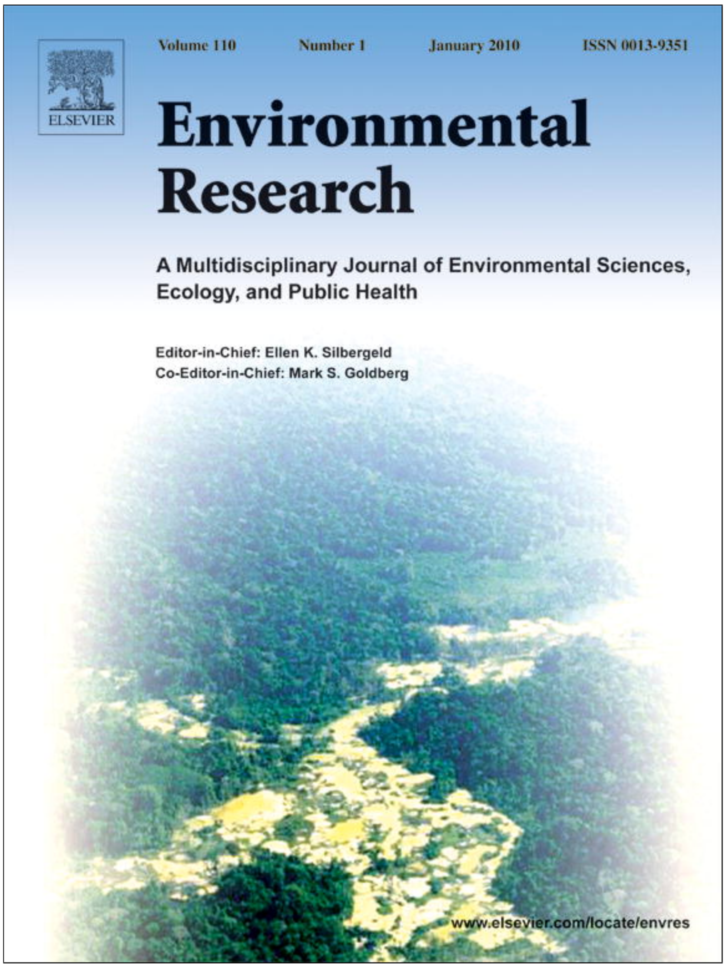
Volume 110
Number 1
January 2010
ISSN 0013-9351
ELSEVIER
Environmental Research
A Multidisciplinary Journal of Environmental Sciences, Ecology, and Public Health
Editor-in-Chief: Ellen K. Silbergeld
Co-Editor-in-Chief: Mark S. Goldberg
www.elsevier.com/locate/envres

Environmental Research 110 (2010) 96–104

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Hair mercury levels, fish consumption, and cognitive development in preschool children from Granada, Spain ☆, ☆☆

Carmen Freire [a,*], Rosa Ramos [a], Maria-Jose Lopez-Espinosa [a,b], Sergi Díez [c,d], Jesús Vioque [e], Ferrán Ballester [b], Mariana-Fátima Fernández [a]

[a] *Laboratory of Medical Investigations, San Cecilio University Hospital, University of Granada, CIBER de Epidemiología y Salud Pública (CIBERESP), 18071 Granada, Spain*
[b] *Centre for Public Health Research (CSISP), Conselleria de Sanitat, Generalitat Valenciana, CIBER de Epidemiología y Salud Pública (CIBERESP), Av Catalunya 21, 46020 Valencia, Spain*
[c] *Environmental Geology Department, ICTJA-CSIC, 08028 Barcelona, Spain*
[d] *Environmental Chemistry Department, IIQAB-CSIC, 08034 Barcelona, Spain*
[e] *Department of Public Health, Universidad Miguel Hernandez (UMH), Ctra. Nacional Alicante, CIBER de Epidemiología y Salud Pública (CIBERESP), 03550 San Juan de Alicante, Spain*

A R T I C L E I N F O

Article history:
Received 10 March 2009
Received in revised form
30 September 2009
Accepted 20 October 2009
Available online 11 November 2009

Keywords:
Hair mercury
Fish consumption
Children
Cognition
Spain

A B S T R A C T

The main source of human exposure to mercury is the consumption of fish contaminated with methylmercury, which may adversely affect early neurodevelopment. This study assessed mercury levels in hair of preschoolers in Spain, where fish consumption is elevated, with the aim of investigating the influence of their fish intake and other factors on mercury exposure, and evaluating their association with cognitive development. A population-based birth cohort from Granada (Spain) was studied at the age of 4 yr. Total mercury (T-Hg) levels were determined in children's hair, and daily fish intake was assessed by a food frequency questionnaire. The McCarthy Scales of Children's Abilities (MSCA) were used to assess children's motor and cognitive abilities. Complete data were gathered on 72 children, and multivariate analyses were performed to evaluate the influence of mercury exposure and fish intake on MSCA outcomes. Mean concentration of T-Hg in hair was 0.96 μg/g (95% confidence interval=0.76; 1.20 μg/g). T-Hg levels were associated with higher frequency of oily fish consumption, place of residence, maternal age, and passive smoking. After adjustment for fish intake, T-Hg levels ≥ 1 μg/g were associated with decrements in the general cognitive (−6.6 points), memory (−8.4 points), and verbal (−7.5 points) MSCA scores. Higher mercury exposure in children from this Mediterranean area was associated with cognitive development delay. Studies on the putative benefits of fish intake during early development should consider mercury exposure from different fish species.

1. Introduction

Mercury is a heavy metal from both natural and anthropogenic sources that is widespread and persistent in the environment (ATSDR, 1999). Once released into the environment, inorganic mercury is deposited in aquatic media and becomes methylmercury (MeHg), its most toxic organic form, which bioaccumulates in marine organisms and is biomagnified through the food chain (Boening, 2000). Humans can be affected by mercury from amalgam fillings, thermometers, fossil-fuel emissions, preservative vaccines, and cosmetic products, among others (Counter and Buchanan, 2004), but the main source of human exposure is consumption of MeHg-contaminated fish (US Environmental Protection Agency, 2009)

MeHg is highly and selectively toxic to the central nervous system, especially in the foetus (Díez, 2009). Thus, chronic prenatal exposure to MeHg may results in neurological disabilities (Jedrychowski et al., 2006; Oken et al., 2005), including language, learning, and attention deficits and, to a lesser degree, motor and visual-spatial impairment (Castoldi et al., 2008). In two longitudinal-cohort studies in the Faroe Islands and New Zealand neurodevelopmental deficits in school-age children were associated with prenatal exposure to MeHg from maternal fish consumption (Crumpt et al., 1998; Grandjean et al., 1997). However, a study in the Seychelles Islands found no adverse effect on children's cognitive development (Myers et al., 2003;

☆ *Funding*: This study was supported by grants from the Consejería de Salud de la Junta de Andalucía [SAS 07/0133], the Spanish Ministry of Health [FIS 07/0252], Spanish Ministry of Science and Innovation (FPU-Program to C.F.; Juan de la Cierva Program-FSE to M.J.L.E.), and the European Commission [CONTAMED FP7-ENV-212502].

☆☆ *Approval by committee for human subjects*: Written informed consent was obtained from parents before the study, which was approved by the Ethics Committee of the San Cecilio University Hospital.

* Corresponding author. Fax: +34 958 249953.
E-mail address: cfreire@ugr.es (C. Freire).

0013-9351/$ - see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.envres.2009.10.005

Palumbo et al., 2000). Hence, the effects of chronic prenatal exposure to MeHg remain unclear and controversial (Oken et al., 2005, 2008). Fish is the primary source of nutrients considered to improve child development, such as omega-3 fatty acids (Cohen et al., 2005; Domingo et al., 2007a, b), and the balance between the benefits and risks of fish consumption has yet to be fully elucidated (Davidson et al., 2008; Oken et al., 2008). Three US studies reported that greater fish consumption in pregnancy was related to higher infant cognition but that higher prenatal mercury levels were associated with lower cognition (Lederman et al., 2008; Oken et al., 2005, 2008).

