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Cognitive Deficit in 7-Year-Old Children with Prenatal Exposure to Methylmercury

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Environmental pollution Food contamination Methylmercury compounds Neuropsychological tests Prenatal exposure delayed effects Preschool child

METHYLMERCURY is a well-established neurotoxicant that can have serious adverse effects on the development and functioning of the human central nervous system, especially when exposure occurs prenatally (45). This pollutant is of considerable public health concern, because it is found in seafood and freshwater fish throughout the world (45). Given the potential threat that methylmercury poses to the optimal development of cognitive functioning, clinicians and regulatory agencies are concerned about the amount of methylmercury a pregnant woman can safely ingest (45,51).

It has been estimated that the risk of fetal brain damage increases when the mercury concentration in maternal scalp hair exceeds a level of 10–20 μg/g (50–100 nmol/g) (45). However, this threshold estimate was based on observations of crude abnormalities in infants whose mothers had ingested bread made from methylmercury-treated grain (11,43). In the

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case of contaminated seafood, exposures approaching or exceeding this limit have been associated with mild or equivocal deficits on developmental or general intelligence tests in prenatally exposed children (14,36,37,46).

We generated a cohort of approximately 1000 births in the Faroe Islands (24) and then conducted neurobehavioral examinations at school age. The methylmercury exposure in the Faroes originates mainly from pilot whale meat (34) that, according to ancient tradition, is shared in the communities where the whales are killed. In this small Nordic community (45,000 inhabitants), large variations in seafood intake occur whereas social differences are limited. We therefore hypothesized that increased methylmercury exposure would be related to decreased neurobehavioral function and that potential confounders in this community would play only a small role.

MATERIALS AND METHODS

Study Design

A cohort of 1022 singleton births was assembled in the Faroe Islands during a 21-month period of 1986–1987 (23,24). Mercury concentrations varied considerably. Fifteen percent of the mothers had hair mercury concentrations above 10 μg/g (50 nmol/g), whereas cord blood concentrations ranged up to 350 μg/l (1.75 μmol/l) [i.e., about 100-fold more than the upper reference limit in Denmark (22)]. However, obvious cases of congenital methylmercury poisoning (29) were not found. Because the effects of fetal childhood exposure to methylmercury are persistent (15,29,39), detailed examination of children with prenatal exposure to this neurotoxicant would be appropriate at school age. At this time, they have developed sufficiently to perform a wide variety of neurobehavioral tests, and they are capable of cooperating for most functional tasks.

The protocol was approved by the ethical review committee for the Faroe Islands. The examinations were conducted by a team of health service professionals who had no access to information on individual exposure levels. Children residing in the Faroes were invited for clinical examinations just before school entry (i.e., between early April and late June in 1993) and, for the youngest children of the cohort, at the same time in 1994. Children currently residing in Denmark were examined in 1994. Seven children had died from causes apparently unrelated to mercury exposure. A total of 917 of the surviving children (90.3%) completed the examinations, 443 of them in 1993. Because of a slightly lower participation rate of children from the capital of Tórshavn, the prenatal mercury exposure levels of the children examined was significantly higher [geometric mean cord blood mercury concentration, 22.8 μg/l (114 nmol/l)] than that of those who did not participate [17.9 μg/l (89 nmol/l)]. Because no other selection bias was apparent, the small attrition would be unlikely to affect a relationship between mercury exposure and neurobehavioral function.

Examination Schedule

Most of the children were examined at the National Hospital in Tórshavn, the capital of the Faroe Islands. All transportation costs were refunded. To facilitate travel for the families, examinations also took place at the two smaller hospitals in the Faroes in 1993, and the following year in both Odense and Copenhagen, Denmark (for families who had moved). Four children were examined during the morning and four during the afternoon at five examination stations, with each station taking up to 60 min. Past medical history, current health status, and social factors were recorded on a self-administered form by the parent accompanying the child (usually the mother). Although translations of the Child Behavior Checklist (1) have not been validated in societies similar to the Faroes, items 8, 10, 13, 22, 23, 25, 34, 37, 45, 50, 57, 81, 82, 89, 95, and 112 relate to neurobehavioral effects previously reported to be associated with methylmercury exposure (29,39) and were therefore incorporated in a questionnaire administered to the mothers.

The physical examination included a functional neurological examination that was developed in Denmark with an emphasis on motor coordination and perceptual–motor performance (41). Visual acuity was determined by Snellen's board and contrast sensitivity by the Functional Acuity Contrast Test (20). Otoscopy and tympanometry were supplemented by audiometry at 4 and 8 kHz. Main emphasis was placed on detailed neurophysiological and neuropsychological tests.

Neurophysiological Tests

Because of abnormalities in evoked potentials recorded in patients with methylmercury poisoning (28,30–32,55), neurophysiological tests not requiring electrical stimulation or long measurement times were conducted. The equipment used included a four-channel electromyograph (Medelec Saphire-4ME), a strain-gauge-type force platform (NEC-Sanei Static Sensograph 1G06, Japan), an electrocardiogram amplifier (NEC-Sanei 1271SP), and two computers (NEC PC9801CV and PC9801LS5).

Pattern reversal visual evoked potentials (VEP) with binocular full-field stimulation were recorded in a darkened room (2). The subjects sat quietly 127 cm from the front of a 17-inch monitor screen and were asked to stare at the center of the screen. The checkerboard pattern on the screen consisted of white and black squares (mean luminance, 371 and 5 cd/m^2 respectively), reversing at a rate of two per second (sampling time, 0.2 ms). Two widths of the squares were used (30′ and 15′). The latencies of one positive and two negative peaks (P100, N75, and N145) were recorded using standard electroencephalography (EEG) electrodes fixed to the skull above the occipital cortex, the forehead, and the left mastoid (ground). Responses were averaged 128 times after amplification and filtration (bandpass, 1–100 Hz), with one replication for each pattern.

