·2)	4·1 (0·2)	4·2 (0·2)	–0·010 (0·018)	0·60	–0·5 to 0·3
Grooved pegboard time (s)§										
Dominant hand¶	74 (15)	91·8 (20·5)	86 (78–100)	88·5 (79–100)	89 (80–100)	87 (78–101)	89 (78–99)	$3·3\times10^{-5}$ ($1·9\times10^{-5}$)	0·08§	91·4 to 98·1
Non-dominant hand¶	81 (16)									
Male		100·1 (18·9)	95 (87–104)	95·5 (86–113)	93 (84–105)	106 (93–113)	98 (89–110)	$6·5\times10^{-5}$ ($2·5\times10^{-5}$)	0·01	101·7 to 112·9
Female		108·2 (29·8)	106 (95–124)	101 (90–113)	107·5 (89–120)	101 (85–120)	98 (90–113)	$-2·5\times10^{-5}$ ($2·6\times10^{-5}$)	0·34	100·0 to 111·3
Trail making time (s)										
A§	25 (9)	33·7 (17·1)	30 (24–40)	29 (22–40)	29 (23–39)	31 (25–39)	31 (23–38)	0·0037 (0·0038)	0·33	32·5 to 37·6
B§	55 (19)	81·5 (49·6)	67 (49–101)	63 (48–90)	65 (52–101)	63·5 (49–91)	62 (47–89)	0·0067 (0·0050)	0·17	79·1 to 96·0
Finger tapping										
Dominant hand	M 40 (5) F 39 (5)	34·0 (5·7)	34 (0·5)	34 (0·4)	33·8 (0·5)	34·7 (0·6)	33·7 (0·5)	–0·050 (0·053)	0·34	–1·5 to 0·5
Non-dominant hand	M 35 (5) F 33 (5)	30·0 (4·6)	29·5 (0·4)	30 (0·4)	30·1 (0·4)	31 (0·5)	29·8 (0·4)	0·016 (0·041)	0·69	–0·6 to 1·0
WRAML design memory	10 (3)	7·7 (2·9)	7·7 (0·2)	7·6 (0·2)	7·9 (0·3)	7·7 (0·3)	7·8 (0·3)	–0·021 (0·029)	0·48	–0·8 to 0·4

Higher scores indicate better performance except for Grooved Pegboard and Trailmaking where higher scores indicate poorer performance. VMI=visual motor integration. WRAML=wide range assessment of memory and learning. *Data from prenatal mercury term in the multiple regression models. †95% CI on the scale of the original measurement. ‡No norm available for the Bruninks-Oseretsky since the complete test was not administered. §The prenatal MeHg by sex main interaction was significant. ¶Where indicated test score was transformed for analysis. Medians and quartiles are reported for untransformed scores. Confidence limits should be compared with the mean test score (third column); if the slope was non-significant the confidence limits straddle this value.

Table 3: **Neurodevelopmental tests for motor, perceptual motor and memory by prenatal exposure**

When the cohort was 42–56 months of age, a home visit was made to administer the Caldwell-Bradley preschool version of the home observation for measurement of the environment. The child's primary caregiver, defined as the family member with whom the child lived for at least 5 days per week hosted the visit (93% of caregivers were the child's biological mother). Primary caregivers were recalled in 1999–2000 and given the family resource scale and the Henderson early learning process scale to measure the quality of stimulation in the home environment. They also completed the matrices subtest of the Kaufman brief intelligence test to determine caregiver intelligence.

The covariates used in the analysis are shown in table 1. They were selected a priori for their known effect on child development and were expected to provide an index of the effectiveness of the assessments. The Hollingshead four-factor socioeconomic score was calculated with a list of Seychellois employment codes. Recent postnatal MeHg exposure was included since it was associated with outcomes in the 66-month assessments. It was measured in a 1-cm segment of hair closest to the scalp on a sample taken at the initial 9-year assessment, and represented about 1 month of recent exposure. The mean postnatal hair concentration was 6·1 μg/g (SD 3·5). Lead, polychlorinated biphenyls, and pesticides were not

