International Journal of Hygiene and Environmental Health 222 (2019) 9–21

Contents lists available at ScienceDirect

International Journal of Hygiene and Environmental Health

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

Prenatal mercury exposure and child neurodevelopment outcomes at 18 months: Results from the Mediterranean PHIME cohort

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ARTICLE INFO

Keywords:
Mercury
Fish
Child neurodevelopment
Bayley scales of infant and toddler development
Developmental motor scales
Cohort study

ABSTRACT

Introduction: Neurotoxicity due to acute prenatal exposure to high-dose of mercury (Hg) is well documented. However, the effect of prenatal exposure to low Hg levels on child neurodevelopment and the question about "safety" of fish-eating during pregnancy remain controversial. International comparisons of Hg concentrations in mother-child biological samples and neurodevelopmental scores embedded in birth cohort studies may provide useful evidence to explore this issue.

Materials and methods: The Mediterranean (Italy, Slovenia, Croatia, and Greece) cohort study included 1308 mother-child pairs enrolled in the Public Health Impact of long-term, low-level, Mixed Element exposure in a susceptible population EU Sixth Framework Programme (PHIME). Maternal hair and venous blood, cord blood and breast milk samples were collected, and total Hg (THg) levels were measured. Demographic and socio-economic information, lifestyles and nutritional habits were collected through questionnaires at different phases of follow-up. Children at 18 months of age underwent neurodevelopmental testing using the Bayley Scales of Infant and Toddler Development, Third Edition (BSID-III). Multivariate linear and logistic regressions were performed, for each country, to assess the association between THg and BSID-III scores, obtaining adjusted β coefficients and odds ratios (ORs). These values were used to conduct a meta-analysis, to explore possible heterogeneity among countries and to obtain combined estimates of the association between THg exposure and BSID-III scores.

Results: Median THg (ng/g) was: 704 in maternal hair, 2.4 in maternal blood, 3.6 in cord blood, and 0.6 in breast milk. THg concentrations were highest in Greece and lowest in Slovenia. BSID-III neurodevelopmental scores

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https://doi.org/10.1016/j.ijheh.2018.07.011
Received 16 February 2018; Received in revised form 13 July 2018; Accepted 19 July 2018

were higher in Croatia and Slovenia. The meta-analysis of multivariate linear models found an overall positive association between language composite score and receptive communication scaled score and increasing THg in maternal hair (n = 1086; β = 0.55; 95%CI: 0.05–1.05 and n = 1075; β = 0.12; 95%CI: 0.02–0.22, respectively). The meta-analysis of logistic regression models showed that the overall adjusted OR between THg in cord blood and suboptimal gross motor score was borderline significant (n = 882; OR = 1.03; 95%CI: 1.00–1.07). Heterogeneity was found across the four sub-cohorts for language composite score in maternal blood, and for fine motor scaled score in cord blood and breast milk. Language composite score and THg concentrations in maternal venous blood were positively related (n = 58; β = 4.29; CI95% (−0.02, 8.60)) in Croatia and an increase of 1 ng/g of THg in maternal venous blood was associated with a reduced risk for children to fall in the lowest quintile of language score by 31% (n = 58; OR = 0.69; CI 95%: 0.37, 1.01). The comparison of β coefficients obtained by multiple linear regression model showed an inverse association between fine motor score and THg concentrations in cord blood for Croatia (n = 54; β = −0.53; CI 95%: −1.10, 0.04) and Slovenia (n = 225; β = −0.25; CI 95%: −0.49, −0.01). In Slovenia THg level in breast milk was associated with suboptimal fine motor performance (n = 195; OR = 5.25; CI 95%: 1.36, 21.10).

Conclusions: This study showed an inverse relation between THg levels and developmental motor scores at 18 months, although the evidence was weak and partially internally and externally inconsistent. No evidence of detrimental effects of THg was found for cognitive and language outcomes at these concentrations and age.

1. Introduction

Neurotoxicity due to acute exposure to high-dose of mercury (Hg) is well known because of poisoning cases in Japan and Iraq (Amin-Zaki et al., 1974; Bose-O'Reilly et al., 2010; Counter and Buchanan, 2004; Diez, 2009). Children of asymptomatic mothers, exposed to high levels of methylmercury (MeHg) during pregnancy, showed several neurodevelopmental abnormalities including delayed onset of motor and language development, impairments of sensory functions, severe mental retardation, seizures and in some cases coma and death (Bose-O'Reilly et al., 2010; Counter and Buchanan, 2004; Davidson et al., 2004; Diez, 2009; Myers and Davidson, 1998; UNEP DTIE Chemicals Branch, 2008). The implications of these findings caused concern: Hg is an element that is ubiquitous in nature and is methylated in the aquatic environment by bacteria to form MeHg (National Research Council (US), Committee on the Toxicological Effects of Methylmercury (2000)). This organic compound bioaccumulates in marine organisms and is biomagnified through the food chain (Myers and Davidson, 1998). Therefore, the primary source of non-occupational Hg exposure is consumption of fish and other seafood, especially predatory species (e.g., tuna, swordfish, shark, bass, king mackerel, tilefish) (Bose-O'Reilly et al., 2010; Diez, 2009; Myers et al., 2007; Oken and Bellinger, 2008).

Several cohort studies have evaluated the effect of pre-natal exposure to MeHg on child neurodevelopment, with conflicting results. The Norwegian Mother and Child Cohort Study (MoBa) found at three years of age a significant association between prenatal MeHg exposure above the 90th percentile and delayed language and communication skills in a low exposed population (Vejrup et al., 2016). In this cohort, a positive association between low levels of prenatal mercury exposure and language and communication skills was found at five years. However, in the highest exposure group a matched sibling analyses suggested that mercury was negatively associated with language skills (Vejrup et al., 2018). Cohort studies conducted in New Zealand (Kjellstrom et al., 1989) and the Faroe Islands, reported in children (6–7 years of age) dose-related neurological deficits associated with prenatal MeHg exposure (by maternal fish intake), such as impairments of attention, language, verbal memory, motor (speed) and visuospatial function and loss of intelligence quotient (IQ) points (Debes et al., 2006; Grandjean et al., 1998, 1997; Holmes et al., 2009). Only weaker deficits were identified concerning motor speed, attention, and language in adolescents of a mean age of 14 years (Debes et al., 2006; Dovydaitis, 2008; Holmes et al., 2009). On the other hand, the Seychelles study did not find alterations of neuropsychological development and IQ at each studied age between 6 months and 24 years, although the Hg/MeHg levels of exposure were similar to those found in the Faroe Islands study (Bose-O'Reilly et al., 2010; Counter and Buchanan, 2004; Davidson et al., 2011; Davidson et al., 2010; Davidson et al., 2008a, 2008b; 2006a, 2006b; 1998; Dovydaitis, 2008; Myers et al., 2007; Strain et al., 2015, 2008; Van Wijngaarden et al., 2017).