Brain stem auditory evoked potentials (BAEP) were measured in comfortably resting subjects (2). Click signals with an intensity of 65 dB HL (0.1-ms impulses of alternating polarity) were presented to the right ear through electromagnetically shielded earphones at rates of 20 and 40 per second independently (sampling time, 0.01 ms); the other ear was masked with white noise of intensity of 45 dB HL. BAEP latencies were recorded using three standard EEG electrodes placed on the vertex, the right mastoid ipsilateral to stimulation, and the left mastoid (ground). The responses were averaged 1024 times after amplification and filtration (bandpass, 200–2,000 Hz), with one replication for each rate.

Postural sway was quantitatively measured in a quiet room using computerized posturography (4,60). The subject was asked to stand quietly on the platform without foam under eyes-open and eyes-closed conditions, and then again with foam in the same manner (sampling duration and time were 60 s and 0.5 ms, respectively). The X (right–left) and Y (anterior–posterior) locations of the body's center of gravity in the horizontal plane were recorded with a sampling interval of 50 ms using a computer connected to the platform via A-D converter. The data were stored on a diskette for analysis, and the area of the sway of the body's gravity center was calculated for all four conditions.

As a measure of autonomic nervous system function, we recorded the coefficient of variation for R–R interpeak intervals (CV_{RR}) on the electrocardiogram (ECG) (3). After the subjects lay quietly supine for 5 min, 300 R–R intervals were measured in real time (sampling time, 1 ms) and stored on a diskette; 100 consecutive R–R intervals with the minimal standard deviation were automatically extracted for calculation of the CV_{RR}.

Neuropsychological Tests

Neuropsychological tests were selected on the basis of a range of considerations (58). Tests were chosen to include tasks that would be affected by the neuropathological abnormalities described in congenital methylmercury poisoning (9,18,29,44,54) and the functional deficits seen in children with early life exposure to neurotoxicants (37,59). The tests also had to be acceptable to the children and their parents, viz. painless, not too time consuming, and appropriate for 7-year-old Faroese children who had not yet begun school. Tests that were likely to provide a high statistical sensitivity (i.e., with a wide range of scores possible without floor or ceiling effects, and acceptable test–retest reliability) were preferred. In addition, test versions standardized in Scandinavian countries were favored. With the partial exception of one test (WISC-R Similarities), the paper-and-pencil tests were administered to all children in a uniform sequence by the same Faroese neuropsychologist. Three computer-assisted tests of increasing difficulty were given in a separate room; the same portable computer (Toshiba 3200) with the recommended joy-stick (40) was used for the examination of all children. Each child was first asked by the examiner about familiarity with computer games, and the answer was rated as "none," "some," or "much." The mood test was administered in connection with the computer-assisted tests. The staff at the latter station was the same throughout 1993, but medical supervision was discontinued after the first week of the 1994 examinations. Two different examiners carried out more than 90% of the Tactual Performance Tests after the physical examination. After an initial test battery had been administered in a pilot study on a group of Faroese children not included in the cohort, some minor changes were made due to considerations of time and feasibility under local conditions. The individual tests are briefly outlined below.

Neurobehavioral Evaluation System (NES) Finger Tapping Test (12,40). In this task, the child tapped a key for 15 s first with the preferred hand for practice, then twice with the preferred hand, then twice with the nonpreferred hand. Then the two keys were tapped with both hands twice for the same interval. Score was the maximum number of taps in each condition. This task is a measure of manual motor ability that focuses specifically on speed.

NES Hand–Eye Coordination Test (12,40). In this test, the child had to follow a sine-wave curve on the computer screen using a joy-stick. Score was average deviation from the stimulus in the best two trials. This test is a measure of manual motor coordination.

Tactual Performance Test (27). On this test the child was placed blindfolded before a formboard that contained six cutout geometrical shapes. In front of the board were the six forms that the child had to place appropriately on the board first with the preferred hand, then the nonpreferred hand, then both hands together. Time to completion of each task was recorded. Some children had insufficient time to complete all tasks, and the score for the preferred hand only was used. This test measures tactile processing.

NES Continuous Performance Test. This test was modified so that the children saw a series of animal silhouettes flashed on the computer screen (12,40). The child was required to press a button every time a cat appeared over a 4-min interval. Scores were the total number of missed responses and the average reaction time during the last 3 min. This test measures vigilance/attention.

Wechsler Intelligence Scale for Children—Revised (WISC-R) Digit Spans (57). Digit spans of increasing length were presented until the child failed both trials in a series of the same length. Score was total number of correct trials forward only. This is a test of attention and tracking.

WISC-R Similarities (57). This verbal task required the child to identify a common category linking two objects or ideas. Scoring was done by WISC-R criteria. This test is generally considered to be a measure of reasoning and cognitive flexibility.

WISC-R Block Designs (57). Children used red and white blocks to replicate 2×2 and 3×3 stimulus designs presented on cards. Scoring was based on the WISC-R criteria combining a basic score for producing a correct design with bonus points for quick performances. This is usually considered to be a test of visuospatial organization and reasoning.

Bender Gestalt Test (50). The children drew the nine Bender figures, first to copy and then from immediate recall. The Göttingen system (50) was used to score the errors in the copying condition, and the number of recognizable figures was summed for the recall score. These results were subsequently scored by two independent psychologists who had no information on the individual children. This visuospatial test has been used extensively as a nonspecific measure of brain damage but may also be informative in regard to the functioning of the right cerebral hemisphere. The Göttingen scoring system has been shown to be particularly sensitive to metal-related dysfunctions (56).

California Verbal Learning Test (Children) (16). A list of 12 words that can be semantically clustered into categories was given over five learning trials, followed by presentation of an interference list, followed by spontaneous and cued recall of the initial list (short recall). Later, spontaneous and cued recall of the list was requested (long or 20-min delayed recall) and a recognition test was administered. Scores used were total correct in each learning and memory condition. This test assesses several components of short-term memory.