	Normal (mean [SD] or normal range)	Overall data (mean [SD])	Data by MeHg in maternal hair (mean [SE])					Regression coefficient (SE)*	p	CI for a 10 μg/g change†
			≤3 (n=135)	>3 to 6 (n=190)	>6 to 9 (n=143)	>9 to 12 (n=87)	>12 (n=88)			
CPT										
Hit reaction time‡	50 (10)	31·6 (14·1)	29·2 (1·3)	33·6 (1·1)	32·2 (1·2)	31·7 (1·7)	29·6 (1·9)	–0·13 (0·16)	0·41	(–4·4 to 1·8)
Attentiveness	50 (10)	57·5 (9·7)	57·3 (0·9)	58·8 (0·8)	56·7 (0·9)	57·2 (1·3)	56·8 (1·1)	–0·0063 (0·10)	0·95	(–2·1 to 2·0)
Risk-taking‡	50 (10)	75·0 (29·7)	74·3 (2·0)	75 (1·7)	77·6 (4·4)	71·7 (2·3)	75·3 (2·5)	0·11 (0·22)	0·60	(–3·1 to 5·4)
CBCL	50 (10)	59·4 (10·2)	59·8 (0·9)	59·4 (0·8)	58·7 (0·9)	58·3 (1·1)	60·9 (1·1)	–0·031 (0·10)	0·76	(–2·3 to 1·7)
CTRS hyperactivity index§	45–55	55·3 (12·8)	52 (44–65)	51·5 (46–65)	52 (45–60)	50 (46–59)	49 (44–60)	–0·0067 (0·0023)	0·004	(49·4 to 54·1)

Higher scores indicate better performance except for the CBCL and TRS where higher scores indicate poorer performance. CPT=continuous performance task. CBCL=Connor's child behaviour checklist. CTRS=Connor's teacher rating scale. *Data from prenatal mercury term in the multiple regression models. †95% CI on the scale of the original measurement. ‡A score that deviates positively or negatively from the norm can be regarded clinically indicative of attention problems. §Where indicated test score was transformed for analysis. Confidence limits should be compared with the mean test score (third column); if the slope was non-significant the confidence limits straddle this value. ¶Medians and quartiles are reported for untransformed scores.

Table 4: **Neurodevelopmental tests for attention and behaviour by prenatal exposure**

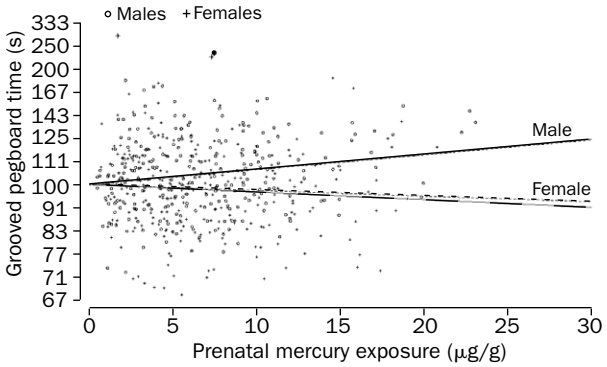

Figure 1: **Association between prenatal MeHg exposure and scores on the groove pegboard with the non-preferred hand**
Test scores have been adjusted for covariates to show the association with mercury concentrations in the multiple regression model. Lines are shown for the model with outliers (dashed) and without outliers (solid). Outliers are indicated by larger symbols. Test scores were transformed for analysis so the y-axis is nonlinear.

included since measured concentrations in Seychelles are low.[10,17] Use of alcohol and tobacco among Seychellois women of childbearing age is low; consequently they were not used as covariates. At enrolment, 5% of the mothers reported occasional alcohol intake during pregnancy and 2% reported tobacco use.

Statistical analysis

The primary analysis included 21 endpoints. We used the main score for most tests, but several measures yielded more than one endpoint. For every endpoint, we did a maximum of three linear-regression analyses for prenatal MeHg exposure using all the covariates defined in table 1. All analyses were done with the SAS system, version 8. Because differential effects on males and females have been reported, every model was run first with and then without a MeHg by sex interaction term for both prenatal and recent postnatal exposure.[10,18–20] If the overall test for both models was not significant at a two-tailed significance level of 0·05, the results of that analysis were deemed negative. We assessed the model with interactions first. If both interactions were significant then results are reported for this model. If neither interaction was significant, we report the model without interactions. If only the prenatal or postnatal interaction were significant, we reran the model, dropping the non-significant interaction term (including only a simple effect), but including the significant interaction term. This happened for four endpoints.