In 2005 Cohen et al., 2005 aggregated results from the Faroe Islands study, the Seychelles Child Development Study, and the New Zealand study showing that a prenatal MeHg exposure sufficient to increase the concentration of mercury in maternal hair at delivery by 1 μg/g decreases intelligence quotient by 0.7 points.

A birth cohort study carried out in Japan suggested that, in neonates of 3 days, Hg levels in maternal hair (median 1.96 μg/g) affect early motor function (Suzuki et al., 2010). In a cohort of Spanish children of 4 years, total mercury concentrations (THg) in hair greater than 1 μg/g were associated with deficits in cognitive abilities. However, in the larger Spanish Infancia y Medio Ambiente (INMA) cohort, the relation between cord blood level of THg and mental and psychomotor development were by and large negative (Llop et al., 2012). Inconsistent results were shown in Boucher et al. (2010), on Canadian Inuit children while no detrimental effects of Hg low level were found in the Avon Longitudinal Study of Parents and Children (ALSPAC). In this cohort maternal and infants' fish intakes resulted related to higher mean developmental scores, suggesting a beneficial effect of fish intake on neurodevelopment (Hibbeln et al., 2007).

The evaluation of the toxic effects of contaminants in fish-eating populations is complicated by the fact that fish is an important source of beneficial nutrients, like polyunsaturated fatty acids (PUFAs), lean protein, iron, iodine, selenium, vitamins, and choline. These nutrients are transferred from the mother to the fetus via placenta and play a positive role in fetal neurodevelopment (Genuis, 2008; Gil and Gil, 2015; Starling et al., 2015). This might explain why the effects on child neurodevelopment of prenatal exposure to low Hg levels, are still unclear, although it has been demonstrated that mercury can cross both the placenta and the blood-brain barrier and it can be measured in umbilical cord blood (Al-Saleh et al., 2016; Grandjean et al., 2005).

The uncertainty about "safety" of fish intake during pregnancy represents an important issue of public health (Genuis, 2008). The Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives (JECFA) in 2004 had fixed a maximum acceptable intake for MeHg of 1.6 μg/kg body weight per week for the general population and for pregnant women to protect the fetus from neurotoxic effects (JECFA, 2004). In the same year, the U.S. Food and Drug Administration (FDA) recommended limiting intake of fish to no more than 12 oz (340 gr) per week. In 2014 the FDA evaluated benefits and concerns of eating fish during pregnancy and breastfeeding and suggested that children of pregnant women who consumed two seafood meals per week (8–12 oz, 227–340 g) could have an additional 3.3 IQ points at 9 years of age (FDA Food and Drug Administration, 2014; McGuire et al., 2016).

To address the uncertainty associated with the health effects of exposure to low-level metals including mercury, the EU Commission Sixth Framework Programme for Research and Technological Development funded the project entitled 'Public health impact of long-term, low-level mixed element exposure in susceptible population strata' (PHIME) (PHIME, 2011). Within the PHIME framework, in 2007, partners from Italy, Slovenia, Croatia and Greece established a prospective mother-child cohort aimed to assess the association between mercury exposure from food consumption during pregnancy and development of the children nervous system. Preliminary results from the Italian PHIME sub-cohort, published by Valent et al. (2013a), showed no association between child neurodevelopment at 18 months of age and prenatal Hg exposure. BSID-III scores were influenced directly by higher child intake of fish, higher maternal IQ, and female child's sex. Also, maternal fish intake and maternal serum LCPUFAs were not associated with neurocognitive abilities. On the other hand, results from the Croatian PHIME sub-cohort, published by Prpić et al. (2017) showed a negative correlation at 18 months between cord blood THg concentration and fine motor skills (rho = −0.22, p = 0.01).

This study aimed to assess at 18 months of age the association of low-level prenatal mercury exposure on neurodevelopment among residents in the four Mediterranean coastal regions of Italy, Slovenia, Croatia and Greece where pregnant women consume different amounts and different species of fish from different origins (Miklavčič et al., 2013). A specific primary objective of this prospective cohort study was to compare the impact of different levels of mercury exposure while taking into account potential confounders or effect modifiers.

2. Material and methods

2.1. Study population

A detailed description of the study protocol has been published previously (Valent et al., 2013b).

In brief, recruitment took place at the Institute for Maternal and Child Health IRCCS Burlo Garofolo in Trieste, Italy (I); at the Maternity Hospital of the University Medical Centre of Ljubljana, Slovenia (S); at the University Hospital of Rijeka, Croatia (C); and at the general regional hospitals of Mytilini (Lesvos), Chios, Samos, and Leros in Greece (G). At recruitment, eligible women were approached for consent after their routine morphologic ultrasound scan between 20 and 22 gestational weeks (I), at routine visits between 34 and 38 gestational weeks (C), or during their hospital stay for delivery (S, C, G). Times for enrollment were chosen according to logistic considerations in each country. In Italy and Croatia, the eligible women provided a maternal urine sample and underwent the Raven's Progressive Matrices Test, a test of general intelligence. At different phases, three questionnaires were administered to mothers: (a) a short questionnaire to identify any excluding conditions and to provide some brief information on family and lifestyles during pregnancy; (b) a long questionnaire to collect information on demographic, socioeconomic and health status, on pregnancy and delivery, on lifestyles and dietary habits; in the end (c) a supplementary questionnaire to update information on the family and child. Biological samples (maternal hair, maternal venous blood, cord blood and breast milk) were collected in each country of the study cohort during different phases. Children at 18 (range 16–20) months of age underwent neurodevelopmental evaluation.