Based on the studies in Faroe, New Zealand, and the Seychelles, the US Environmental Protection Agency (EPA) adopted a reference dose (RfD) for MeHg of 0.1 μg/kg bw/day (USEPA, 2001). This RfD is based on neurodevelopmental effects associated with in utero exposure to MeHg from the maternal diet and is related to a maternal hair mercury concentration of 1.0 μg/g (NRC, 2000). Among the few studies addressing postnatal exposure and potential neurodevelopmental impairment, contradictory results were published by two cross-sectional studies from Brazil (Grandjean et al., 1999; Tavares et al., 2005), and another study in the Faroe Islands found no association between higher hair mercury levels in children and delayed neurodevelopment (Murata et al., 2002).

Scalp hair has been widely used as a biomarker of human mercury exposure (McDowell et al., 2004). Mercury in the hair correlates with mercury in the brain, and MeHg accounts for approximately 70–80% of total mercury (T-Hg) in hair (Cernichiari et al., 1995). Although investigations of mercury exposure and postnatal neurodevelopment have largely relied on concentrations in maternal hair, it has been suggested that children's hair mercury levels reflect exposure to the same dietary mercury sources as the mother (Marques et al., 2007).

Spain is considered one of the largest consumers of fish in Europe (Welch et al., 2002) and the second in the world (Ministerio de Sanidad y Consumo, 2009). The Mediterranean basin contains important cinnabar deposits, and long-lived predatory fish have higher mercury content in the Mediterranean than elsewhere (Renzoni et al., 1998).

The potential sub-clinical effects on neurodevelopment of low-level exposure to MeHg from contaminated fish remain unclear. The objectives of this study were to investigate exposure to mercury in preschool children from Granada, Spain, by quantifying hair T-Hg levels, to examine the influence of fish intake during infancy and other factors on this T-Hg exposure, and to evaluate their combined effect on cognitive and motor development at 4 yr of age in a cross-sectional analysis of a birth cohort.

2. Methods

2.1. Study area and population

The study sample was drawn from a cohort established in the province of Granada in South-eastern Spain (Freire et al., 2009) within the INMA (Environment and Childhood) study (Ribas-Fitó et al., 2006). The survey was conducted in the area served by the San Cecilio University Hospital (population, 512 000 inhabitants; 50 municipalities). The study area is located in a Mediterranean province with little industrial activity that is mostly devoted to agriculture practices and services.

From 2000 to 2002, 700 eligible mother–son pairs registered at the San Cecilio University Hospital were enrolled after delivery. The inclusion and exclusion criteria were published elsewhere (Lopez-Espinosa et al., 2007). A medical follow-up of the children was carried out 4 yr after delivery, including interviews, examination of growth assessment, neuropsychological evaluation, and biological sample collection. Between 2005 and 2006, 1 out of 3 mothers were randomly contacted and invited to participate in the cognitive testing of their children. A total of 220 boys were evaluated. Complete outcome data, information on hair T-Hg concentration, and frequency of fish intake were available for a subset of 72 subjects. No differences in study characteristics were found between this subset and the children without T-Hg measurements (Table 1). Written informed consent was obtained from parents before the study, which was approved by the Ethics Committee of our Hospital.

Table 1
Characteristics of study population.

	Children included (n=72)	Children not included (n=138)[a]
Child		
Place of residence (%)		
Rural	13	18
Sub-urban	21	21
Metropolitan	45	54
Urban	21	15
Weight at 4 yr (kg)	18.6	19.2
Height at 4 yr (cm)	105.0	105.1
Passive smoking at 4 yr (%)		
No	54	61
Yes	46	39
Age at evaluation (months)	51	51
Mother		
Age at delivery (yr)	33.5	33.0
Educational level (%)		
Only primary school	15	20
Secondary school	70	63
University	15	17
Occupational status (%)		
Unemployed	47	42
Employed	53	58
Parity at child's birth (%)		
0	43	40
1	40	42
≥2	17	18
Breastfeeding (weeks) (%)		
<2	8	18*
2–15	47	36
16–27	39	38
≥28	6	8
Smoking during pregnancy (%)		
No	78	75
Yes	22	25

[a] Children with data up to the age of 4-yr visit but not included in the analysis because of missing data on total hair mercury (T-Hg) concentration or fish intake at 4 yr.

* $p<0.05$ (difference between children included and not included).

2.2. Mercury exposure

A minimum of 10 mg of hair from the occipital scalp was collected for each child, placing samples in a plastic bag and storing them at room temperature until their dispatch to the Environmental Chemistry Department (IIQAB) of the CSIC (Barcelona, Spain) for analysis. T-Hg was analyzed using the AMA-254 analyzer from the Leco Corp. (Praha, Czech Republic) following a previously described method (Díez et al., 2007). Hair strands (5–6 cm) were weighed in a nickel boat and analyzed by catalytic combustion, preconcentration by gold amalgamation, thermal desorption, and atomic absorption spectrometry. The analytical procedure was validated by analyzing two human hair Certified Reference Material (CRM), the NIES CRM No. 13 and the IAEA-086, both obtained from the National Institute of Environmental Studies, Environmental Agency of Japan and the International Atomic Energy Agency (IAEA; Vienna, Austria), respectively. The limit of detection (LOD) for T-Hg was 0.0027 μg/g and limit of quantification (LOQ) was 0.009 μg/g.

The contribution of MeHg to T-Hg was assessed by analyzing MeHg in the 23 hair samples with the highest T-Hg levels, using a previously reported method (Díez and Bayona, 2002; Montuori et al., 2004). Briefly, the hair samples were digested in a 350 μl nitric acid solution (100 °C). Nitric acid (60%) was purchased from Quimivita (Barcelona, Spain). A cooled aliquot was transferred to a glass vial with an acetate buffer solution and dipentylmercury (as internal standard) and phenylmercury (as ethylation quality control). Finally, after derivatization with aqueous $NaBEt_4$, extraction was accomplished using solid-phase microextraction. The final detection was carried out using a gas chromatograph equipped with a cold-vapour atomic fluorescence spectrometry system. NIES CRM No. 13 was used to validate the method. LOD for MeHg was 0.040 μg/g and LOQ was 0.080 μg/g. Digestion recoveries were 75 ± 11%.

2.3. Fish consumption

A semi-quantitative food frequency questionnaire (FFQ) of 95 items was administered at the 4-yr visit to assess children's usual dietary intake. Parents were asked how often, on average, their child had consumed each food item over the past 12 months. The FFQ was an adaptation of the Harvard questionnaire (Willet et al., 1985) validated in adult Spanish populations (Vioque, 2006). The original food list was modified to include foods frequently eaten by children, and serving sizes were reduced to avoid potential intake overestimation. The questionnaire had nine possible responses, ranging from "never or less than once per month" to "six or more per day", and included four fish-intake items: assorted fried fish (generally white fish species, e.g., very small hake fish) (1 small serving), boiled or grilled white fish (e.g., hake, sole, golden bream) (1 serving), boiled or grilled oily fish (e.g., tuna, swordfish, salmon, anchovy) (1 serving), and canned fish (most of them small-medium size, e.g., tuna, mackerel, sardine). Frequency of consumption of each fish item was converted to average daily intake in grams.