Every analysis included an assessment of residuals as a check on the assumptions of normally distributed errors with constant variance. If the assumptions seemed to be violated, we used power transformations to stabilise the variance and produce more normally distributed errors. For every analysis, we assessed the model for statistical outliers (scores with standardised residual values >3 or <–3). All models with outliers were rerun without the outliers and the results with and without outliers were compared and are reported.

We also assessed every regression model for the effects of influential points, identified by deleting each point in the data set individually from the analysis and calculating the resulting standardised change in the regression coefficient for prenatal MeHg exposure. The regression analysis for all primary endpoints was repeated without influential points to determine whether the original results were dependent upon such points. The final analysis included influential points that were not also outliers.

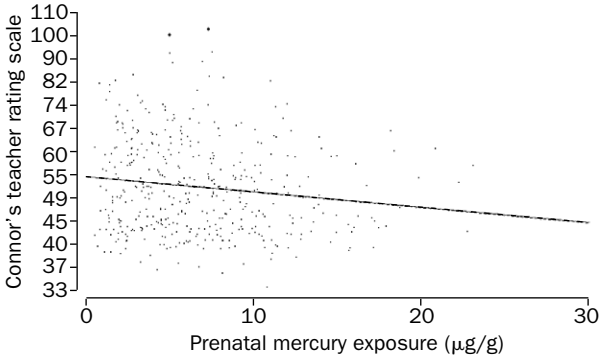

Figure 2: **Association between prenatal MeHg exposure and scores on the attention deficit hyperactivity index of the Connor's teacher rating scale**
Test scores have been adjusted for covariates to show the association with mercury concentration in the multiple regression model. Lines are shown for the model with (dashed) and without (solid) outliers. Outliers are indicated by black ovals. Test scores were transformed for analysis so the y-axis is non-linear.

Role of the funding source

The sponsors of the study approved the study design but had no other involvement in the study design, data collection, data analysis, data interpretation, or writing of this report.

Results

The mean prenatal total MeHg exposure was 6·9 μg/g (SD 4·5). The correlation coefficient between prenatal and postnatal exposure was –0·08 (p=0·04).

Intraclass correlation coefficients were computed for each of the 13 subtests of the WISC III. This test was chosen for reliability computations since it was the most difficult component to administer. Comparisons between WISC-III subtest scores obtained by pairs of testers (n=45) ranged from 0·90 to 1·00. Agreement between each tester and one of the team psychologists (n=37) ranged from 0·81 to 1·00.

The mean age at testing was 107 months (SD 4). For data presentation, the mean (SD) for each endpoint was computed by prenatal mercury exposure groupings. These results are shown in table 2 (cognition and achievement domains), table 3 (motor, perceptual-motor, and memory domains), and table 4 (attention and behaviour domains). In cases where the endpoint was transformed for analysis, the median and quartiles have been included in the tables,

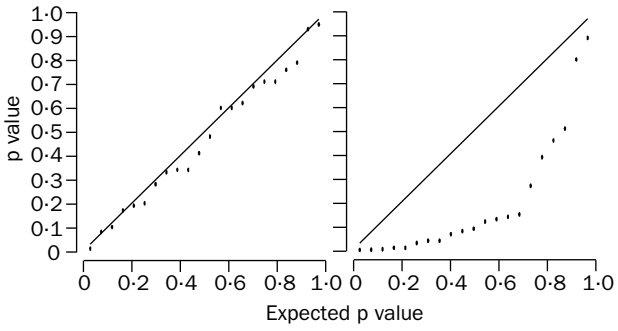

Figure 3: **Probability plots of p values for the prenatal exposure effect (left) and the home observation covariate (right) from 21 primary regression analyses**
The degree to which p values conform to the null hypothesis line shows the consistency of the data with the overall null hypothesis.

rather than the mean. Seychellois children's scores on most endpoints compared favourably with US norms. The variability of the tests was as expected and they seemed to discriminate well among cohort children. The WISC III and the Boston naming test (table 2) were both affected by cultural variation, with lower means for Seychellois children than for US controls. However, the variability associated with these and other endpoints was consistent with test expectations, suggesting that all tests discriminated well among cohort children. Seychellois children did substantially better than the US norms on the Woodcock-Johnson letter-word recognition test, measuring scholastic achievement in reading.