Only children born during or after week 37 of gestation who had at least one measure of THg exposure and underwent the Bayley Scales of Infant and Toddler Development at 18 ± 2 months were included in the analysis of the present paper.

2.2. Ethics

The research protocol was approved by the Ethics Committees of the University of Udine, of the Institute for Maternal and Child Health IRCCS Burlo Garofolo, of the Clinical Center of Rijeka, of the Institute of Child Health of Athens and by the National Ethics Committee of the Republic of Slovenia. All aspects of the study, including ethics, were monitored annually by the European Commission.

2.3. Mercury exposure

In the present study, THg concentration in biological samples was the main exposure of interest. THg concentrations were measured in maternal hair, maternal blood, umbilical cord blood and/or cord tissue and breast milk from lactating women. Hair samples, approximately 1 g of hair cut close to the occipital area, were stored in transparent mercury-free plastic bags in a dark and uncontaminated place and periodically sent to Jozef Stefan Institute (JSI) of Ljubljana for analysis. Aliquots of blood, cord tissue, and breast milk samples were stored in freezers (below −24 °C) and then sent or transported in a frozen state (on dry ice) to the laboratories for analysis.

THg in biological samples was determined by cold vapor atomic absorption spectrometry (CVAAS) using three different analytical procedures, depending on matrix type and the study group (period of sampling). All measurements were made under strict quality control procedures and gave comparable results. In addition, the JSI laboratory participated in a series of inter-laboratory comparisons organized within the PHIME project. Three inter-comparisons used lyophilized samples of human blood from non-exposed persons, people occupationally exposed to elemental Hg, fish eaters while the fourth study used fresh blood from the general population. The obtained values were in good agreement with the assigned values (Miklavčič et al., 2013).

The analytical procedures used are described in detail elsewhere (Akagi, 1997; Horvat et al., 1991; Miklavčič et al., 2013, 2011). The estimated analytical precision of the measurements was less than 10%, the limit of detection (LOD) of the procedures was < 0.1 ng/mL in blood and cord blood and < 1 ng/g in hair.

MeHg in hair was measured by gas chromatography–electron capture detection (GC-ECD), whereas MeHg in cord blood was measured by a cold vapor atomic fluorescence detector (CVAFS). The estimated analytical precision of the measurements was 12%, the LODs were 0.2 ng/g in hair, and 0.02 ng/g in blood. The analytical procedures are described in detail by Miklavčič et al. (2013). MeHg measurements were performed on a restricted group of subjects, however, since among subjects with MeHg data, the ratio of MeHg to THg was very high (median, 0.99 in hair and 0.90 in cord blood), we assumed that in the majority of cohort members most Hg was methylated and, therefore, THg could be used as a proxy for MeHg.

2.4. Evaluation of child neurodevelopment (study outcome)

Child neurodevelopment was assessed at 18 months of age (range, 16–20 months) by using the Bayley Scales of Infant and Toddler Development, Third Edition (BSID-III) which measures the major areas of child development. The testing was conducted in each study centers by pediatricians or psychologists who had attended the same BSID-III Accreditation Workshop. In Italy, inter-rater reliability was very high for the BSID-III cognitive score (intraclass correlation coefficient [ICC] 0.98, 95% CI: 0.97–0.99), language score (ICC 0.99, 95% CI: 0.99–1.00), and motor score (ICC 0.93, 95% CI: 0.90–0.97) (Valent et al., 2013a).

As outcomes, we considered the composite scores - cognitive (COG), language (LAN) and motor (MOT) - and the scaled scores receptive communication (RC), expressive communication (EC), fine motor (FM) and gross motor (GM). The scaled scores represent a child's performance on a subtest relative to his or her same age peers and were derived from the standardization of total raw score (the sum of the number of points earned for a subtest) for child's age in days at test administration. For each composite score, the distribution of scaled score (or the sum of scaled scores, RC plus EC and FM plus GM) was

Table 1
General characteristics of 1308 mothers and their children.