Boston Naming Test (35). On this test children were presented with line drawings of objects that they were then asked to name. If no correct response was produced in 20 s, a semantic cue was provided describing the type of object represented. If a correct response still did not occur, a phonemic cue consisting of the first two letters of the name of the object was presented. Scores of this language test were total correct without cues and total correct with semantic and phonemic cues.

Nonverbal Analogue Profile of Mood States (38). Cartoon pictures of faces depicting various mood states (happy, tired, afraid, angry, energetic, sad, confused, tense) were presented with a nonverbal response scale consisting of a vertical 10-cm line between the neutral face and the one depicting a mood state. Score was the distance in centimeters from the neutral face. Two composite scores were calculated as the sum of the two positive moods (happy and energetic) and the sum of the six negative moods. This test is an experimental measure of mood.

Exposure Data

As the primary indicator of prenatal exposure to methylmercury, we used the mercury concentration in cord blood (Table 1). Almost all mercury in the blood was methylmercury (24). The cord blood concentration measured in μg/l (nmol/l) averages approximately five times the maternal hair concentration in μg/g (nmol/g) (24). Mercury analyses of hair samples obtained at 12 months of age were also available (26). A high quality of these analyses has been documented (24,26).

In addition, hair obtained from the clinical examination at age 7 years was analyzed for mercury. For this purpose, we used flow-injection cold-vapor atomic absorption spectrometry after digestion of the hair sample in a microwave oven (13,26). The total analytical imprecision for this analysis was estimated to be 3.2% and 4.2% at mercury concentrations of 4.8 and 11.7 μg/g, respectively. Accuracy was ensured by using certified reference material CRM 397 (BCR, Brussels, Belgium) as quality control material; the mercury concentration found averaged 11.68 μg/g, compared to the assigned value of 11.93 ± 0.77 μg/g. The high quality of this analysis is comparable to previous data (24,26). Results in μg may be converted to nmol by multiplying by 5.0.

As increased exposure to polychlorinated biphenyls (PCBs) may occur in the Faroes from ingestion of blubber from pilot whales (6), concentrations of major PCB congeners were determined in 436 stored samples of umbilical cords from the 443 children examined in 1993. Due to the extent of this analytical effort, the samples were split between two laboratories. After homogenization and clean-up, major PCB congeners were measured by similar methods involving capillary gas chromatography with electron-capture detector (8,52). Quality control procedures in both laboratories included analyses of Community Bureau of Reference (BCR) certified reference material (CRM) 350, and comparison was further secured by analysis of bovine umbilical cords spiked with representative PCB congeners at two concentration levels. Adjustment for incomplete recovery of PCB congeners was not attempted, as the two laboratories and individual analytical runs differed only little in this respect. In accordance with previous findings in the Faroes (25), the sum of the three major congeners (138, 153, and 180) multiplied by 2.0 was used as a measure of the total PCB concentration.

Statistical Analysis

Because most of the neurobehavioral test results and the logarithmic transformation of the exposure parameters approximated a Gaussian distribution, parametric tests were used. For some neuropsychological outcome variables that deviated from this distribution, other transformations were attempted. The importance of mercury exposure as a risk indicator for neurobehavioral dysfunction was determined in multiple regression analyses. For reason of uniformity, two-sided p-values are given throughout.

Age (median, 6.83 years) and sex were considered obligatory independent variables in all regression analyses. The presence of strabismus and eye-glasses was used to stratify the VEP data, and BAEP results were stratified according to the presence of current middle ear infection. Body sway was adjusted for height.

For the neuropsychological test results, maternal cognitive function was considered an obligatory independent variable, as represented by the score on Raven's Progressive Matrices (49) obtained in connection with the examination of the child (N = 848). Additional covariates that might affect neuropsychological functions were chosen from empirical and theoretical considerations (17,42). Obstetric parameters were not associated with mercury exposure, except birth weight, which increased at higher mercury concentrations (23). Major medical risk factors for neurobehavioral dysfunction (i.e., low birth weight, small-for-date, and history of head trauma and meningitis) each occurred in a small number of children and did not show any relationship with mercury exposure. For practical reasons, they were combined into a single risk parameter. Other potential predictors, such as neonatal jaundice (as documented by serum-bilirubin analyses), and duration of breastfeeding (26), were not related to mercury exposure and were not further considered. Maternal smoking (60% were nonsmokers) and alcohol use (76% were abstainers) during pregnancy were known from a questionnaire filled in at the time of parturition (24). Current information on potential confounders was obtained from a detailed questionnaire filled in by the mother. For the socially highly homogeneous Faroese society where social status scales from other countries are not appropriate, we used vocational or professional education of each parent and the employment status of the father as supplementary indicators of social background. The child's acquaintance with computers and computer games was considered an obligatory independent variable for the computer-assisted tests. The major risk factors are listed in Table 2. The instances of observer differences were also taken into regard.

Regression models were initially developed separately for boys and girls, and an interaction parameter between sex and mercury exposure was considered in joint models. In addition to age, sex, and maternal Raven score, important predictors were identified for each neuropsychological test outcome by backward elimination (one-sided $p < 0.1$). Predictors that were important for more than three neuropsychological tests were included in the regression models for all of these tests. This procedure ensured that related test outcomes were adjusted for the same covariates, although adjustment for unrelated covariates may cause an increase in the error term for the mercury regression coefficient. For each outcome variable, the effect of addition of further covariates was also tested.

In further explorations of the relationship between mercury exposure and neurobehavioral dysfunction, regression equations with the same covariates were determined after exclusion of children whose mothers had a hair mercury concentration of 10 μg/g or more at the time of parturition. Additional analyses were also carried out for the total group with the hair mercury concentrations as the mercury exposure variable. Also, similar regression models were calculated for the children examined in 1993 where the PCB concentration was

TABLE 1

MERCURY EXPOSURE PARAMETERS* FOR 917 FAROESE CHILDREN FOLLOWED FROM BIRTH TO 7 YEARS OF AGE

Mercury Concentration	N	Geometric Average	Interquartile Range	Correlation With Blood
Cord blood (μg/l)	894	22.9	13.4–41.3	(1.00)
Hair (μg/g)				
Mother at parturition	914	4.27	2.6–7.7	0.78
Child, 12 months	527	1.12	0.69–1.88	0.48
Child, 7 years	903	2.99	1.7–6.1	0.33

*To convert μg to nanomoles, multiply by 5.0.

available for inclusion as an independent variable. For the computer-assisted tests, regression models were developed for the two examination years separately due to the limited medical supervision during the second year. Differences between these models were tested by *F*-tests, and joint models were developed by backward elimination ($p < 0.1$) of interaction parameters (i.e., product terms with examination year).