Significant two-tailed overall model F statistics ($p<0·05$) resulted from the regression analysis for 20 of the 21 models with outliers removed. The squared multiple correlation coefficients for these models ranged between 5% and 24% with most values between 10 and 20%. Significant associations between prenatal MeHg exposure and performance were found for two endpoints, and both needed transformation for analysis. There was a significant decrease in performance on the grooved pegboard time for the non-dominant hand in males (table 3; figure 1). This task required an average of 100 s for cohort participants to complete and a 95% CI for a 10 μg/g change in exposure ranged from 1 to 13 s. The variability for this task within the reference population was 19 s, suggesting the effect was small. The association for the same task using the dominant hand was not significant. There was a significant improvement of the hyperactivity index of the Connor's teacher-rating scale (table 4; figure 2) as prenatal MeHg increased. A 10 μg/g increase in exposure would result in a drop of between 1 and 6 points (95% CI) in the hyperactivity index.

	Caregiver IQ	Maternal	Chid's age	Home				Socioeconomic score				
				Overall	Low	Med	High	Overall	Unskill	Semi-skill	Skill	Professional
WISC III full scale IQ	0·14 (0·03)*	0·20 (0·08)†	–2·08 (1·48)	*	–1·61 (0·67)	–0·40 (0·64)	2·02 (0·68)	*	–2·61 (0·8)	0·28 (0·75)	–0·20 (0·84)	2·53 (1·06)
CVLT												
Short delay	0·001 (0·003)	–0·01 (0·01)	–0·61 (0·16)*		–0·11 (0·07)	0·05 (0·06)	0·06 (0·07)	†	–0·17 (0·08)	–0·0004 (0·08)	–0·05 (0·09)	0·22 (0·11)
Long delay	0·0001 (0·003)	–0·002 (0·01)	–0·57 (0·16)*		–0·05 (0·07)	–0·04 (0·06)	0·09 (0·07)	†	–0·26 (0·08)	0·03 (0·08)	0·10 (0·09)	0·13 (0·11)
Boston naming test total score	0·03 (0·02)‡	0·06 (0·04)‡	1·45 (0·73)†	*	–1·10 (0·31)	0·13 (0·29)	0·97 (0·31)	*	–0·94 (0·36)	–0·34 (0·34)	0·22 (0·38)	1·06 (0·48)
W-J test												
Letter-word recognition	0·44 (0·13)*	0·64 (0·32)†	13·7 (8·03)	*	1·37 (2·73)	–7·60 (2·52)	6·23 (2·7)		–3·69 (3·18)	–0·34 (2·94)	1·08 (3·29)	2·95 (4·14)
Applied problems	0·24 (0·05)*	0·20 (0·12)*	0·96 (3·00)		–1·21 (1·02)	–0·79 (0·93)	2·00 (1·02)	*	–3·62 (1·17)	0·66 (1·11)	–1·03 (1·24)	3·98 (1·56)
VMI	0·11 (0·04)*	0·03 (0·09)	–3·99 (1·86)†		–1·03 (0·78)	–0·68 (0·73)	1·71 (0·78)		–1·33 (0·91)	0·98 (0·86)	0·86 (0·96)	–0·52 (1·2)
B-0 test of motor development	0·03 (0·02)‡	–0·01 (–0·04)	2·42 (0·88)*		–0·004 (0·38)	–0·36 (0·35)	0·36 (0·38)		–0·14 (0·44)	–0·43 (0·42)	0·32 (0·46)	0·26 (0·58)
Haptic discrimination test	0·01 (0·01)	–0·02 (0·01)	–0·37 (0·29)		–0·03 (0·12)	0·06 (0·12)	–0·03 (0·12)		0·01 (0·14)	0·01 (0·14)	–0·08 (0·15)	0·07 (0·19)
Grooved pegboard Dominant hand§	–1·9 (0·63)	–3·7 (1·5)	–92 (31)		28 (13)	4·5 (0·12)	–32 (13)	†	1·3 (15)	20 (14)	20 (16)	2·7 (20)
Non-dominant hand§	1·4 (0·6)	–02·1 (1·4)	–100 (29)		36 (12)	–1·0 (11)	–35 (12)	*	15 (14)	4·6 (13)	–2·8 (15)	–17 (19)
Trail-making												
A	–0·003 (0·001)†	–0·004 (0·003)	–0·30 (0·06)*		0·03 (0·03)	–0·02 (0·02)	–0·01 (0·03)		0·0001 (0·03)	0·01 (0·03)	0·01 (0·03)	–0·02 (0·04)
B	–0·01 (0·002)*	–0·01 (0·004)	–0·35 (0·08)*	*	0·05 (0·03)	0·04 (0·03)	–0·09 (0·03)		0·04 (0·04)	0·05 (0·04)	0·03 (0·04)	–0·12 (0·05)
Finger tapping												
Dominant hand	0·02 (0·02)	0·06 (0·04)	3·33 (0·85)*		–0·76 (0·36)	0·09 (0·33)	0·67 (0·36)		–0·31 (0·42)	–0·30 (0·39)	0·45 (0·44)	0·16 (0·56)
Non-dominant hand	0·003 (0·01)	0·05 (0·03)	2·05 (0·67)*	*	–0·98 (0·28)	0·28 (0·26)	0·70 (0·28)		–0·27 (0·33)	–0·42 (0·31)	0·40 (0·35)	0·29 (0·44)
WRAML design memory	0·03 (0·01)*	0·02 (0·02)	–0·22 (0·47)		–0·43 (0·20)	0·05 (0·19)	0·38 (0·20)		–0·25 (0·23)	–0·03 (0·22)	0·08 (0·24)	0·19 (0·31)
CPT												
Hit reaction	0·13 (0·05)†	0·20 (0·12)‡	0·99 (2·12)	†	–2·61 (1·03)	0·92 (0·96)	1·69 (1·03)		0·33 (1·22)	0·10 (1·14)	–0·56 (1·26)	0·13 (1·57)
Attentiveness	–0·05 (0·03)	–0·17 (0·08)†	3·87 (1·39)*		1·31 (0·68)	–0·37 (0·64)	–0·93 (0·68)		0·003 (0·8)	0·95 (0·75)	–0·63 (0·83)	–0·32 (1·03)
Risk-taking	–0·16 (0·07)†	0·26 (0·16)	3·18 (2·9)		2·84 (1·14)	–0·75 (1·32)	–2·09 (1·42)		0·12 (1·67)	0·39 (1·55)	0·46 (1·72)	–0·97 (2·14)
CBCL	–0·06 (0·03)	–0·32 (0·08)*	1·58 (1·48)		0·70 (0·68)	0·68 (0·64)	–1·38 (0·69)		–0·21 (0·79)	–0·44 (0·76)	–0·13 (0·84)	0·77 (1·06)
CTRS hyperactivity index	–0·001 (0·001)	0·0003 (0·002)	–0·05 (0·03)‡		–0·01 (0·02)	0·01 (0·01)	0·004 (0·02)		0·02 (0·02)	0·02 (0·02)	–0·01 (0·02)	–0·02 (0·02)