2.4. Covariates

Information on maternal socio-demographic characteristics, reproductive history, parity, smoking during pregnancy, duration of breastfeeding, children's passive smoking, weight and length at birth and at 4 yr, age at which the children were introduced to the different food groups, and detailed residential address was gathered at delivery and the follow-up visit. The children's place of residence was classified as urban (city of Granada, 236 000 inhabitants), metropolitan (towns of > 20 000 inhabitants in city residential belt), sub-urban (town of 10 000–20 000 inhabitants), or rural (< 10 000 inhabitants).

2.5. Neuropsychological assessment

Cognitive and motor abilities were assessed using a standardized Spanish adaptation of the McCarthy Scales of Children's Abilities (MSCA) (McCarthy, 1972), which gives standardized test scores for five domains (quantitative, verbal,

Table 2
Total hair mercury (T-Hg) concentrations (μg/g) and general cognitive scores by characteristics of study population (n=72), INMA-Granada cohort, Southern Spain, 2000–2006.

	N(%)	T-Hg (μg/g)			General cognitive score[a]		
		GM	95% CI	p-value[b]	AM	95% CI	p-value[c]
Child							
Area of residence at 4 yr				0.03			0.01
Rural	9 (13)	0.49	0.19; 1.23		100.3	89.6; 111.0	
Sub-urban	15 (21)	0.89	0.44; 1.80		99.5	91.8; 107.1	
Metropolitan	33 (45)	0.95	0.75; 1.22	*	95.7	91.0; 100.5	
Urban	15 (21)	1.55	1.01; 2.36	**	110.1	102.1; 118.0	*
Weight at 4 yr (kg)				0.64			0.61
< 18	31 (43)	1.06	0.75; 1.51		99.1	93.3; 102.9	
18–21	28 (39)	0.90	0.60; 1.37		101.8	95.7; 107.9	
> 21	13 (18)	0.85	0.55; 1.29		101.0	91.4; 110.5	
Height at 4 yr (cm)				0.59			0.67
< 100	10 (14)	1.30	0.74; 2.27		96.4	88.4; 104.3	
100–108	41 (57)	0.91	0.66; 1.27		101.0	96.5; 105.5	
> 108	21 (29)	0.90	0.60; 1.36		100.0	92.5; 107.6	
Passive smoking at 4 yr				0.02			0.78
No	39 (54)	0.78	0.59; 1.03		99.6	94.8; 104.4	
Yes	33 (46)	1.21	0.84; 1.75	**	100.6	95.5; 105.7	
School term of evaluative				0.44			0.25
3rd year, 3rd term	29 (41)	0.97	0.67; 1.14		96.3	90.6; 102.1	
4th year, 1st term	19 (26)	1.07	0.67; 1.71		101.4	95.2; 107.7	
4th year, 2nd term	19 (26)	0.76	0.49; 1.18		102.3	95.9; 108.7	
4th year, 3rd term	5 (7)	1.41	0.48; 4.15		108.2	84.8; 131.6	
Mother							
Age at delivery (yr)				0.06			0.12
< 32	33 (46)	1.21	0.96; 1.55		97.2	92.1; 102.2	
≥ 32	39 (54)	0.78	0.55; 1.12	*	102.5	97.8; 107.2	
Educational level				0.54			0.03
Only primary school	11 (15)	1.19	0.72; 1.97		95.5	86.6; 104.3	
Secondary school	50 (70)	0.85	0.64; 1.13		98.9	95.6; 102.1	
University	11 (15)	1.32	0.76; 2.28		110.2	94.9; 125.5	**
Occupation status				0.48			0.07
Unemployed	34 (47)	0.90	0.65; 1.24		96.8	93.0; 100.6	
Employed	38 (53)	1.01	0.73; 1.40		103.0	97.5; 108.5	*
Parity at child's birth				0.10			0.02
0	31 (43)	0.80	0.54; 1.20		103.1	98.0; 108.1	
1	29 (40)	0.93	0.69; 1.25		94.6	89.5; 99.7	*
≥ 2	12 (17)	1.61	0.93; 2.79	**	105.6	95.5; 115.7	
Breastfeeding (weeks)				0.58			0.58
< 2 weeks	6 (8)	1.21	0.31; 4.70		92.3	82.6; 102.1	
2–15 weeks	34 (47)	0.98	0.70; 1.39		100.2	94.6; 105.8	
16–27 weeks	28 (39)	0.87	0.61; 1.23		101.4	95.9; 106.9	
≥ 28 weeks	4 (6)	1.07	0.34; 3.37		101.9	92.6; 111.1	
Smoking during pregnancy				0.84			0.45
No	56 (78)	0.97	0.76; 1.24		99.5	95.4; 103.7	
Yes	16 (22)	0.91	0.49; 1.69		102.0	96.6; 107.4	

GM: geometric mean; AM: arithmetic mean; CI: confidence interval.
*$p < 0.1$; **$p < 0.05$ for test of difference in means with the first category as reference.

[a] Mean score for the general cognitive is 100, with a standard deviation of 15.
[b] p-value for Mann–Whitney or Kruskal–Wallis test.
[c] p-value for t-test or ANOVA.

memory, perceptual-performance, and motor). A general cognitive score (GCS), which estimates global intellectual function (Jacobson et al., 1990), was calculated by combining the verbal, perceptual-performance, and quantitative scores. Two specifically trained neuropsychologists administered the MSCA in the Pediatrics Department of our Hospital. A strict protocol was applied to avoid inter-observer variability (Júlvez et al., 2007), including inter-observer training and three sets of quality controls (inter-observer reliability-tests) during the field work. The inter-observer variability was $< 5\%$. Neuropsychologists were blinded to the degree of the child's mercury exposure.

In accordance with previous INMA studies (Freire et al., 2009; Júlvez et al., 2007), MSCA items were reorganized into sub-scales for tasks highly associated with specific neurocognitive functions: verbal memory, working memory, memory span, gross motor, fine motor, and executive functions.

2.6. Statistical analysis

Because hair T-Hg and MeHg levels were not normally distributed, the Spearman test was used for their correlation and for correlation between T-Hg and fish intake (g/day). Neurodevelopment scores followed a normal distribution. The geometric mean (GM) of T-Hg levels and arithmetic mean (AM) of the GCS, with 95% confidence intervals (CI), were used for descriptive analysis. T-Hg levels were transformed into natural logarithms (log T-Hg) to improve normality. Neurodevelopmental outcomes were standardized to a mean of 50 points, with a standard deviation (SD) of 10. GCS were standardized to a mean of 100 and SD of 15. Non-parametric tests were used to compare log T-Hg values as a function of children's and maternal variables. Differences in the GCS were evaluated with the Student' s *t*-test or ANOVA.

Multivariate regression analyses were used to assess the association of fish consumption and other factors with T-Hg concentrations and MSCA scores. T-Hg exposure was dichotomized based on the EPA RfD-related hair concentration of 1 μg/g. Association of fish intake and other factors with T-Hg levels was therefore explored by linear regression and by logistic regression, evaluating variables associated with T-Hg levels at a *p*-value < 0.2 in bivariate analysis.

In order to assess the overall effect of T-Hg and fish intake on neurodevelopment, a model was first built for the GCS, adjusting for covariates that might influence child neurodevelopment: evaluator (1st or 2nd), child's school term at evaluation, place of residence, maternal age, parity, mother's education and occupational status, and fish intake (total and different fish types). The same models were constructed for the other psychological scores. MSCA score models were rerun with T-Hg dichotomous measures. Analyses were performed using SPSS version 15.0 (SPSS Inc., Chicago, IL, US) and STATA version 9.0 statistical packages.