	Total (n = 1308)	Croatia (n = 127)	Greece (n = 302)	Italy (n = 607)	Slovenia (n = 272)	*p*-value[a]
Mother's age at delivery, mean ± std (median)	31.4 ± 5.1 (32)	30.0 ± 4.5 (30)	28.9 ± 6.3 (29)	33.3 ± 4.3 (33)	30.4 ± 4.1 (30)	< 0.001
Maternal BMI before pregnancy, mean ± std (median)	23.2 ± 4.5 (22.5)	23.2 ± 4.5 (22.2)	23.5 ± 5.2 (22.9)	22.8 ± 3.8 (22.2)	23.8 ± 4.8 (22.1)	< 0.001
Weight gain during pregnancy, mean ± std (median)	13.9 ± 5.4 (14)	16.1 ± 5.7 (16)	13.5 ± 6.7 (13)	13.5 ± 4.4 (13)	13.9 ± 5.2 (14)	< 0.001
Mother's occupation, n (%):						
Employed on maternity	880 (68.0)	113 (91.1)	68 (55.6)	458 (76.5)	241 (88.9)	
Employed worker	130 (10.0)	–	77 (25.6)	52 (8.7)	1 (0.4)	
Housewife	184 (14.2)	3 (2.4)	133 (44.2)	47 (7.8)	1 (0.4)	
Other condition	101 (7.8)	8 (6.5)	23 (7.6)	42 (7.0)	28 (10.3)	< 0.001
Mothers' marital status, n (%):						
Married/Living together	1227 (94.4)	124 (97.6)	298 (98.7)	542 (90.3)	263 (97.1)	
Widow/single/never married/Separated/divorcing	73 (5.6)	3 (2.4)	4 (1.3)	58 (9.7)	8 (2.9)	< 0.001
Mother's educational level, n (%):						
Elementary and middle school	265 (20.3)	74 (58.7)	73 (24.2)	100 (16.5)	18 (6.6)	
High school	560 (43.0)	17 (13.5)	166 (55.2)	293 (48.4)	84 (31.0)	
University degree	478 (36.7)	35 (27.8)	62 (20.6)	212 (35.0)	169 (62.4)	< 0.001
Home size, n (%):						
< 50 mq	108 (8.3)	15 (12.2)	12 (4.0)	41 (6.8)	40 (14.7)	
50–100 mq	812 (62.7)	73 (59.3)	175 (58.1)	406 (67.7)	158 (58.1)	
> 100 mq	376 (29.0)	35 (28.5)	114 (37.9)	153 (25.5)	74 (27.2)	< 0.001
Number of cigarettes smoked, by mother, during pregnancy, mean ± std (median)	162.4 ± 591.4 (0)	150.0 ± 477.7 (0)	210.6 ± 683.2 (0)	144.2 ± 559.6 (0)	154.7 ± 596.4 (0)	0.127
Alcoholic drinks per week during pregnancy, mean ± std (median)	1.5 ± 2.8 (0.5)	1.2 ± 2.2 (0.3)	1.6 ± 2.7 (0.6)	1.5 ± 2.8 (0.3)	0.8 ± 1.4 (0.3)	0.013
Fish consumption of mother per week during pregnancy, mean ± std (median)	1.4 ± 1.2 (1)	1.8 ± 1.1 (1.5)	1.7 ± 1.3 (1.5)	1.5 ± 1.2 (1.1)	0.9 ± 0.9 (0.6)	< 0.001
Children's sex, n (%):						
Male	634 (48.6)	69 (54.3)	130 (43.5)	306 (50.4)	129 (47.4)	
Female	671 (51.4)	58 (45.7)	169 (56.5)	301 (49.6)	143 (52.6)	0.124
Birth weight (g), mean ± std (median)	3418.9 ± 458.5 (3410)	3583.7 ± 385.7 (3580)	3317 ± 418.3 (3300)	3418.9 ± 452.5 (3400)	3450.8 ± 516.3 (3475)	< 0.001
Breastfeeding months, mean ± std (median)	8.4 ± 6 (8)	5.8 ± 5.3 (4)	4.2 ± 5 (2)	10.1 ± 5.9 (10)	8.9 ± 5.3 (9)	< 0.001
Child intake of homogenized fish (number of months with at least one portion per week), mean ± std (median)	2.1 ± 4.1 (0)	1.3 ± 3.4 (0)		2.7 ± 4.5 (0)	0.9 ± 2.5 (0)	< 0.001
Child intake of fresh fish (number of months with at least one portion per week), mean ± std (median)	7.7 ± 4.2 (8)	6.4 ± 4.9 (7.0)	7.6 ± 2.2 (7)	9.1 ± 4 (10)	4.7 ± 4.4 (5)	< 0.001
Number of children living in home (excluding the newborn), n (%):						
None	689 (52.7)	71 (55.9)	116 (38.4)	355 (58.5)	147 (54.0)	
One or more	619 (47.3)	56 (44.1)	186 (61.6)	252 (41.5)	125 (46.0)	< 0.001
Daycare attendance at 18 month, n (%):						
Member of the family or other people not included in the family	970 (74.2)	116 (91.3)	293 (97.0)	378 (62.3)	183 (67.3)	
Kindergarden	338 (25.8)	11 (8.7)	9 (3.0)	229 (37.7)	89 (32.7)	< 0.001

[a] Differences among countries were assessed by the Chi Square test (for categorical variables) and the Kruskall Wallis test (for continuous variables).

used to derive corresponding percentiles which were converted to composite scores. Higher are the composite or the scaled scores, and better is the child's performance (neurodevelopment). Scaled scores range from 1 to 19 and composite scores from 40 to 160.

2.5. Potential explanatory variables

The potential explanatory variables considered in this study were: mother's age at delivery, maternal body mass index before pregnancy; weight gain, smoking habits, alcohol and fish consumption of mother during pregnancy; as socio-economic indicator we used occupational and marital status, educational level, home size (surface in m^2 of the house in which mother lived) of mother during pregnancy; child's gender and birth weight in grams, child's fresh and homogenized fish consumption until 18 months of age, breastfeeding history until 18 months of age, number of children living in home and daycare attendance at age 18 months. Fish consumption during pregnancy was collected soon after delivery using a detailed 138-item questionnaire adapted from a validated food frequency questionnaire (FFQ) (Valent et al., 2013b). The questionnaire included seven quantitative questions on fish, which addressed the frequency of consumption of 150-g servings of fish, crustaceans, and mollusks (cooked according to different recipes), and fish in oil. For each fish item, conversion from categories of consumption into continuous intakes of fish servings was done by assigning to each category a consumption level equal to the median value for that category (e.g., 2–4 times/week became three times/

Table 2
Distributions of THg concentrations (ng/g) in different biological samples and BSID-III composite and scaled score of children assessed at 18 months.