Values are regression coefficients (SE). WISC=Wechsler intelligence scale for children. CVLT=California verbal learning test. W-J=Woodcock-Johnson test of achievement. VMI=visual motor integration. B-0=Bruininks-Oseretsky. WRAML=wide range assessment of memory and learning. CPT=continuous performance test. CBCL=children's behaviour checklist. CTRS=Connors Teacher Rating Scale. *p<0·01. †p<0·05. ‡p<0·10. †Where indicated test score was transformed for analysis. §Values are $\times 10^{-5}$.

Table 5: **Social and environmental covariate effects**

Seychellois children were functioning at the upper limit of the normal range for this test.

The large number of endpoints raised concern about multiplicity and so we investigated the actual distributions of the p values for the prenatal exposure for consistency with the overall null hypothesis of no association. If the overall null hypothesis were true, the p values should be uniformly distributed from zero to one. We assessed this graphically by plotting the p values against idealised values from the uniform distribution. The distribution of p values was consistent with the expected values, and provides support for the overall null hypothesis of no association (figure 3). For comparison we plotted the p values for the home observation for measurement of the environment, a covariate from the same series of analyses that was associated with the endpoints.

Table 5 shows the associations between endpoints and selected social and environmental covariates. These factors have well established associations with child development and were expected to provide an index of the effectiveness of the assessments in ascertaining developmental status of the children. The data suggest that the effects of these factors on the endpoints were consistent with established associations. For example, socioeconomic score, early home environment scores, and maternal IQ were consistently associated with outcomes for neurocognitive endpoints but only occasionally with outcomes on motor tasks. Measures of later home environment, such as the family resource scale and the Henderson early learning process scale had limited effect, as would be expected for normally developing children at this age, and are not included.