3. Results

Mean age of the children at testing was 51 months (range=48–57 months). T-Hg and MeHg were quantified in all hair samples (n=72 and 23, respectively). GM of T-Hg concentrations was 0.96 μg/g (95% CI=0.8; 1.2 μg/g) and median was 1.04 μg/g. Mean MeHg concentration was 1.81 μg/g (95% CI=1.4; 2.3 μg/g). Spearman test showed that T-Hg and MeHg levels in the paired samples were highly correlated (r=0.88; $p < 0.001$). Fried and boiled/grilled white fish were the most frequently consumed (76% and 65% of children consumed ≥ 1 serving/week of fried and white fish, respectively). Child GCS ranged from 53.9 to 138.1 points. Bivariate association of T-Hg with psychological outcomes was only significant for working memory function (r=0.27; p=0.02).

T-Hg concentrations were significantly higher among urban children, passive smokers, and those with ≥ 2 siblings (Table 2). The GCS was also higher among urban children and among the children of employed mothers and those with university

Table 3
Total hair mercury (T-Hg) concentrations (μg/g) and general cognitive scores by fish consumption, INMA-Granada cohort, Southern Spain, 2000–2006.

	N(%)	T-Hg (μg/g)			General cognitive score[a]		
		GM	95% CI	*p*-value[b]	AM	95% CI	*p*-value[c]
Child's age at fish introduction				0.05			0.78
≤8 months	14 (19)	1.24	0.72; 2.15		102.0	92.9; 111.1	
9 months	33 (46)	1.12	0.81; 1.55		99.5	93.8; 105.2	
≥10 months	25 (35)	0.67	0.45; 0.99	**	99.8	94.8; 104.7	
Total fish consumption (g/day)				0.25			0.07
<25	18 (25)	0.66	0.38; 1.13		107.3	102.6; 111.9	
25–50	32 (45)	1.18	0.85; 1.65		96.1	89.9; 102.3	**
51–75	11 (15)	1.10	0.63; 1.92		100.0	91.4; 108.6	
>75	11 (15)	0.84	0.47; 1.48		100.0	92.7; 107.4	
Frequency of fish consumption							
All types of fish				0.50			0.02
<3 servings per week	28 (39)	0.83	0.53; 1.31		104.9	99.7; 110.1	
≥3 servings per week	44 (61)	1.04	0.82; 1.33		97.0	92.6; 101.4	**
Oily fish				0.01			0.72
Rarely/never	17 (23)	0.58	0.34; 0.99		100.5	94.2; 106.9	
1–3 servings per month	20 (28)	1.57	1.08; 2.29	**	97.8	90.5; 105.2	
≥1 serving per week	35 (49)	0.92	0.68; 1.24		101.1	96.0; 106.3	
Canned fish				0.07			0.19
Rarely/never	31 (43)	0.73	0.52; 1.03		97.9	92.6; 103.3	
1–3 servings per month	20 (28)	1.22	0.83; 1.77	**	98.3	91.2; 105.5	
≥1 serving per week	21 (29)	1.14	0.71; 1.84	*	104.9	99.0; 110.9	
White fish				0.36			0.71
Rarely/never	12 (17)	1.19	0.68; 2.06		96.9	89.2; 104.6	
1–3 servings per month	13 (18)	1.25	0.64; 2.45		98.9	90.0; 107.8	
1 serving per week	29 (40)	0.83	0.57; 1.23		102.4	95.8; 108.9	
≥2 servings per week	18 (25)	0.85	0.58; 1.26		99.3	93.9; 104.7	
Fried fish (varied)				0.07			0.12
Rarely/never	9 (13)	0.53	0.30; 0.94		105.6	96.3; 115.0	
1–3 servings per month	8 (11)	1.18	0.49; 2.82	**	103.5	90.7; 116.2	
1 serving per week	36 (50)	0.95	0.69; 1.31	**	101.3	96.5; 106.2	
≥2 servings per week	19 (26)	1.20	0.75; 1.86	**	93.6	86.6; 100.6	

*$p < 0.1$; **$p < 0.05$ for test of difference in means with the first category as reference.
GM: geometric mean; AM: arithmetic mean; CI: confidence interval.

[a] Mean score for the general cognitive is 100, with a standard deviation of 15.
[b] *p*-value for Mann–Whitney or Kruskal–Wallis test.
[c] *p*-value for *t*-test or ANOVA.

Table 4
Association of children's fish consumption and other characteristics with total hair mercury (T-Hg) concentrations (μg/g), INMA-Granada cohort, Southern Spain, 2000–2006[a]

	T-Hg (μg/g)[b]		≥1 μg/g T-Hg[c]	
	Exp(β)	95% CI[¥]	OR	95% CI
Area of residence				
Sub-urban	1.55	0.75; 3.22	6.12	0.57; 65.34
Metropolitan	1.85	0.98; 3.51**	4.76	0.56; 40.41
Urban	3.37	1.64; 6.93***	20.76	1.79; 240.12**
Maternal age (<32 yr)	1.63	0.40; 0.94**	5.65	1.41; 22.72**
Passive smoking at 4 yr	1.45	0.92; 2.29*	3.74	0.95; 14.79**
Oily fish consumption				
1–3 servings per month	2.86	1.56; 5.25***	12.91	1.40; 118.95**
≥1 serving per week	2.01	1.10; 3.66**	8.41	1.03; 68.54**
Canned fish consumption				
1–3 servings per month	1.12	0.66; 1.91	1.90	0.40; 9.09
≥1 serving per week	1.39	0.83; 2.31	2.09	0.45; 9.63
White fish consumption				
1–3 servings per month	0.94	0.46; 1.92	0.44	0.04; 5.00
1 serving per week	0.66	0.34; 1.29	0.28	0.02; 3.21
≥2 servings per week	0.67	0.32; 1.40	0.15	0.01; 1.96
Fried fish consumption				
1–3 servings per month	0.76	0.30; 1.94	1.87	0.08; 42.77
1 serving per month	1.13	0.57; 2.25	1.24	0.14; 11.16
≥2 servings per week	1.11	0.52; 2.34	1.51	0.14; 16.15

*$p<0.1$; **$p<0.05$; ***$p<0.001$.
CI: confidence interval; [¥]confidence interval for Exp(β); OR: Odds ratio.

[a] Multivariate models taking as reference group children living in rural areas, children with mothers ≥32 yr old, not living with smokers, and children consuming less than 1 serving per month of oily, canned, white, and fried fish.
[b] Linear regression model with log T-Hg level as dependent variable.
[c] Logistic regression model with dichotomous T-Hg level (<1 μg/g, ≥1 μg/g) as dependent variable.

education. Table 3 shows that T-Hg concentrations were significantly lower in children who started eating fish later (≥10 months). T-Hg levels were not associated with total fish consumption (g/day) ($p>0.10$); however, they were significantly associated with canned fish intake (g/day) (r=0.24; p=0.04) (data not shown). The GCS was associated with the frequency of total fish intake, finding a lower score for children consuming ≥3 servings/week. A higher frequency of oily, fried or canned fish consumption was associated with T-Hg levels, but not with the GCS.

In the multivariate analysis, area of residence, maternal age, passive smoking, and frequency of consumption of oily fish were independently associated with T-Hg levels, but the frequency of consumption of the other types of fish were not (Table 4). Children consuming 1–3 serving/month or ≥1 serving/week of oily fish had around 3- or 2-fold higher T-Hg levels, respectively, compared to children rarely or never consuming oily fish. T-Hg concentrations were 3-fold higher among urban children in comparison to rural children. Interaction between area of residence and oily fish consumption was not significant.

A detrimental effect of T-Hg exposure (≥1 μg/g) on memory and verbal memory was found (7.2 and 8.8-point decreases, respectively) after adjusting for frequency of total fish intake (Table 5). Fish intake ≥3 servings/week was associated with decreases (about 7 points) in the GCS, perceptual-performance score, and executive function scores. Both T-Hg and fish intake had a negative effect on most outcomes. After adjustment of consumption frequency of the four fish types, T-Hg exposure remained negatively associated with memory (−8.4 points) and verbal (−7.5 points) areas, and with the GCS (−6.6 points) (Table 6). Fried fish consumption was also negatively associated with the verbal score, whereas canned fish intake was positively associated with the GCS. In general, cognitive functions were positively related to oily and canned fish intake and negatively related to white and fried fish intake.