In addition, we used the Peters–Belson approach (10) to calculate adjusted outcome variables based on data from the children with a mercury exposure in the lowest quartile [i.e., cord blood mercury concentrations below 15 μg/l (75 nmol/l)]. Regression coefficients associated with a *p*-value below 0.1 (backward elimination) within this control group were used to adjust the outcome data for all children. The adjusted values were then entered into simple regression equations with the cord blood mercury concentration as the only independent variable. In the presence of confounding, this method may provide an unbiased estimate of the true effect of mercury exposure (10).

TABLE 2

GEOMETRIC AVERAGES FOR CORD BLOOD MERCURY CONCENTRATIONS (IN μg/l) IN RELATION TO POSSIBLE PREDICTORS OF NEUROBEHAVIORAL PERFORMANCE

Predictor	*N*	Mercury in Cord Blood	*p*-Value
Alcohol during pregnancy			
Occasionally	212	19.8	0.032
None	681	23.9	
Smoking during pregnancy			
Yes	352	22.3	0.295
No	541	23.7	
Sex			
Boy	449	23.9	0.127
Girl	445	21.9	
Risk factors in past medical history			
Yes	128	22.4	0.784
No	766	22.9	
Current residence			
Faroe Islands	834	23.4	0.025
Denmark	60	16.1	
Mother Faroese			
Yes	849	23.8	<0.001
No	41	10.4	
Mother unskilled			
Yes	472	24.6	0.01
No	411	21.2	
Father unskilled			
Yes	268	23.9	0.35
No	616	22.5	
Father employed			
Yes	739	23.4	0.110
No	151	20.7	
Child's computer acquaintance			
None	284	23.1	0.371
Some	359	23.7	
Much	243	21.5	
Day care			
Yes	467	20.5	<0.001
No	427	25.8	
Older siblings at home			
Yes	586	24.2	0.005
No	308	20.4	
Younger siblings at home			
Yes	506	22.4	0.35
No	382	23.6	

RESULTS

Relation of Mercury Exposure to Other Predictors

Mercury concentrations in cord blood were most closely associated with the concentrations in maternal hair at parturition and less so with later exposure measurements (Table 1). The child's age at examination was poorly correlated with the logarithmic transformation of the cord blood mercury concentration ($r = 0.04$, $p = 0.33$). Also, maternal age at parturition showed a poor correlation with cord blood mercury ($r = 0.05$, $p = 0.12$). However, mercury in cord blood was significantly related to several other factors that may act as predictors of neurobehavioral function (Table 2). Some of these associations are probably due to the lower exposure to mercury in the capital area of Tórshavn, where pilot whale is landed only rarely, whereas access to education and day care is easier. A significant association between mercury and maternal Raven score ($r = -0.13$, $p < 0.001$) was at least partially due to the average score being 0.7 points higher in Tórshavn. Although most women were abstainers during pregnancy, the 217 women (23.8%) who had occasionally ingested alcoholic beverages showed lower cord blood mercury concentrations (Table 2). Again, although alcohol intake is limited among Farose women, alcohol is more easily available in Tórshavn. Any confounding bias due to this association would most likely be small and toward the null hypothesis (23).

Physical Examination

The children were generally found to be in good health. A total of seven children had neurological diagnoses (mainly epilepsy) and were excluded from all statistical calculations. As expected for this age group (41), the individual tasks on the neurological examination were successfully completed by the majority of the children. The total number of tests completed with optimal performance was unrelated to the mercury exposure. Among the three most difficult tests that were performed optimally by less than 60% of the children, reciprocal motor coordination and simultaneous finger movement showed no relation to mercury exposure. However, in the finger opposition test, the 465 children with optimal performance had a geometric mean mercury concentration of 21.8 μg/l (109 nmol/l), compared to 23.9 μg/l (119 nmol/l) in the 425 with questionable or deficient performance ($p = 0.04$).

Visual acuity was not associated with mercury exposure. Contrast sensitivity was generally excellent but unrelated to mercury concentrations. The 82 children with current middle ear infection had slightly increased mercury exposure levels. These children were excluded in statistical analyses involving auditory performance. A weak positive association between audiometric hearing thresholds and mercury exposure was not significant after the exclusion.

Responses to the questions from the Child Behavior Checklist were not associated with the degree of mercury exposure. Also, other questionnaire information on current health status did not reveal any clear associations with mercury.

Neurophysiological Tests

Almost all of the children completed all electrophysiologic tests (Table 3). Girls showed significantly shorter latencies of

TABLE 3

RESULTS OF NEUROPHYSIOLOGICAL TESTS IN CHILDREN WITHOUT NEUROLOGICAL DISEASE AND THE ADJUSTED REGRESSION COEFFIENT FOR THE LOGARITHMIC TRANSFORMATION OF THE CORD BLOOD MERCURY CONCENTRATION

Test	*N*	Mean	Interquartile Range	Regression Coefficient	*p*-Value
Visual evoked potential latencies at 15′ (ms)*					
N75	813	76.1	73.0–79.0	0.21	0.70
P100	813	110.4	105–115	−0.75	0.33
N145	813	147.3	140–154	−0.99	0.37
Brain stem auditory evoked potential latencies at 40 Hz (ms)†					
I	824	2.04	1.88–2.21	0.043	0.10
III	824	4.28	4.09–4.47	0.053	0.06
V	824	6.17	6.01–6.32	0.059	0.01
Computerized posturography (area in cm^2)					
Eyes open, no foam	898	7.15	4.46–8.73	−0.04	0.90
Eyes closed, no foam	892	12.60	6.96–15.1	−1.54	0.09
Eyes open, with foam	896	11.15	7.36–13.1	−0.43	0.40
Eyes closed, with foam	891	18.16	11.0–21.7	−0.19	0.86
Heart rate variability (coefficient of variation in %)					
R–R interval	889	8.33	5.12–10.8	−0.39	0.29