	N	Mean	SD	Min	20th percentile	40th percentile	Median	60th percentile	80th percentile	Max	*p*-value[a]
THg in maternal hair during pregnancy (ng/g)	1282	997.0	1035.1	16.9	337	566	704	881	1456	13520.0	
Croatia	127	967.9	1176.6	24	288	555	662	782.6	1175	8710	
Greece	295	1408.5	1146.9	55	550	960	1097	1371.5	2003	8973	
Italy	605	1062.1	1029.0	16.9	436	641.5	788	938.5	1475	13520	
Slovenia	255	381.0	297.1	24	127.5	251.5	317	378.5	565.5	1880	< 0.001
THg in maternal blood during pregnancy (ng/g)	733	3.2	3.4	0.1	1.0	2.0	2.4	2.8	4.4	39.6	
Croatia	126	3.2	2.9	0.6	1.2	1.9	2.3	3.0	4.5	20.5	
Italy	607	3.2	3.5	0.1	1.0	2.0	2.4	2.8	4.4	39.6	0.355
THg in cord blood (ng/g)	1078	5.2	5.0	0.1	1.5	2.8	3.6	4.6	7.8	33.1	
Croatia	122	5.1	5.1	0.5	1.4	2.7	3.7	4.7	7.7	32.3	
Greece	247	7.7	6.0	0.5	2.8	4.8	6.2	7.4	11.4	33.1	
Italy	458	5.6	4.8	0.1	2.1	3.4	4.0	5.2	7.9	32.8	
Slovenia	251	2.1	1.6	0.2	0.8	1.3	1.7	1.9	3.0	10.0	< 0.001
THg in breast milk (ng/g)	819	0.4	1.2	0.0	0.1	0.2	0.2	0.2	0.4	28.3	
Croatia	75	0.3	0.4	0.0	0.1	0.1	0.2	0.2	0.3	2.4	
Greece	36	1.7	2.9	0.0	0.2	0.4	0.7	1.1	2.0	11.5	
Italy	492	0.3	1.3	0.0	0.1	0.2	0.2	0.2	0.3	28.3	
Slovenia	216	0.3	0.3	0.0	0.1	0.2	0.2	0.2	0.4	2.9	< 0.001
Cognitive composite score	1302	106.9	11.3	65	100	105	105	110	115	145	
Croatia	126	107.5	13.2	80	95	100	105	110	120	145	
Greece	300	101.4	10.9	65	95	100	100	105	110	135	
Italy	604	106.3	8.2	75	100	105	105	110	115	130	
Slovenia	272	114.1	12.7	70	105	110	115	115	125	145	< 0.001
Language composite score	1302	100.3	12.0	47	91	97	100	103	109	141	
Croatia	126	108.0	14.2	65	97	103	109	112	118	141	
Greece	300	97.2	12.5	47	86	94	97	100	109	132	
Italy	604	97.8	8.6	47	91	97	97	100	106	121	
Slovenia	272	105.5	13.6	56	94	103	106	109	115	141	< 0.001
Receptive Communication scaled score	1300	11.1	2.5	1	9	10	11	11	13	19	
Croatia	126	12.2	2.7	3	10	12	12	13	14	19	
Greece	300	10.0	2.5	1	8	9	10	11	12	16	
Italy	604	10.6	1.6	1	9	10	11	11	12	14	
Slovenia	270	12.7	2.8	5	10	12	13	14	15	19	< 0.001
Expressive Communication scaled score	1301	9.0	2.2	1	7	8	9	9	11	18	
Croatia	126	10.4	2.9	4	8	10	10	11	13	17	
Greece	299	9.0	2.2	1	7	8	9	10	11	17	
Italy	604	8.6	1.8	1	7	8	9	9	10	14	
Slovenia	272	9.2	2.4	3	7	8	9	9	11	18	< 0.001
Motor composite score	1302	102.6	8.5	61	97	100	103	103	110	142	
Croatia	126	108.3	10.1	85	100	107	107	110	115	142	
Greece	300	98.8	8.4	61	94	97	100	100	107	121	
Italy	604	101.5	5.8	67	97	100	100	103	107	115	
Slovenia	272	106.5	9.6	61	100	103	107	110	112	136	< 0.001
Fine Motor scaled score	1302	11.5	1.8	3	10	11	11	12	13	19	
Croatia	126	11.8	2.1	8	10	11	12	12	14	19	
Greece	300	10.9	2.0	3	10	10	11	11	13	18	
Italy	604	11.4	1.4	6	10	11	11	12	13	16	
Slovenia	272	12.3	2.1	4	11	12	12	13	14	19	< 0.001
Gross Motor scaled score	1298	9.3	1.5	1	8	9	9	10	10	19	
Croatia	126	10.8	2.2	4	9	10	10	11	12	19	
Greece	299	8.7	1.4	3	8	9	9	9	10	12	
Italy	603	9.0	1.0	1	8	9	9	9	10	12	
Slovenia	270	9.9	1.6	4	9	10	10	10	11	15	< 0.001

[a] Differences among Countries were assessed by the Kruskall Wallis test.

week). Overall fish intake was calculated by summing the estimated weekly intake of all fish types (Valent et al., 2013b).

2.6. Statistical analysis

THg concentrations in different biological samples and BSID-III scores were described, for each country, by arithmetic means and standard deviations (SDs), quintiles, median and minimum-maximum ranges. The other continuous variables are presented as means, medians, and SDs. Categorical variables are presented as number and percentages. Differences among countries for general characteristics of the enrolled population, for distributions of THg concentrations and for the BSID-III scores were assessed by the Kruskal Wallis test (for continuous variables) and the $\chi 2$ test (for categorical variables). The relation between THg concentrations and BSID-III scores was explored with linear regression analysis. Logistic regression analysis was conducted as sensitivity analysis. Linear regression models included BSID-III scores as dependent continuous variables assuming that the neurodevelopmental scores decreased proportionally with increasing THg concentrations. THg was $\log_2$ transformed because of its skewed distribution. However, models with no logarithmic transformation were also built for sensitivity analysis (data not shown). Logistic regression was conducted to evaluate the hypothesis that the probability of suboptimal development (Golding et al., 2016), measured as a dichotomous variable derived from the distribution of the BSID-III score, depended on the increasing THg concentration. Given that the Italian sub-cohort was the largest, the lower end of each Italian BSID-III score distribution closest to 20% was defined as suboptimal development (Golding et al., 2016), and considered as a cut-off to dichotomize the corresponding scores in all countries. Separate models were built for each neurodevelopmental

Table 3
Meta-analysis of the association between BSID-III scores and THg concentrations in maternal hair, maternal blood, cord blood and breast milk, when no heterogeneity by country was found.