4. Discussion

4.1. Mercury exposure

Levels of T-Hg in the hair of these children from Granada, Spain, were higher than those reported in other young populations from North-eastern Spanish areas with important chemical and fishing-industry activities (Batista et al., 1996; Nadal et al., 2005; Torrente et al., 2005), much higher than concentrations found in children from US and Germany (Pesch et al., 2002; McDowell et al., 2004), but lower than levels reported in heavily exposed children from Brazil, Faroe, and Seychelles Islands (Grandjean et al., 1997; Myers et al., 2003; Tavares et al., 2005). T-Hg hair levels in the present population were similar to those found in Spanish children living near a chlor-alkali plant (Montuori et al., 2006) and in Menorca (Mediterranean island) (Díez et al., 2008). Some of these authors reported that the children's hair mercury levels were highly influenced by their fish consumption (Kruzikova et al., 2008; McDowell et al., 2004; Montuori et al., 2006; Pesch et al., 2002).

The present study found an association between T-Hg levels and higher frequency of oily fish intake, place of residence, maternal age, and passive smoking. Oily fish intake proved to have the greatest influence on T-Hg levels, in accordance with findings in Valencia, a Spanish Mediterranean coastal area, where high levels of T-Hg in cord blood were reported; white fish was the most frequently consumed group by the Valencian women but T-Hg exposure was associated with intake of oily fish (Ramón et al., 2008). Mercury exposure in populations from other Mediterranean areas has also been associated with fish consumption (Elhamri et al., 2007; Gibičar et al., 2006). A recent study (Martí-Cid et al., 2007) estimated a T-Hg daily intake of 6.5 μg/day from fish consumption by children in North-eastern Spain. T-Hg concentrations were found to be higher in long-lived predatory fish, such as swordfish (1.59–2.22 μg/g fresh weight) and tuna

Table 5
Adjusted effect of total hair mercury (T-Hg) concentrations (μg/g) and total fish intake on child neurodevelopment outcomes at age of 4 yr in 72 children from the INMA-Granada cohort, Southern Spain, 2000–2006[a]

Psychological outcomes	T-Hg (μg/g)[b]		≥ 1 μg/g T-Hg[c]	
	β	95% CI	β	95% CI
General cognitive				
T-Hg	−2.09	−5.72; 1.54	−5.61	−11.74; 0.53*
Fish intake ≥ 3 sv/week	−6.60	−12.76; −0.45**	−7.01	−13.03; −0.98**
Quantitative				
T-Hg	0.73	−3.32; 4.79	−1.37	−8.33; 5.60
Fish intake ≥ 3 sv/week	−5.77	−12.64; 1.10	−5.66	−12.50; 1.17*
Memory				
T-Hg	−2.45	−6.77; 1.87	−7.19	−14.44; 0.06**
Fish intake ≥ 3 sv/week	−4.81	−12.13; 2.50	−5.29	−12.41; 1.83
Verbal				
T-Hg	−2.62	−7.04; 1.80	−6.54	−14.03; 0.95*
Fish intake ≥ 3 sv/week	−4.03	−11.52; 3.46	−4.53	−11.88; 2.83
Perceptual-performance				
T-Hg	−1.66	−5.92; 2.59	−3.56	−10.85; 3.72
Fish intake ≥ 3 sv/week	−7.12	−14.33; 0.08**	−7.43	−14.59; −0.28**
Motor				
T-Hg	−2.05	−6.20; 2.11	−4.01	−11.12; 3.11
Fish intake ≥ 3 sv/week	−2.61	−9.65; 4.43	−2.98	−9.97; 4.01
Executive function				
T-Hg	−0.63	−4.28; 3.02	−2.67	−8.90; 3.56
Fish intake ≥ 3 sv/week	−7.36	−13.54; −1.18**	−7.49	−13.61; −1.37**
Memory span				
T-Hg	−2.81	−6.87; 1.25	−5.75	−12.68; 1.81*
Fish intake ≥ 3 sv/week	−4.60	−11.49; 2.28	−5.12	−11.93; 1.68
Verbal memory				
T-Hg	−3.60	−8.76; 1.56	−8.80	−17.51; −0.09**
Fish intake ≥ 3 sv/week	−3.18	−11.93; 5.57	−3.86	−12.41; 4.69
Working memory				
T-Hg	3.36	−0.63; 7.35*	1.40	−5.61; 8.41
Fish intake ≥ 3 sv/week	−4.05	−10.80; 2.71	−3.49	−10.38; 3.40
Gross motor				
T-Hg	−1.09	−5.25; 3.07	1.59	−5.56; 8.74
Fish intake ≥ 3 sv/week	0.09	−6.95; 7.14	−0.06	−7.01; 6.96
Fine motor				
T-Hg	−1.03	−5.46; 3.41	−3.72	−11.28; 3.84
Fish intake ≥ 3 sv/week	−1.69	−9.20; 5.82	−1.90	−9.33; 5.52

*$p < 0.1$; **$p < 0.05$; ***$p < 0.001$; CI: confidence interval.
Mean score for the general cognitive is 100, with a standard deviation (SD) of 15. Mean score for the rest of psychological outcomes is 50, with a SD of 10.

[a] Simultaneous adjusted effect of T-Hg levels and frequency of fish intake (≥ 3 servings per week), controlling for school term of evaluation, psychologist, place of residence, maternal age, parity, and mother's educational level and occupation status.
[b] Each row is a different multivariate model with T-Hg levels on log-transformed scale.
[c] Each row is a different multivariate model with T-Hg levels as dichotomous variable (< 1 μg/g, ≥ 1 μg/g).

(0.38–0.58 μg/g), followed by red mullet (0.14–0.36 μg/g) and hake (0.12–0.29 μg/g). T-Hg levels in swordfish exceeded the European Union limit (0.5 μg/g) (Directive 2001/22/EC).

Although there is little industrial activity in our study area, water pollution with mercury has been reported from other local anthropogenic sources, including agricultural pesticides, waste water effluents, and metal waste dumps, mainly near urban locations (Cabrera-Vique et al., 2007). The present results show about 3-fold higher T-Hg hair levels in the urban versus rural children. As previously reported in German children (Pesch et al., 2002), passive smoking was found to influence T-Hg exposure, probably due to the presence of elemental mercury in tobacco smoke (Rickert and Kaiserman, 1994). However, exposure to mercury appeared to be mainly due to fish consumption in our study population, since T-Hg content in hair was highly correlated with MeHg levels, reflecting exposure to its organic form.

4.2. Effects on neurodevelopment

A concentration of T-Hg ≥ 1 μg/g in hair was associated with decrements in MSCA general cognitive, memory, and verbal areas, in regression models that controlled for the consumption frequency of four different fish groups and selected confounders. Higher fish intake was found to adversely affect the GCS, perceptual-performance, and executive function scores. However, the effects of fish consumption on neurodevelopment appeared to depend on the type of fish, e.g., a higher consumption of canned fish showed a positive effect on the GCS. Our results support the findings of some studies that higher mercury exposure in young children is associated with lower cognition, even at relatively low-exposure levels (Oken et al., 2005).