*Children with glasses and strabismus excluded.
†Children with middle ear infection on the right ear excluded.

evoked potentials than boys, but age was of minimal importance. Children with strabismus or wearing eye-glasses had longer VEP latencies and were therefore excluded from the regression analyses. Both in the 15′- and 30′-conditions, some peak latencies tended to decrease at higher mercury exposure levels. For BAEP, children with current middle ear infection on the right ear were excluded from the regression analyses. Peak I at 40 Hz was slightly delayed at increased prenatal mercury exposures, and the delays for peaks III and V were significant (Table 3). However, interpeak latencies showed no association with mercury. The same pattern was seen with the 20-Hz condition where the regression coefficients were 0.056 ($p = 0.02$) and 0.046 ($p = 0.04$) for peaks III and V, respectively. After adjustment for sex, height, and age, body sway showed slight negative associations with mercury exposure in all four conditions. Although not significant, this tendency suggests less body sway at higher exposures. The relative variability of the R–R interval on the electrocardiogram decreased only slightly with increased mercury exposure.

Neuropsychological Tests

The neuropsychological test battery was completed by most children (Table 4). Failure to complete the paper-and-pencil tests within the 60 min available and to complete the Tactual Performance Test within 20 min most frequently occurred in the youngest children. Despite ample time for the mood test, this test seemed to be the most difficult one for the children to comprehend; the geometric average cord blood mercury concentration was 29.5 μg/l (147 nmol/l) in the 85 children who failed or refused this test, compared to 22.3μg/l (111 nmol/l) in the children who completed the test ($p = 0.003$).

Most of the results approximated a Gaussian distribution, but for WISC-R Block Designs a square-root transformation was used, as was a logarithmic transformation of the number of missed responses on the Continuous Performance Test. The test results were generally associated with age and maternal Raven score, whereas the significance of sex and other predictors varied. On most tests, children with obstetric or postnatal risk factors performed more poorly than the rest of the children. Occasional alcohol intake during pregnancy was unexpectedly associated with improved performance in the children; because alcohol intake was also associated with lower mercury exposures, this parameter was not included in the regression models. Children in daycare showed better results, particularly on the Boston Naming Test, Bender Visual Gestalts, and WISC-R Block Designs. Both maternal and paternal vocational or professional training, and paternal employment also appeared to be important predictors in most of the models. These covariates were therefore included in the final models for all neuropsychological test outcomes (Table 4). For five outcome variables, an additional predictor remained significant ($p < 0.1$) after backward elimination. Addition of these variables to the models changed the mercury effect only slightly, although the *p*-value for Digit Spans decreased to 0.04. Models for boys and girls were similar, and no interaction between sex and mercury exposure was identified.

On most computer-assisted test outcomes, performance was highly influenced by acquaintance with computer games. However, the models for the second year data, where medical supervision was lacking, differed from those for the first year. When the Finger Tapping Test and the Hand–Eye Coordination test data from the 2 years were pooled, the computer acquaintance by year interaction term was the only interaction term that remained in the model after backward elimination ($p < 0.1$). As computer acquaintance was scored by the examiner, a difference between the 2 years was deemed plausible. However, for the CPT reaction time and missed responses, several additional interaction parameters remained in the model after backward elimination with a *p*-value criterion of 0.1. The effect of mercury was significant for the first year, but not for the second year alone, where the residual was larger. When the data for the 2 years were merged, the joint mercury regression coefficients for missed responses and reaction time were 0.02 ($p = 0.55$) and 17.6 ($p = 0.01$), respectively. Because supervision was stringent only during the first year, these data

TABLE 4

RESULTS OF NEUROPSYCHOLOGICAL TESTS IN CHILDREN WITHOUT NEUROLOGICAL DISEASE AND ADJUSTED REGRESSION COEFFICIENTS (BETAS) FOR THE LOGARITHMIC TRANSFORMATION OF THE CORD BLOOD MERCURY CONCENTRATION

Test	N	Mean	Interquartile Range	Multiple Regression		Peters–Belson Adjustment		Low-Level Exposure*	
				Beta	p-Value	Beta	p-Value	Beta	p-Value
NES2 Finger Tapping (maximum in 15 s)									
Preferred hand	901	42.9	39–47	−1.10	0.05	−1.18	0.03	−0.68	0.29
Other hand	901	41.2	37–45	−0.39	0.46	−0.37	0.47	−0.13	0.83
Both hands	895	55.5	47–64	−1.67	0.14	−1.86	0.08	−0.62	0.63
NES2 Hand–Eye Coordination (average of best two trials)									
Error score	897	2.60	2.41–2.80	0.034	0.19	0.033	0.20	0.033	0.28
Tactual Performance Test (s)									
Preferred hand	852	466	231–583	−14.3	0.63	−18.8	0.60	−11.3	0.76
NES2 Continuous Performance Test (first year only)									
Ln total missed responses	431	6.79	2–10	0.12	0.02	0.14	0.007	0.21	0.0005
Average reaction time (ms)	428	759	705–809	40.3	0.001	38.2	0.0002	46.9	0.0003
Wechsler Intelligence Scale for Children—Revised									
Digit Spans	889	3.8	3–5	−0.27	0.05	−0.27	0.05	−0.31	0.05
Similarities	746	7.4	5–10	−0.05	0.90	0.14	0.70	0.65	0.15
Squareroot Block Designs	888	14.6	7–20	−0.17	0.11	−0.25	0.02	−0.13	0.27
Bender Visual Motor Gestalt Test									
Errors on copying	895	29.4	26–33	0.67	0.15	1.04	0.03	0.71	0.19
Reproduction	841	3.0	2–4	−0.25	0.10	−0.16	0.31	−0.43	0.02
Boston Naming Test									
No cues	866	25.0	21–28	−1.77	0.0003	−1.66	0.0007	−1.42	0.01
With cues	865	27.5	24–31	−1.91	0.0001	−1.82	0.0002	−1.57	0.005
California Verbal Learning Test (Children)									
Learning	879	27.9	22–34	−1.25	0.12	−1.30	0.11	−1.55	0.10
Short-term reproduction	867	4.0	2–6	−0.57	0.02	−0.63	0.009	−0.74	0.009
Long-term reproduction	837	4.4	2–7	−0.55	0.05	−0.64	0.02	−0.56	0.08
Recognition	830	10.4	10–12	−0.29	0.15	−0.28	0.15	−0.22	0.34
Nonverbal Analogue Profile of Mood States									
Average positive moods	825	59.8	48.0–80.5	2.61	0.31	2.39	0.34	3.66	0.20
Average negative moods	825	36.0	16.3–52.6	−0.04	0.99	0.17	0.94	1.83	0.51