We have focused on prenatal exposures, with postnatal hair concentration of MeHg a covariate in the analysis for prenatal effects. In a few tests this analysis suggested an adverse association with postnatal exposure in females. Since postnatal exposure differs substantially from prenatal exposure and since males are thought to be more susceptible, the interpretation of these findings is unclear. Analyses for the entire postnatal period are still in progress.

19 regression analyses revealed between one and five outlier scores involving a total of 40 different participants. In all cases, the association between prenatal MeHg exposure and the endpoint was the same, irrespective of whether outliers were included. For the two endpoints with a significant prenatal MeHg effect (Connor's teacher rating scale [two outliers] and the grooved pegboard non-dominant hand [three outliers]), the outlier scores all had prenatal MeHg concentrations of 7·5 μg/g or less and low performance.

Every model had between 0 and 3 influential points, defined as a score that may have affected the slope of the regression line but did not reach the status of an outlier. We report results with influential points included. However, the models with both interactions were re-run without influential points included, and in no case did the results for prenatal exposure change.

Discussion

Two of 21 endpoints were associated with prenatal MeHg exposure and developmental outcomes at 9 years of age. One association involved diminished performance (grooved pegboard non-dominant hand in males only) and the other an enhancement (hyperactivity index of the Connors teacher rating scale). As indicated by the distribution of p values in figure 3, both these outcomes are probably due to chance.

Results of studies of prenatal exposure to MeHg from seafood consumption in the Faeroe Islands[21] and New Zealand[22,23] have shown adverse neuropsychological outcomes in school-aged children. The difference in findings from these studies and the Seychelles study has been investigated in two reviews[6,7] and several explanations have been proposed,[24] including the power of the studies to detect subtle differences. Assessment of standard power curves for the two studies shows that the power of the Seychelles study was only slightly less than that of the Faeroe Islands and both are substantially greater than any previous study. Our original power calculations estimated a 90% chance of detecting a five-point difference on the Bayley scales of infant development with every 10 μg/g increase in MeHg.

These outcomes might differ because of the cellular effect of very different concentrations of MeHg in the seafood consumed by these populations. The presence of cellular mechanisms in mammals that detoxify Hg raises the possibility that a larger bolus dose with a meal might behave differently than a small dose.[25] In Seychelles, the seafood consumed has a lower concentrations of MeHg than in the other two populations. The mean concentration of organic Hg in whale meat (the main source of MeHg in the Faeroe Islands) was 1·6 μg/g (SD 0·4).[26] In New Zealand the shark muscle consumed in the popular take-out food of fish and chips had a mean Hg concentration of 2·2 μg/g with some samples more than 4 μg/g.[27] By contrast, the Seychellois consume many different species of ocean fish, the mean MeHg content of which averages 0·3 μg/g (with 97·5 % of the samples below 0·7 μg/g).[12]

Cord blood was used as the monitoring medium in the Faeroe Islands and the investigators argued that it is a more sensitive biomarker for prenatal MeHg exposure than concentrations in hair.[21] However, maternal hair has been the biological monitor of choice in most studies of prenatal exposure and was used in the New Zealand study.[3] Moreover, hair and blood concentrations are closely correlated, and hair can recapitulate exposure during the entire period of pregnancy.[12]

The tests used and the age at testing also differed between the studies.[6] However, our test battery included both global and domain-specific items and nearly all the tests reported previously had shown an association with MeHg.[21,23] Moreover, most of the tests gave results that were normally distributed and scores that were similar to those in Western countries, and were sensitive enough to detect the expected effects of covariates. The tests should have been sensitive enough to detect MeHg effects if they were present. A difference in age at testing is no longer a viable explanation since our previous findings at 66 months of age are now extended to 9 years, thus bracketing the 6 and 7 years ages used in the other studies.

One factor unique to the Faeroe Islands study is the consumption of whale meat and blubber. Whale blubber has high concentrations of polychlorinated biphenyls and other persistent organic pollutants and the meat has concentrations of inorganic Hg similar to MeHg.[24,26] These factors would not explain the associations reported from New Zealand.