Previous studies reported adverse effects of prenatal mercury exposure on cognitive development (Chevrier et al., 2009; Cohen et al., 2005; Grandjean et al., 1997; Jedrychowski et al., 2006;

Table 6
Association of total hair mercury (T-Hg) concentrations (μg/g) and intake of different fish types (servings/week) with child neurodevelopment test scores at age of 4 yr in 72 children from the INMA-Granada cohort, Southern Spain, 2000–2006[a]

Psychological outcomes	T-Hg (μg/g)[b]		≥ 1 μg/g T-Hg[c]	
	β	95% CI	β	95% CI
General cognitive				
T-Hg	−2.85	−6.69; 0.99	−6.60	−13.04; −0.15**
Oily fish	1.02	−6.39; 8.42	1.55	−5.76; 8.85
Canned fish	7.98	0.28; 15.68**	7.68	0.16; 15.20**
White fish	−3.32	−11.09; 4.46	−3.60	−11.23; 4.03
Fried fish	−4.70	−12.34; 2.94	−5.07	−12.51; 2.37
Quantitative				
T-Hg	−0.65	−4.96; 3.66	−2.76	−10.10; 4.57
Oily fish	0.65	−7.67; 8.96	0.93	−7.38; 9.24
Canned fish	2.36	−6.28; 11.00	2.36	−6.20; 10.91
White fish	−5.54	−14.27; 3.18	−5.83	−14.52; 2.85
Fried fish	4.13	−4.45; 12.70	4.12	−4.35; 12.58
Memory				
T-Hg	−2.79	−7.36; 1.77	−8.40	−15.96; −0.83**
Oily fish	1.97	−6.84; 10.78	2.74	−5.84; 11.31
Canned fish	4.55	−4.61; 13.71	4.35	−4.47; 13.17
White fish	−1.83	−11.08; 7.42	−2.45	−11.40; 6.50
Fried fish	−4.15	−13.23; 4.94	−4.40	−13.13; 4.33
Verbal				
T-Hg	−2.72	−7.21; 1.77	−7.50	−14.99; −0.02**
Oily fish	4.20	−4.46; 12.86	4.86	−3.64; 13.36
Canned fish	7.95	−1.05; 16.95*	7.72	−1.02; 16.47*
White fish	−2.36	−11.45; 6.73	−2.84	−11.72; 6.03
Fried fish	−8.02	−16.95; 0.91*	−8.31	−16.96; 0.34**
Perceptual – performance				
T-Hg	−2.62	−7.20; 1.97	−3.91	−11.77; 3.95
Oily fish	−3.53	−12.37; 5.32	−3.32	−12.23; 5.60
Canned fish	6.70	−2.52; 15.87	6.28	−2.89; 15.45
White fish	−1.91	−11.19; 7.38	−1.78	−11.09; 7.53
Fried fish	−2.06	−11.19; 7.06	−2.54	−11.62; 6.54
Motor				
T-Hg	−2.41	−6.86; 2.04	−4.16	−11.76; 3.45
Oily fish	0.47	−8.11; 9.06	0.74	−7.89; 9.36
Canned fish	5.15	−3.78; 14.07	4.81	−4.06; 13.68
White fish	−0.64	−9.65; 8.37	−0.62	−9.63; 8.38
Fried fish	−2.52	−11.37; 6.34	−2.92	−11.70; 5.86

*$p < 0.1$; **$p < 0.05$; ***$p < 0.001$; CI: confidence interval.
Mean score for the general cognitive is 100, with a standard deviation (SD) of 15. Mean score for the rest of psychological outcomes is 50, with a SD of 10.

[a] Simultaneous adjusted effect of T-Hg levels and frequency of consumption of different types of fish (≥ 1 serving per week), controlling for school term of evaluation, psychologist, place of residence, maternal age, parity, and mother's educational level and occupational status.
[b] Each row is a different multivariate model with T-Hg levels on log-transformed scale.
[c] Each row is a different multivariate model with T-Hg levels as dichotomous variable (< 1 μg/g, ≥ 1 μg/g).

Lederman et al., 2008; Oken et al., 2005, 2008). However, postnatal exposure was not related to impaired neurodevelopment in some cross-sectional studies (Murata et al., 2002; Tavares et al., 2005), calling attention to the differential effects of prenatal and postnatal exposure. Children can be exposed to mercury from various sources, including breast milk and weaning foods. However, reports of an association between hair mercury levels in mothers and children during the first months of life suggest that children ingest similar doses of MeHg to their mothers and may reflect family exposure through fish consumption (Kim et al., 2008; Marques et al., 2007). Hair can be used to estimate mercury exposure over extended periods of time, including fetal exposure (Cernichiari et al., 1995). Hence, the T-Hg hair levels in our study may be considered a proxy for both prenatal and postnatal exposure. An assessment of prenatal exposure of Spanish newborns (Díez et al., 2008) found a significant maternal transfer of T-Hg to the newborn and concluded that fish consumption during pregnancy was the best predictor of neonatal mercury concentrations. In this context, it has been proposed that early postnatal effects of low-exposures to MeHg may not emerge until later in life (Rice, 1996). Nevertheless, our study was able to establish a significant negative association between mercury exposure and child cognition, specifically with verbal and memory areas. Similar brain function domains were found to be affected by prenatal mercury exposure in children in the Faroes (language and memory) (Grandjean et al., 1997), in the US (visual memory) (Oken et al., 2005), and in the Amazon (visuospatial organization) (Chevrier et al., 2009; Cordier et al., 2002).

4.3. Risks and benefits of fish consumption

A moderate intake of low-contaminated fish during pregnancy is considered to benefit children's neurodevelopment and visual function (Daniels et al., 2004; Oken et al., 2005). The harmful effect of fish intake on cognition detected in our sample may be partially explained by contaminants other than mercury in fish, such as polychlorinated biphenyls (PCBs), which have been linked to neurological damage (Ribas-Fitó et al., 2001). Despite the small sample size, we found a positive association between cognition and intakes of oily or canned fish, suggesting that neurodevelopmental effects depend on the fish species. Oily and canned fish groups include species that may be rich in omega-3 fatty acids, while white and fried fish groups may have a lower content of beneficial nutrients. Hence, research into the effects of mercury on child development should consider the different types of fish consumed by the mothers and children (Cohen et al., 2005; Domingo et al., 2007a).

The present study shows that mercury exposure in children from a Mediterranean area is related to fish intake. It is the first report on neurodevelopmental outcomes associated with mercury exposure and fish consumption in Spanish children. Given the widespread consumption of fish in Spain, it is important to accurately determine the associated risk to children's health of low-level mercury exposure.

Acknowledgments

The authors are grateful to Richard Davies for editorial assistance. The results would not have been achieved without the selfless collaboration of the study participants and staff at the Pediatrics Department of the San Cecilio University Hospital.

References

Agency for Toxic Substances and Disease Registry (ATSDR), 1999. Toxicological profile for mercury. Agency for Toxic Substances and Disease Registry, Atlanta, GA. Available: 〈http://www.atsdr.cdc.gov/toxprofiles/tp46.html〉 [accessed September 2009].

Batista, J., Schuhmacher, M., Domingo, J.L., Corbella, J., 1996. Mercury in hair for a child population from Tarragona Province, Spain. Sci. Total Environ. 193, 143–148.

Boening, D.W., 2000. Ecological effects, transport, and fate of mercury: a general review. Chemosphere 40, 1335–1351.

Cabrera-Vique, C., Ruiz-López, M.D., Javier, F., 2007. Mercury in waters from South-eastern Spain: possible sources of pollution. Ars. Pharm. 48, 37–53.

Castoldi, A.F., Johansson, C., Onishchenko, N., Coccini, T., Roda, E., Vahter, M., Ceccatelli, S., Manzo, L., 2008. Human developmental neurotoxicity of methylmercury: impact of variables and risk modifiers. Regul. Toxicol. Pharmacol. 51, 201–214.