*Maternal hair-mercury below 10 μg/g.

were chosen for development of the final regression model (Table 4).

The multiple regression analyses almost uniformly indicated decreased function with increased prenatal mercury exposure level (Table 4). WISC-R Similarities was the last test performed by the psychologist during the first 2 months of examinations, and a high proportion of the youngest children failed to complete it due to lack of time. For the children older than 6 years and 10 months, where attrition was only 20% during this initial period, the regression coefficient for mercury exposure was −1.86 ($p = 0.04$). This tendency was not seen when the test was carried out by a technician at the neurophysiological test station.

The regression coefficients in Table 4 can be compared to those obtained for age (in years). For the Boston Naming Test with cues, the regression coefficient for age was 4.1. Accordingly, a regression coefficient close to −2 for the logarithmic transformation of the mercury concentration (Table 4) would mean that a 10-fold increase in mercury exposure was associated with a decreased score on this test similar to a developmental delay in this age group of about 5 months. Likewise, the effects of a 10-fold increase in mercury exposure were similar to age-associated delays of about 8 months in WISC-R Digit Spans forward and 6–7 months in short-term reproduction on the California Verbal Learning Test (Children). For reaction time, a 10-fold increase in mercury exposure resulted in a delay corresponding to 4–5 months of age. For a doubling in mercury exposure, these delays should be multiplied by 0.3 (log 2).

Most regression coefficients obtained by Peters–Belson adjustment were greater than those obtained by multiple regression analysis (Table 4) and were associated with lower *p*-values. This result is in accordance with the expectation, as fewer predictors were significant at the 0.1 level in the subset of children with the lowest exposure. However, the error terms for mercury could still increase, as the covariate adjustment based on this subset could be less precise. For the Similarities test, the Tactual Performance Test, and the mood scale, *p*-values for the association with mercury exposure remained much above 0.05.

Feasibility and psychometric properties of the tests in this population would be likely to affect the association of the test results with risk factors. Main emphasis should therefore be placed on tests completed by the majority of the children and with the widest variability in scores. For further analysis, we therefore selected one test with appropriate psychometric properties that reflected each of the following brain functions: motor function (Finger Tapping with preferred hand), attention (CPT reaction time), visuospatial performance (error score on the Bender Visual Motor Gestalt Test), language (Boston Naming Test score after cues), and memory (long-delay recall on the California Verbal Learning Test). These tests are also among those that show the most convincing effect of mercury in the regression analyses (Table 4). A small decrease in average performance in these tests may also be reflected in an increased proportion of children with poor performance. After Peters–Belson adjustment for covariates, children with scores in the lowest quartile were identified, and their distribution between quartile groups of mercury exposure is shown in Fig. 1. In accordance with Table 4, the results show that a significant trend is apparent for the attention, language, and memory tests.

Because a maternal hair mercury concentration of 10 μg/g (50 nmol/g) may be interpreted as an upper limit for "safe" exposure (45), multiple regression analyses with the cord blood mercury concentration as independent variable were repeated for children below this limit. Although this restriction diminished the number of observations by 15% and narrowed the range of blood mercury concentrations, the regression coefficients and p-values were quite similar to those obtained for the group as a whole (Table 4). This finding is in accordance with the trends illustrated in Fig. 1 for the lower three quartiles of exposure.

Relation to Other Exposure Tests

The maternal hair mercury concentration showed regression coefficients that were generally lower and less significant than the ones obtained with cord blood mercury as exposure indicator. However, for Finger-Tapping, the hair mercury concentration was a better predictor, especially for the both-hands condition where the regression coefficient was –2.69 ($p = 0.02$). Although the child's own hair mercury concentrations were lower (Table 1), the concentration at age 12 months was a significant predictor for Finger Tapping with both hands and CPT reaction time, whereas the concentration at the time of examination was significantly associated with CPT reaction time, Block Designs, and Bender Visual Motor Gestalt errors. All of these associations also suggested decreased performance at increased mercury exposures, but after adjustment for the cord blood mercury concentration, the hair mercury measures were not significantly related to any dysfunction.