	Meta-analysis Linear regression			Meta-analysis Logistic regression		
	N	Overall β[a]	95%CI	N	Overall OR[c]	95%CI
Cognitive composite score:						
THg in maternal hair (ng/g)	1083	0.20[a]	(-0.29, 0.69)	1081	0.99[a]	(0.97, 1.00)
THg in maternal blood[b] (ng/g)	636	−0.09[a]	(-0.61, 0.43)	634	0.98[a]	(0.93, 1.03)
THg in cord blood (ng/g)	892	0.13[a]	(-0.39, 0.64)	891	0.98[a]	(0.95, 1.01)
THg in breast milk (ng/g)	734	−0.01[a]	(-0.53, 0.50)	738	1.07[a]	(0.90, 1.28)
Language composite score:						
THg in maternal hair (ng/g)	1086	0.55[b]	(0.05, 1.05)	1084	0.99[g]	(0.98, 1.01)
THg in cord blood (ng/g)	896	0.25[b]	(-0.29, 0.78)	895	0.98[g]	(0.95, 1.02)
THg in breast milk (ng/g)	735	0.26[b]	(-0.26, 0.79)	739	0.94[g]	(0.74, 1.19)
Motor composite score:						
THg in maternal hair (ng/g)	1083	−0.12[c]	(-0.47, 0.22)	1074	1.01[h]	(0.99, 1.02)
THg in maternal blood[b] (ng/g)	636	0.11[c]	(-0.25, 0.48)	628	0.99[h]	(0.94, 1.06)
THg in cord blood (ng/g)	892	−0.11[c]	(-0.47, 0.25)	848	1.00[h]	(0.97, 1.04)
THg in breast milk (ng/g)	734	−0.01[c]	(-0.38, 0.36)	733	1.09[h]	(0.86, 1.39)
Receptive Communication scaled score:						
THg in maternal hair (ng/g)	1075	0.12[d]	(0.02, 0.22)	1083	0.99[b]	(0.97, 1.00)
THg in maternal blood[b] (ng/g)	628	−0.02[d]	(-0.12, 0.08)	635	1.00[b]	(0.94, 1.07)
THg in cord blood (ng/g)	887	0.12[d]	(-0.08, 0.32)	861	0.99[b]	(0.96, 1.02)
THg in breast milk (ng/g)	728	0.08[d]	(-0.02, 0.19)	732	0.96[b]	(0.74, 1.23)
Expressive Communication scaled score:						
THg in maternal hair (ng/g)	1272	0.04[e]	(-0.06, 0.13)	1084	1.00[i]	(0.98, 1.01)
THg in maternal blood[b] (ng/g)	727	0.13[e]	(-0.22, 0.48)	635	0.98[i]	(0.92, 1.04)
THg in cord blood (ng/g)	1070	0.01[e]	(-0.09, 0.11)	895	1.01[i]	(0.98, 1.04)
THg in breast milk (ng/g)	809	0.01[e]	(-0.09, 0.10)	739	0.90[i]	(0.68, 1.20)
Fine Motor scaled score						
THg in maternal hair (ng/g)	1082	−0.03[f]	(-0.11, 0.06)	985	1.01[l]	(0.99, 1.02)
THg in maternal blood[b] (ng/g)	636	0.05[f]	(-0.04, 0.15)	542	1.01[l]	(0.96, 1.08)
Gross Motor scaled score:						
THg in maternal hair (ng/g)	1081	−0.01[a]	(-0.07, 0.05)	1072	1.00[m]	(0.99, 1.01)
THg in maternal blood[b] (ng/g)	635	−0.02[a]	(-0.08, 0.05)	627	1.01[m]	(0.95, 1.07)
THg in cord blood (ng/g)	890	−0.03[a]	(-0.09, 0.03)	882	1.03[m]	(1.00, 1.07)
THg in breast milk (ng/g)	732	0.09[a]	(-0.07, 0.24)	692[d]	0.95[m]	(0.75, 1.20)

[a]Adjusted for mother's educational level, child's gender, child's birth weight (grams), child's consumption of fresh fish.
[b]Adjusted for mother's educational level, child's gender, child's consumption of fresh fish, number of children living in home.
[c]Adjusted for child's gender, child's birth weight (grams), breastfeeding history.
[d]Adjusted for mother's educational level, child's gender, child's consumption of fresh fish, number of children living in home, home size.
[e]Adjusted for mother's educational level, child's gender, number of children living in home.
[f]Adjusted for mother's educational level, child's gender, child's birth weight (grams), breastfeeding history, mother's fish consumption during pregnancy.
[g]Adjusted for mother's educational level, child's gender, child's consumption of fresh fish.
[h]Adjusted for mother's occupation, child's gender, child's birth weight (grams), child's consumption of fresh fish, breastfeeding history, day care attendance at 18 months.
[i]Adjusted for mother's educational level, child's gender, child's consumption of fresh fish, breastfeeding history.
[l]Adjusted for child's gender, mother's smoking habits, mother's weight gain during pregnancy, breastfeeding history, day care attendance at 18 months.
[m]Adjusted for child's gender, child's birth weight (grams), child's consumption of fresh fish.
[a] THg concentrations (ng/g) were $\log_2$ transformed in multiple linear regression.
[b] Meta-analysis was conducted between Italy and Croatia, the only countries that had maternal blood samples.
[c] The ORs were estimated each 100 ng/g for THg concentrations in maternal hair.
[d] Meta-analysis was conducted only among Italy, Greece, and Slovenia. In Croatia only 4 children were in the lowest quintile and had valid THg measurements in breast milk, therefore the logistic model was not applied.

score and stratified by country. Multivariate linear and logistic regression models were built to assess the association between THg and BSID-III score, adjusting for potential confounding variables. Among the covariates considered as potential confounders, only those associated with at least one BSID-III outcome ($p < 0.15$), were included in the final models (data not shown). Given that THg concentrations were $\log_2$ transformed, in linear regression models the β coefficient corresponded to the variation in BSID-III score associated with the doubling in THg levels (i.e., the lower the β coefficient, the worse the effect of THg concentration). In logistic regression models, the odds ratio (OR) corresponded to the risk of a suboptimal development associated with the THg levels (i.e., the higher the OR, the worse the effect of THg concentration). Meta-analysis procedures were performed to assess combined estimates of the association between each of the seven BSID-III scores and the pre and post-natal mercury exposure from the four countries. Adjusted β coefficients and ORs obtained by multivariate models were used. The presence of heterogeneity among the four countries was explored. Estimates of association were computed using weighted regression, and the weights were the inverse of the local variances, i.e., according to the fixed-effect model. The heterogeneity, I-squared (I2), was calculated under the fixed-effect hypothesis and if the heterogeneity was detected ($I2 > 50\%$), the random-effect was applied. When no heterogeneity was found, only the overall estimates were shown. In case of heterogeneity, the adjusted β coefficients and ORs for each country were shown in forest plots. SAS (version 9.4 SAS Institute INC., Cary, N.C., USA) and STATA (StataCorp. 2013. Stata Statistical Software: Release 13. College Station, TX: StataCorp LP) were used for the statistical analysis.