Our study has potential limitations. As with any observational study, enrolment could have been biased. We sought to prevent such bias by offering participation to all children who reached the target age, and comparisons of enrolled children with those not participating did not show any bias. The best biomarker for prenatal exposure to MeHg is also controversial. In studies of neuro-developmental outcomes, choosing the appropriate

covariates affecting child development is crucial to get valid results from the statistical models. We included a standard array of covariates known to be related to developmental outcomes that were selected a priori. A-priori selection was preferred to alternative data-driven approaches to avoid the possibility of bias and type-1 errors. Because children were enrolled when they were 6 months old, information about pregnancy, birth, and feeding was obtained retrospectively and might not have been as accurate as if it had been gathered at delivery. We also did not assess nutritional covariates such as selenium or polyunsaturated fatty acids. We are now investigating a new cohort in Seychelles to address this issue. Different results might have been obtained with different developmental tests, but we designed our tests to assess developmental domains known to be affected by prenatal MeHg poisoning and included most tests administered in previous studies. Finally, the choice of a different statistical approach might have led to different results. However, secondary analyses of our earlier studies with other statistical models have been consistent with conclusions based on the primary analysis.[29,30]

In summary, the Seychelles Child Development Study longitudinal assessments at 9 years of age indicate no detectable adverse effects in a population consuming large quantities of a wide variety of ocean fish. These results are consistent with our earlier findings in the same children when examined at 6, 19, 29, and 66 months of age. In Seychelles, fetal exposure was continuous through frequent consumption of ocean fish containing concentrations of MeHg comparable to those consumed by the general population in the USA. We recorded effects from covariates known to affect child development, but did not find an association with prenatal mercury. We believe this finding is relevant to public health measures and that the Seychelles could serve as a sentinel population for fish consumers.

Contributors

G Myers, P Davidson, C Cox, T Clarkson, C Shamlaye, and D Palumbo designed and helped inplement the study. C Shamlaye and G Myers managed the study in Seychelles and Rochester, respectively. P Davidson and D Palumbo trained testers and did on site reliabilities. E Cernichiari analysed mercury and managed the data office. J Sloane-Reeves scored tests, trained testers, and with E Cernichiari managed the database. C Cox, G Wilding, J Kost, and L-S Huang were responsible for statistical analysis. G Myers, P Davidson, C Cox, and T Clarkson interpreted the data and wrote the report.

Conflict of interest statement

None declared.

Acknowledgments

This research was supported by Grants R01-ES10219; R01-08442; ES-01247, and T32 ES-007271 from the US National Institutes of Health; the Food and Drug Administration, USDHHS, and by the Ministry of Health, Republic of Seychelles.

References

1 Harada Y. Congenital (or fetal) Minamata disease. In: Study Group of Minamata Disease eds. Minamata Disease. Japan; Kumamoto University, 1968: 93–118.

2 Choi BH, Lapham LW, Amin-Zaki L, Saleem T. Abnormal neuronal migration, deranged cerebral cortical organization and diffuse white matter astrocytosis of human fetal brain: a major effect of methylmercury poisoning in utero. *J Neuropathol Exp Neurol* 1978; **87:** 719–33.

3 WHO. Environmental Health Criteria 101 Methylmercury. Geneva: World Health Organization, 1990.

4 Cox C, Clarkson TW, Marsh DO, Amin-Zaki L, Tikriti S, Myers GJ. Dose-response analysis of infants prenatally exposed to methylmercury: an application of a single compartment model to single-strand hair analysis. *Environ Res* 1989; **31:** 640–49.

5 Daviglus ML, Stamler J, Orenica AJ, et al. Fish consumption and the 30-year risk of fatal myocardial infarction. *N Engl J Med* 1997; **336:** 1046–53.

6 National Institute of Environmental Health Sciences. Workshop report on scientific issues relevant to assessment of health effects from exposure to methylmercury. (Raleigh, November 18–20, 1998). http://ntp-server.niehs.nih.gov/main_pages/PUBS/MethMercWkshpRpt.html#MeHgTOC (accessed Oct 8, 2001).

7 National Research Council. Toxicological effects of methylmercury. Washington, DC: National Academy Press, 2000: 1–344.

8 Marsh DO, Clarkson TW, Myers GJ, et al. The Seychelles study of fetal methylmercury exposure and child development: Introduction. *Neurotoxicology* 1995; **16:** 583–96.

9 Myers GJ, Marsh DO, Davidson PW, et al. Main neurodevelopmental study of Seychellois children following in utero exposure to methylmercury from a maternal fish diet: Outcome at six months. *Neurotoxicology* 1995; **16:** 653–64.