The geometric mean PCB concentration of the 435 umbilical cords was 1.12 ng/g wet weight (interquartile range, 0.57–1.55 ng/g). The lipid content averaged 2.2 mg/g. The PCB concentration was significantly associated with the cord blood mercury concentration, but the correlation was better ($r = 0.41$) for the wet weight PCB concentration than for the lipid-adjusted concentration ($r = 0.31$). Four neuropsychological

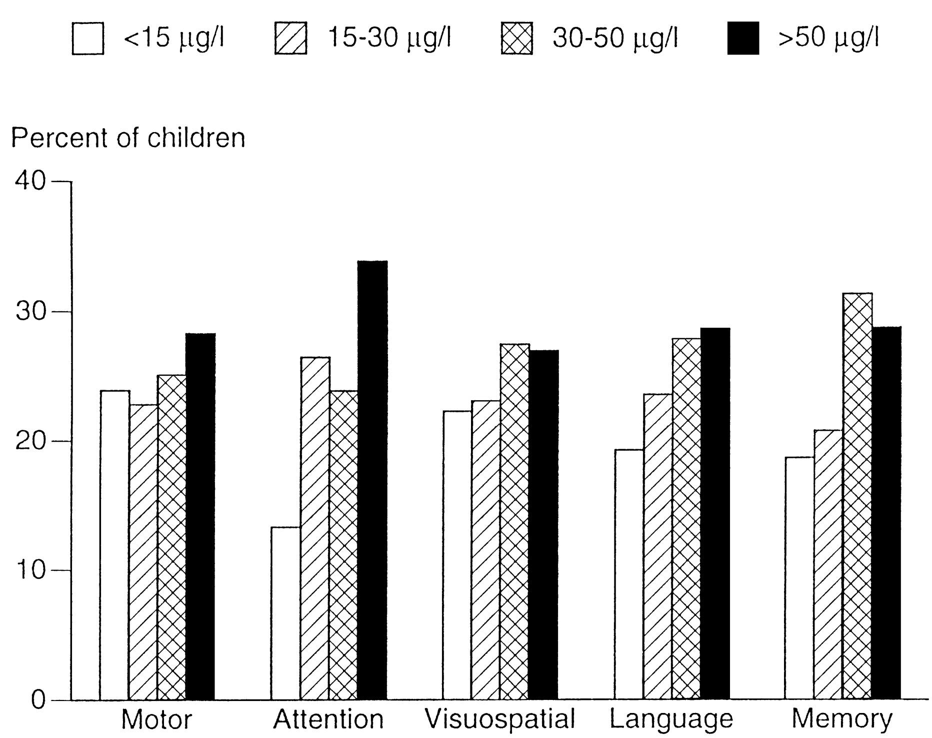

FIG. 1. Prenatal mercury exposure levels (in quartile groups) of Faroese children with scores in the lowest quartile after adjustment for confounders. For each of five major cognitive functions, one neuropsychological test with a high psychometric validity was selected. Motor: NES2 Finger Tapping with preferred hand (p-value for trend, 0.23). Attention: Reaction time on the NES2 Continued Performance Test ($p = 0.003$). Visuospatial: Bender Visual Motor Gestalt Test error score ($p = 0.16$). Language: Boston Naming Test score after cues ($p = 0.02$). Memory: California Verbal Learning Test (Children) long-delay recall ($p = 0.004$).

outcome variables showed an association with the logarithmic transformation of the wet weight PCB concentration ($p < 0.1$) before adjustment for mercury. For comparison, nine outcome variables showed mercury-associated decrements in this subset of children. For the four tests that were associated with both neurotoxicant exposures, regression analyses were repeated with mercury alone and with both of the two exposure parameters included as independent variables (Table 5). PCB had almost no effect on the magnitude of the regression coefficients for mercury, except for the Boston Naming Test, although, due to increases in error terms, three of the mercury regression coefficients were no longer significant. Also, the regression coefficients for mercury adjusted for PCB are not significantly different from those not adjusted, and the *p*-value for removal of both exposure parameters is quite similar to the *p*-value for mercury alone. Nonetheless, especially for the Boston Naming Test, the PCB concentration appeared to be an important predictor.

DISCUSSION

This study is one of the largest and most intensive performed so far to elucidate the possible neurobehavioral effects of prenatal exposures to neurotoxicants. Each child went through 5 h of detailed examinations, and the clinical team spent almost 6 months in the field. The very high participation rate suggests that any selection bias would be limited and unlikely to affect the relationship between mercury exposure and neurobehavioral performance in this study. Although multiple comparisons were done, almost all associations with the neuropsychological results point in the same direction. Because several genetic, social, and environmental factors can affect neurobehavioral function (17,42), covariates suspected of being relevant in the Faroese society (Table 2) were considered in the regression analyses. The maternal Raven score was the most important confounder, although it seemed to be independent of seafood habits. Pilot whale consumption and mercury exposure are less extensive in the capital area of Tórshavn, and slight adjustments resulted from inclusion of covariates that differ locally. Maternal alcohol intake during pregnancy was not associated with any detectable adverse effects and was therefore not further considered. Tradition, religious beliefs, and the ban of alcohol in shops and restaurants are important reasons for the alcohol intake in the Faroes being the lowest among the Nordic countries, with an average annual alcohol consumption for Faroese women of 1.24 l in 1991 (P. W., unpublished results). Confounding bias therefore appears to be of limited importance in this study performed in this relatively homogeneous community with limited potentials for other neurotoxic exposures. Some statistical imprecision or even overadjustment may have resulted from the inclusion of covariates with uncertain influence on neuropsychological test results. This possibility is supported by the lower *p*-values for several regression coefficients obtained after Peters–Belson adjustment, which is likely to be more accurate (10). On multiple regression analysis, 9 out of 20 neuropsychological measures showed mercury-associated deficits at a one-tailed statistical significance level of 0.05 (Table 4). After Peters–Belson adjustment, decreased performance on 11 tasks was significantly related to increased mercury exposure.

As the methylmercury exposure originates from seafood, including pilot whale, the significance of other components of marine food must be considered. In addition to methylmercury, seafood also contains essential nutrients that could act as effect modifiers. Thus, an increased intake of n-3 fatty acids from marine food is thought to protect against low birth weight (48), a known risk factor for neurobehavioral dysfunction (17,42). The positive association between mercury exposure and birth weight seen in this cohort (23) could therefore bias the study toward the null hypothesis. Further, experimental evidence suggests that n-3 fatty acids may protect against some forms of neurotoxicity (7). In addition, optimal development of the visual system depends on an ample supply of these essential nutrients (5). Signs of methylmercury toxicity to the visual system (29,39) were not observed in the Faroese children. Their excellent contrast sensitivity and the tendency of decreased VEP latencies may rather point to a beneficial effect of seafood. Accordingly, the reliance on a seafood diet could perhaps compensate for or counteract some adverse effects due to methylmercury, whether from pilot whale or other seafood.