3. Results

3.1. Descriptive analyses

In total, 2189 (C = 233; G = 466; I = 900; S = 590) pregnant women were enrolled in the cohort, while 1308 subjects remained in the study and provided information for this analysis. Compared with those who remained under observation, mothers withdrawn from the study were more likely to hold an “Elementary and Middle school degree” (28.0% vs 20.8%), less likely to hold a “University degree”, (31.3% vs 36.5%, p = 0.013) and less likely to live in a house they

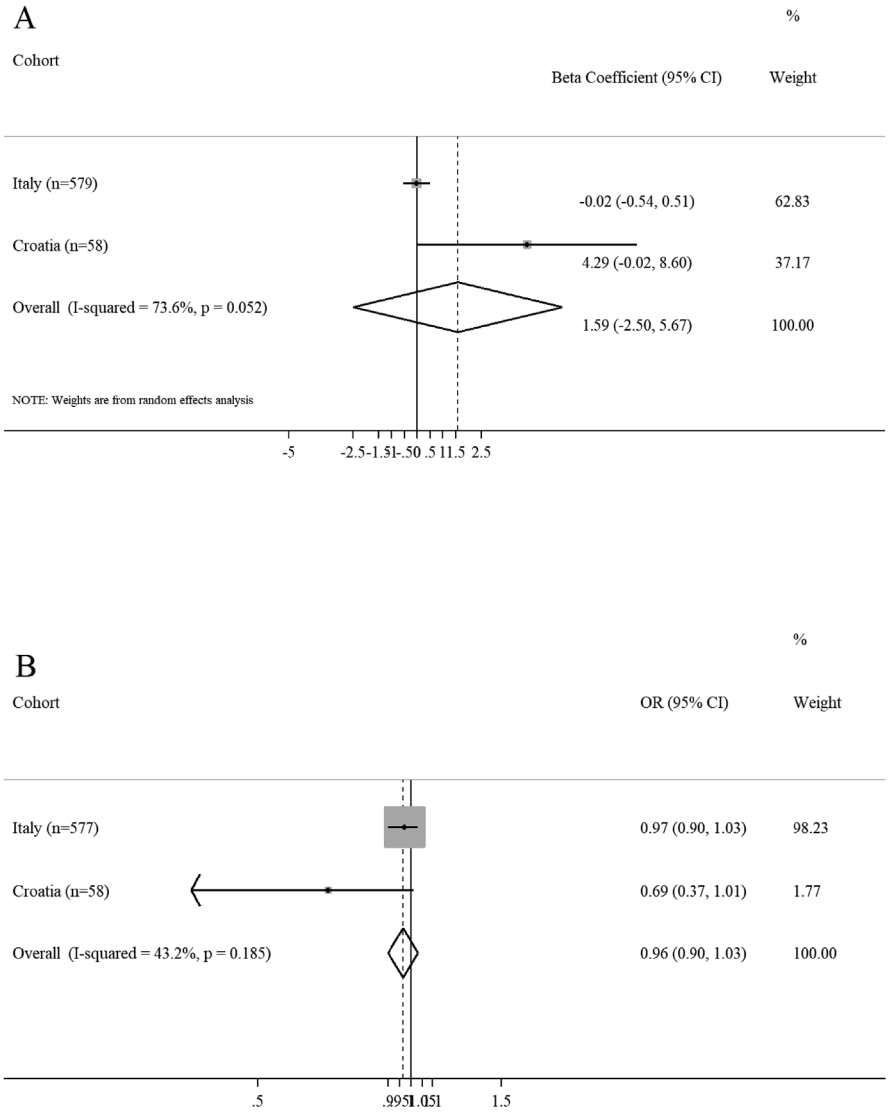

Fig. 1. Meta-analysis of the multiple linear regression (A) and of the multiple logistic regression (B) between LAN composite score and THg concentrations in maternal blood.

For multiple linear regression THg concentrations are $\log_2$ transformed.

A. Adjusted for mother's educational level, child's gender, child's consumption of fresh fish, number of children living in home.

B. Adjusted for mother's educational level, child's gender, child's consumption of fresh fish.

owned (64.1% vs 71.5%, p = 0.008). On the other hand, marital status, employment status, and age at delivery were similar.

The characteristics of mother-child pairs in the four countries are shown in Table 1. Mother's age at delivery, employment and marital status, and educational level were significantly different among the four countries. In Slovenia, the maternal intake of fish (servings/week) during pregnancy was the lowest. Italian mothers introduced fresh fish consumption in the diet of their children earlier than the mothers of the other three countries. Duration of breastfeeding (number of months of breastfeeding up to 18 months) appears more prolonged in I and S than in C and G.

The distribution of THg concentrations and the BSID-III composite and scaled scores by country is presented in Table 2. Mean concentrations of THg in maternal hair, cord blood, and breast milk were higher in the Greek sub-cohort. The lowest mean levels of THg in maternal hair and cord blood were found in Slovenia. Mean composite and scaled score were higher in Croatian and Slovenian children. The distribution of BSID-III scores, as continuous and dichotomous variables, and unadjusted comparisons of differences by mother's and child's characteristics are shown in Appendix.