Cernichiari, E., Toribara, T.Y., Liang, L., Marsh, D.O., Berlin, M.W., Myers, G.J., Cox, C., Shamlaye, C.F., Choisy, O., Davidson, P., 1995. The biological monitoring of mercury in the Seychelles study. Neurotoxicology 16, 613–628.

Chevrier, C., Sullivan, K., White, R.F., Comtois, C., Cordier, S., Grandjean, P., 2009. Qualitative assessment of visuospatial errors in mercury-exposed Amazonian children. Neurotoxicology 30, 37–46.

Cohen, J.T., Bellinger, D.C., Shaywitz, B.A., 2005. A quantitative analysis of prenatal methyl mercury exposure and cognitive development. Am. J. Prev. Med. 29, 353–366.

Cordier, S., Garel, M., Mandereau, L., Morcel, H., Doineau, P., Gosme-Seguret, S., Josse, D., White, R., Amiel-Tison, C., 2002. Neurodevelopmental investigations among methylmercury-exposed children in French Guiana. Environ. Res. 89, 1–11.

Counter, S.A., Buchanan, L.H., 2004. Mercury exposure in children: a review. Toxicol. Appl. Pharmacol. 198, 209–230.

Crumpt, K.S., Kjellstrom, T., Shipp, A.M., Silvers, A., Stewart, A., 1998. Influence of prenatal mercury exposure upon scholastic and psychological test performance: benchmark analysis of a New Zealand cohort. Risk Anal. 18, 701–713.

Daniels, J.L., Longnecker, M.P., Rowland, A.S., Golding, J., ALSPAC Study Team, 2004. University of Bristol Institute of Child Health. Fish intake during pregnancy and early cognitive development of offspring. Epidemiology 15, 394–402.

Davidson, P.W., Strain, J.J., Myers, G.J., Thurston, S.W., Bonham, M.P., Shamlaye, C.F., Stokes-Riner, A., Wallace, J.M., Robson, P.J., Duffy, E.M., Georger, L.A., Sloane-Reeves, J., Cernichiari, E., Canfield, R.L., Cox, C., Huang, L.S., Janciuras, J., Clarkson, T.W., 2008. Neurodevelopmental effects of maternal nutritional status and exposure to methylmercury from eating fish during pregnancy. Neurotoxicology 29, 767–775.

Díez, S., Bayona, J.M., 2002. Determination of methylmercury in human hair by ethylation followed by headspace solid-phase microextraction-gas chromatography-cold-vapour atomic fluorescence spectrometry. J. Chromatogr. A 963, 345–351.

Díez, S., Montuori, P., Querol, X., Bayona, J.M., 2007. Total mercury in the hair of children by combustion atomic absorption spectrometry (Comb-AAS). J. Anal. Toxicol. 31, 144–149.

Díez, S., Delgado, S., Aguilera, I., Astray, J., Pérez-Gómez, B., Torrent, M., Sunyer, J., Bayona, J.M., 2008. Prenatal and early childhood exposure to mercury and methylmercury in Spain, a high-fish-consumer country. Arch. Environ. Contam. Toxicol. doi:10.1007/s00244-008-9213-7.

Díez, S., 2009. Human health effects of methylmercury exposure. Rev. Environ. Contam. Toxicol. 198, 111–132.

Directive 2001/22/EC of 8 March 2001: laying down the sampling methods and the methods of analysis for the official control of the levels of lead, cadmium, mercury and 3-MCPD in foodstuffs.

Domingo, J.L., Bocio, A., Falcó, G., Llobet, J.M., 2007a. Benefits and risks of fish consumption Part I. A quantitative analysis of the intake of omega-3 fatty acids and chemical contaminants. Toxicology 230, 219–226.

Domingo, J.L., Bocio, A., Martí-Cid, R., Llobet, J.M., 2007b. Benefits and risks of fish consumption Part II. RIBEPEIX, a computer program to optimize the balance between the intake of omega-3 fatty acids and chemical contaminants. Toxicology 230, 227–233.

Elhamri, H., Idrissi, L., Coquery, M., Azemard, S., El Abidi, A., Benlemlih, M., Saghi, M., Cubadda, F., 2007. Hair mercury levels in relation to fish consumption in a community of the Moroccan Mediterranean coast. Food Addit. Contam. 24, 1236–1246.

Freire, C., Abril, A., Fernandez, M.F., Ramos, R., Estarlich, M., Manrique, A., Aguirre, A., Ibarluzea, J., Olea, N., 2009. Urinary 1-hydroxypyrene and PAH exposure in 4-year-old Spanish children. Sci. Total Environ. 407, 1562–1569.

Freire, C., Ramos, R., Puertas, R., Lopez-Espinosa, M.J., Julvez, J., Aguilera, I., Cruz, F., Fernández, M.F., Sunyer, J., Olea, N., 2009. Association of traffic-related air pollution with cognitive development in children. J. Epidemiol. Community Health, doi:jech.2008.084574.

Gibičar, D., Horvat, M., Nakou, S., Sarafidou, J., Yager, J., 2006. Pilot study of intrauterine exposure to methylmercury in Eastern Aegean islands, Greece. Sci. Total Environ. 367, 586–595.

Grandjean, P., Weihe, P., White, R.F., Debes, F., Araki, S., Yokoyama, K., Murata, K., Sorensen, N., Dahl, R., Jorgensen, P.J., 1997. Cognitive deficit in 7-year-old children with prenatal exposure to methylmercury. Neurotoxicol. Teratol. 19, 417–428.

Grandjean, P., White, R.F., Nielsen, A., Cleary, D., de Oliveira Santos, E.C., 1999. Methylmercury neurotoxicity in Amazonian children downstream from gold mining. Environ. Health Perspect. 107, 587–591.

Jacobson, J., Jacobson, S., Humphrey, H., 1990. Effects of in utero exposure to polychlorinated biphenyls and related contaminants on cognitive functioning in young children. J. Pediatr. 116, 38–45.

Jedrychowski, W., Jankowski, J., Flak, E., Skarupa, A., Mroz, E., Sochacka-Tatara, E., Lisowska-Miszczyk, I., Szpanowska-Wohn, A., Rauh, V., Skolicki, Z., Kaim, I., Perera, F., 2006. Effects of prenatal exposure to mercury on cognitive and psychomotor function in one-year-old infants: epidemiologic cohort study in Poland. Ann. Epidemiol. 16, 439–447.

Júlvez, J., Ribas-Fitó, N., Torrent, M., Forns, M., Garcia-Esteban, R., Sunyer, J., 2007. Maternal smoking habits and cognitive development of children at age 4 years in a population-based birth cohort. Int. J. Epidemiol. 36, 825–832.

Kim, S.A., Jeon, C.K., Paek, D.M., 2008. Hair mercury concentrations of children and mothers in Korea: implication for exposure and evaluation. Sci. Total Environ. 402, 36–42.

Kruzikova, K., Modra, H., Kensova, R., Skocovska, B., Wlasow, T., Svoboda, T., Svobodova, Z., 2008. Mercury in human hair as an indicator of the fish consumption. Neuroendocrinol. Lett. 29, 675–679.

Lederman, SA, Jones, RL, Caldwell, KL, Rauh, V, Sheets, SE, Tang, D, Viswanathan, S., Becker, M., Stein, J.L, Wang, R.Y., Perera, F.P., 2008. Relation between cord blood mercury levels and early child development in a World Trade Center cohort. Environ. Health Perspect. 116, 1085–1091.

Lopez-Espinosa, M.J., Granada, A., Carreno, J., Salvatierra, M.T., Olea-Serrano, F., Olea, N., 2007. Organochlorine pesticides in placentas from Southern Spain and some related factors. Placenta 28, 631–638.