Other components of seafood could potentially bias the findings in the opposite direction and erroneously increase an apparent effect of mercury exposure. Thus, prenatal exposure to other contaminants such as PCBs has been linked to adverse effects on the nervous system (although the potential confounding due to methylmercury exposure was not determined in these studies) (21,33). Whale blubber with high concentrations of organochlorine compounds (6) may be eaten separately by some Faroese or with dried fish or whale meat. Thus, the average PCB concentration in human milk from the Faroes is considerably increased compared to levels otherwise

TABLE 5

REGRESSION COEFFICIENTS (BETAS) FOR EFFECTS OF LOGARITHMIC TRANSFORMATIONS OF MERCURY BEFORE AND AFTER ADJUSTMENT FOR PCB CONCENTRATIONS ON NEUROPSYCHOLOGICAL TEST RESULTS DURING THE FIRST YEAR

	Before Adjustment		After Adjustment for PCB			
				p-Values		
Neuropsychological Test	Beta	*p*-Value	Beta	Mercury	PCB	Both
Continuous Performance Test						
Average reaction time (ms)	39.3	<0.001	37.8	0.002	0.64	0.001
Boston Naming Test						
No cues	−1.58	0.04	−1.04	0.21	0.16	0.05
With cues	−2.03	0.007	−1.36	0.10	0.08	0.008
Califorania Verbal Learning Test (Children)						
Long-term reproduction	−0.99	0.03	−0.78	0.11	0.26	0.05

reported from Scandinavia (25). Although statistically significant, the association between mercury in cord blood and PCB in cord tissue showed substantial scatter. In a subsequent study of almost 200 births (unpublished findings), we have found a similar association between mercury in cord blood and PCB in the mother's milk. These results are meaningful, because it would be very unlikely that similar proportions of fish, whale meat, and whale blubber were consumed by all mothers. Dietary questionnaire information (24) also shows considerable variation in food preferences. The regression analyses for almost one half of the children (Table 5) showed that inclusion of PCB exposure barely affected the regression coefficients for mercury, except with regard to the Boston Naming test. However, PCB is not known to specifically affect language, and the association could therefore be spurious. Because specific PCB congeners and other organochlorine contaminants would probably be closely associated with the total PCB concentration, these contaminants would also be unlikely sources of possible confounding. Given the well documented neurotoxicity of methylmercury (9,15,18,44,45,54), these considerations suggest that the relationships found in the present study could very well be causal.

The mercury concentration in hair has been used in the past as the most convenient exposure parameter. Although the mercury concentration may be affected by external contamination, hair color, hair treatment, and growth rate (53), the ease of hair sampling and storage has made this parameter useful for field studies. The distance of the hair sample from the root will determine the time period reflected by the analysis. Laboratory quality is also of concern; the quality of the maternal hair mercury determinations in the present study (24) was carefully ensured by the laboratory that was also responsible for the measurements of hair samples from another population study (14,46).

The cord blood concentration directly reflects the exposure of the fetus and was therefore used as the primary exposure measure in this study. With a biological half-life of methylmercury in blood of up to 2 months (45), this parameter reflects mainly exposures during the last trimester of gestation. This exposure indicator appeared to be the best predictor of neurobehavioral dysfunction, with the possible exception of motor speed, where the maternal hair mercury concentration showed larger regression coefficients. As the concentration in hair is more likely to reflect exposure levels occurring further in the past, motor function could be hypothesized to be more sensitive to mercury exposure during earlier parts of gestation. Of considerable interest is also the fact that the child's hair mercury concentrations at age 1 year and 7 years were much less useful as risk predictors. Although effects of postnatal mercury exposures cannot be excluded, this observation is in accordance with the particular neurotoxic potential of prenatal exposure.

The results in Table 4 must also be considered in the light of the psychometric properties of the individual tests. The computer-assisted neuropsychological tests record minute differences in the responses. Compared to scores such as the reproduction score on the Bender test (which showed only nine possibilities from 0 to 8), they are therefore statistically superior in detecting subtle neurobehavioral dysfunction. Further, some tests (Similarities, Hand–Eye Coordination, and mood) were too difficult for many children and were therefore less likely to reveal slight degrees of neurotoxicity in the children who managed to complete the tests. On the mood test, actual failure to complete the test was associated with increased mercury exposure, thus causing a bias in the results obtained from those who managed to complete the test. Other tests, including those shown in Fig. 1, exhibited appropriate psychometric characteristics for detection of neurotoxic effects in this population, and the associations with mercury exposure are therefore likely to be valid.

In contrast, the functional neurological examination and the neurophysiological measures appeared to be less sensitive to mercury-related dysfunctions. Although clinical effects have been recorded in a previous population study (19), the methods available are probably too crude and nonspecific to detect subtle dysfunctions in children. Among the neurophysiological tests, BAEP showed mercury-associated delays of peaks III and V (Table 3), but interpeak latencies were not related to mercury exposure. Similarly, past experience with neurophysiological measures in patients with serious methylmercury poisoning have not consistently shown results outside expected ranges (28,30–32,55).

Overall, the results suggest that several domains of brain function may be affected by prenatal methylmercury exposure. The findings (especially those involving language) suggest that this exposure has widespread effects on cerebral function, and they are consistent with the literature (9,15,18, 44,54) reporting widespread neuropathological involvement in prenatal methylmercury poisoning. A discernible, insidious effect seems to be present below a limit of 10 μg/g (50 nmol/g) for mercury in maternal hair. Although test scores obtained by most of the highly exposed children were mainly within the range seen in the rest of the children, regression coefficients suggest that a doubling in mercury exposure may cause a developmental delay of approximately 2 months for several functions. Such decrements in average cognitive function, especially if permanent, could well be of societal significance in the populations affected (47).

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