10 Davidson PW, Myers GJ, Cox C, et al. Effects of prenatal and postnatal methylmercury exposure from fish consumption on neurodevelopment: outcomes at 66 months of age in the Seychelles Child Development Study. *JAMA* 1998; **280:** 701–07.

11 Davidson PW, Myers GJ, Cox C, et al. Longitudinal neurodevelopment study of Seyuchellois children following in utero exposure to methylmercury from fish ingestion: outcomes at 19 and 29 months. *Neurotoxicology* 1995; **16:** 677–88.

12 Cernichiari E, Toribara TY, Liang L, et al. The biological monitoring of mercury in the Seychelles Study. *Neurotoxicology* 1995; **16:** 613–28.

13 Phelps RW, Clarkson TW, Kershaw TG, Wheatley B. Interrelationships of blood and hair mercury concentrations in a North American population exposed to methylmercury. *Arch Environ Health* 1980; **35:** 161–68.

14 Lapham LW. Cernichiari E, Cox C, et al. An analysis of autopsy brain tissue from infants prenatally exposed to methylmercury. *Neurotoxicology* 1995; **16:** 689–704.

15 Davidson P, Abbott S, Gershenfeld J. Influence of exploration time on haptic and visual matching of complex shape. *Perception Psychophysics* 1974; **14:** 539–43.

16 Davidson PW, Palumbo D, Myers GJ, et al. Neurodevelopmental outcomes of Seychellois children from the pilot cohort at 108 months following prenatal exposure to methylmercury from a maternal fish diet. *Environ Res* 2000; **84:** 1–11.

17 Davidson PW, Myers GJ, Cox C, Cernichiari E, Clarkson TW, Shamlaye C. In reply (letter to the editor). *JAMA* 1999; **281:** 897.

18 Marsh DO, Clarkson TW, Cox C, Myers GJ, Amin-Zaki L, Al-Tikriti S. Fetal methylmercury poisoning. *Arch Neurol* 1987; **44:** 1017–22.

19 McKeown-Eyssen GE, Ruedy J, Neims A. Methyl mercury exposure in northern Quebec II. Neurologic findings in children. *Am J Epidemiol* 1983; **118:** 470–79.

20 Sager PR, Aschner N, Rodier PM. Persistent differential alterations in developing cerebellar cortex of male and female mice after methylmercury exposure. *Dev Brain Res* 1984; **12:** 1–11.

21 Grandjean P, Weihe P, White RF, et al. Cognitive deficit in 7-year-old children with prenatal exposure to methylmercury. *Neurotoxicol Teratol* 1997; **19:** 417–28.

22 Kjellstrom T, Kennedy P, Wallis S, Mantell C. Physical and mental development of children with prenatal exposure to mercury from fish. Stage 1: preliminary tests at age 4. Solna, Sweden: National Swedish Environmental Board Report No 3080: 1986.

23 Kjellstrom T, Kennedy P, Wallis S, et al. Physical and mental development of children with prenatal exposure to mercury from fish. Stage 2: interviews and psychological tests at age 6. Solna, Sweden: National Swedish Environmental board Report 3642; 1989.

24 Dourson ML, Wullenweber AE, Poirier. Uncertainties in the reference dose for methlmercury. *Neurotoxicology* 2001; **22:** 677–89.

25 Clarkson TW. The three modern faces of mercury. *Environ Health Perspect* 2002; **110** (suppl)**:** 11–23.

26 Julshamin K, Andersen A, Ringdal O, Morkore J. Trace elements intake in the Faroe Islands 1: element levels in edible parts of pilot whales (*Globicephalus meleanus*). *Sci Total Environ* 1987; **65:** 53–62.

27 Mitchell JW, Kjellstrom TE, Reeves RL. Mercury in takeaway fish in New Zealand. *N Z Med J* 1982; **95:** 112–14.

28 Cernichiari E, Brewer R, Myers GJ, et al. Monitoring methylmercury during pregnancy: maternal hair predicts fetal brain exposure. *Neurotoxicology* 1995; **16:** 705–10.

29 Axtell CD, Myers GJ, Davidson PW, et al. Semiparametric modeling of age at achieving developmental milestones after prenatal exposure to methylmercury in the Seychelles Child Development Study. *Environ Health Perspect* 1998; **106:** 559–64.

30 Axtell CD, Cox C, Myers GJ, et al, The 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. *Environ Res Sec A* 2000; **84:** 12–19.