Marques, R.C., Dórea, J.G., Bastos, W.R., Malm, O., 2007. Changes in children hair-Hg concentrations during the first 5 years: maternal, environmental and iatrogenic modifying factors. Regul. Toxicol. Pharmacol. 49, 17–24.

Martí-Cid, R., Bocio, A., Llobet, J.M., Domingo, J.L., 2007. Intake of chemical contaminants through fish and seafood consumption by children of Catalonia, Spain: health risks. Food Chem. Toxicol. 45, 1968–1974.

McCarthy, D., 1972. In: Manual for the McCarthy Scales of Children's Abilities. Psychological Corporation, New York, NY (Spanish adaptation: Madrid, Spain. TEA Ediciones, S.A., 1996).

McDowell, M.A., Dillon, C.F., Osterloh, J., Bolger, P.M., Pellizzari, E., Fernando, R., Montes de Oca, R., Schober, S.E., Sinks, T., Jones, R.L., Mahaffey, K.R., 2004. Hair mercury levels in US children and women of childbearing age: reference range data from NHANES 1999–2000. Environ. Health Perspect. 112, 1165–1171.

Ministerio de Sanidad y Consumo. 2009. Available: 〈http://www.msc.es/ciudadanos/proteccionSalud/infancia/alimentacion/tema1.htm〉 [acceded September 2009].

Montuori, P., Jover, E., Alzaga, R., Díez, S., Bayona, J.M., 2004. Improvements in the methylmercury extraction from human hair by headspace solid-phase microextraction followed by gas-chromatography cold-vapour atomic fluorescence spectrometry. J. Chromatogr. A 1025, 71–75.

Montuori, P., Jover, E., Díez, S., Ribas-Fitó, N., Sunyer, J., Triassi, M., Bayona, J.M., 2006. Mercury speciation in the hair of pre-school children living near a chlor-alkali plant. Sci. Total Environ. 369, 51–58.

Murata, K., Budtz-Jørgensen, E., Grandjean, P., 2002. Benchmark dose calculations for methylmercury-associated delays on evoked potential latencies in two cohorts of children. Risk Anal. 22, 465–474.

Myers, G.J., Davidson, P.W., Cox, C., Shamlaye, C.F., Palumbo, D., Cernichiari, E., Sloane-Reeves, J., Wilding, G.E., Kost, J., Huang, L.S., Clarkson, T.W., 2003. Prenatal methylmercury exposure from ocean fish consumption in the Seychelles child development study. Lancet 361, 1686–1692.

Nadal, M., Bocio, A., Schuhmacher, M., Domingo, J.L., 2005. Monitoring metals in the population living in the vicinity of a hazardous waste incinerator. Levels in hair of children. Biol. Trace Elem. Res. 104, 203–213.

National Research Council (NRC), 2000. In: Committee on the Toxicological Effects of Methylmercury. National Academies Press, Wahshington, DC.

Oken, E., Wright, R.O., Kleinman, K.P., Bellinger, D., Amarasiriwardena, C.J., Hu, H., Rich-Edwards, J.W., Gillman, M.W., 2005. Maternal fish consumption, hair mercury and infant cognition in a U.S. cohort. Environ. Health Perspect. 113, 1376–1380.

Oken, E., Radesky, J.S., Wright, R.O., Bellinger, D.C., Amarasiriwardena, C.J., Kleinman, K.P., Hu, H., Gillman, M.W., 2008. Maternal fish intake during pregnancy, blood mercury levels, and child cognition at age 3 years in a US cohort. Am. J. Epidemiol. 167, 1171–1181.

Palumbo, D.R., Cox, C., Davidson, P.W., Myers, G.J., Choi, A., Shamlaye, C., Sloane-Reeves, J., Cernichiari, E., Clarkson, T.W., 2000. Association between prenatal exposure to methylmercury and cognitive functioning in Seychellois children: a reanalysis of the McCarthy Scales of Children's Ability from the main cohort study. Environ. Res. 84, 81–88.

Pesch, A., Wilhelm, M., Rostek, U., Schmitz, N., Weishoff-Houben, M., Ranft, U., Idel, H., 2002. Mercury concentrations in urine, scalp hair, and saliva in children from Germany. J. Expo. Anal. Environ. Epidemiol. 12, 252–258.

Ramón, R., Murcia, M., Ballester, F., Rebagliato, M., Lacasaña, M., Vioque, J., Llop, S., Amurrio, A., Aguinagalde, X., Marco, A., León, G., Ibarluzea, J., Ribas-Fitó, N., 2008. Prenatal exposure to mercury in a prospective mother–infant cohort study in a Mediterranean area, Valencia, Spain. Sci. Total Environ. 392, 69–78.

Renzoni, A., Zino, F., Franchi, E., 1998. Mercury levels along the food chain and risk for exposed populations. Environ. Res. 77, 68–72.

Ribas-Fitó, N., Sala, M., Kogevinas, M., Sunyer, J., 2001. Polychlorinated biphenyls (PCBs) and neurological development in children: a systematic review. J. Epidemiol. Community Health 55, 537–546.

Ribas-Fitó, N., Ramón, R., Ballester, F., Grimalt, J.O., Marco, A., Olea, N., Posada, M., Rebagliato, M., Tardón, A., Torrent, M., Sunyer, J., INMA study group, 2006. Child health and the environment: the INMA Spanish study. Paediatr. Perinat. Epidemiol. 20, 403–410.

Rice, D.C., 1996. Evidence for delayed neurotoxicity produced by methylmercury. Neurotoxicology 17, 583–596.

Rickert, W.S., Kaiserman, M.J., 1994. Levels of lead, cadmium, and mercury in Canadian cigarette tobacco as indicators of environmental change: results from a 21 year study (1968–1988). Environ. Sci. Technol. 28, 924–927.

Tavares, L.M., Camara, V.M., Malm, O., Santos, E.C., 2005. Performance on neurological development tests by riverine children with moderate mercury exposure in Amazonia, Brazil. Cad. Saude Publica 21, 1160–1167.

Torrente, M., Colomina, M.T., Domingo, J.L., 2005. Metal concentrations in hair and cognitive assessment in an adolescent population. Biol. Trace Elem. Res. 104, 215–221.

US Environmental Protection Agency (USEPA), 2001. In: Water quality criterion for the protection of human health: methylmercury. EPA Offices of Sciences and Technology, Washington.

US Environmental Protection Agency (USEPA), 2009. Mercury. Human exposure. Available: 〈http://www.epa.gov/mercury/exposure.htm〉 accessed September .

Vioque, J., 2006. Validez de la evaluación de la ingesta dietética. In: Serra Majem, L., Aranceta Bartrina, J. (Eds.), Nutrición y Salud Pública. Métodos, bases científicas y aplicaciones. Masson-Elsevier, Barcelona, pp. 199–210.

Welch, A.A., Lund, E., Amiano, P., Dorronsoro, M., Brustad, M., Kumle, M., Rodríguez, M., Laceras, C., Janzon, L., Jansson, J., Luben, R., Spencer, E.A., Overvad, K., Tjønneland, A., Clavel-Chapelon, F., Linseisen, J., Klipstein-Grobusch, K., Benetou, V., Zavitsanos, X., Tumino, R., Galasso, R., Bueno-De-Mesquita, H.B., Ocké, M.C., Charrondière, U.R., Slimani, N., 2002. Variability of fish consumption within the 10 European countries participating in the European Investigation into Cancer and Nutrition (EPIC) study. Public Health Nutr. 5, 1273–1285.

Willet, W.C., Sampson, L., Stampfer, M.J., Rosner, B., Brain, C., Witschi, J., Hennekens, C.H., Speizer, F.E., 1985. Reproducibility and validity of a semiquantitative food frequency questionnaire. Am. J. Epidemiol. 122, 51–65.