3.2. Meta-analysis

Table 3 shows the overall (all countries combined) results of the meta-analysis, using both β coefficients and ORs obtained by the fully adjusted multivariate models, between each BSID-III score and pre- and post-natal THg exposure, when no heterogeneity by country was found. Considering the meta-analysis of multivariate linear models, an overall positive association was found between LAN and RC and increasing THg in maternal hair (n = 1086; β = 0.55; 95%CI: 0.05–1.05 and n = 1075; β = 0.12; 95%CI: 0.02–0.22, respectively). The overall adjusted OR between THg in cord blood and suboptimal GM score was borderline significant (n = 882; OR = 1.03; 95%CI: 1.00–1.07). In details the number of children with a suboptimal GM score was: 2 in Croatia (n = 53), 72 in Greece (n = 180), 98 in Italy (n = 424) and 34 in Slovenia (n = 225).

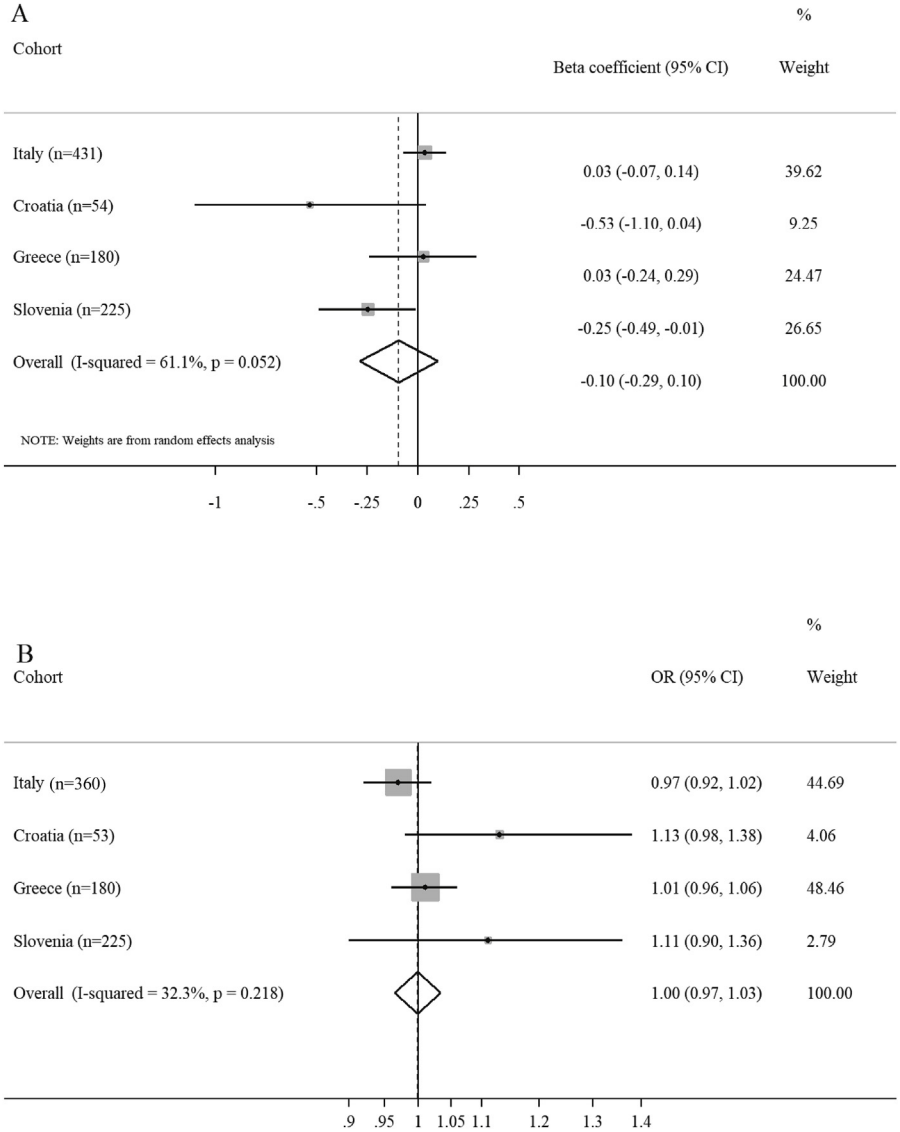

Fig. 2. Meta-analysis of the multiple linear regression (A) and of the multiple logistic regression (B) between FM scaled score and THg concentrations in cord blood.

For multiple linear regression THg concentrations are $\log_2$ transformed.

A. Adjusted for mother's educational level, child's gender, child's birth weight (grams), breastfeeding history, mother's fish consumption during pregnancy.

B. Adjusted for child's gender, mother's smoking habits during pregnancy, mother's weight gain during pregnancy, breastfeeding history, day care attendance at 18 months.

Heterogeneity by country was found for the relation between LAN and THg in maternal blood (Fig. 1), and for FM and THg in cord blood (Fig. 2) and breast milk (Fig. 3). The association, measured by the multivariate linear regression, between LAN and $\log_2$(THg) concentrations in maternal blood, is shown in Fig. 1A. Samples of maternal venous blood were collected only in Italy and Croatia. In Croatia, such an association was positive and borderline significant (n = 58; β = 4.29; 95%CI: −0.02, 8.60). Consistent results are also obtained in Croatia by multivariate logistic regression (Fig. 1B), where the THg concentrations in maternal venous blood were associated with a reduced risk for children to fall in the lowest quintile of LAN of 31% (n = 58; OR = 0.69; 95%CI: 0.37, 1.01). Instead, in Italy LAN was not associated with THg concentrations in maternal venous blood neither in linear nor in logistic regression models. A borderline statistically significant, inverse association between FM and $\log_2$(THg) concentrations in cord blood is shown in Fig. 2A for Croatia (n = 54, β = −0.53; 95%CI: −1.10, 0.04) and Slovenia (n = 225; β = −0.25; 95%CI: −0.49, −0.01). The associations of THg in breast milk with FM are displayed in Fig. 3A (linear regression) and Fig. 3B (logistic regression). The linear regression models did not show a significant association for any country (Fig. 3A). From the logistic regression models (Fig. 3B), for Slovenia, higher THg levels in breast milk were associated with higher risk of suboptimal FM (n = 195; OR = 5.25; IC95%: 1.36, 21.10).

4. Discussion

THg concentrations found in biological samples of our Eastern Mediterranean study were much lower than those reported in the Faroe and Seychelles studies (Myers et al., 2007; Grandjean et al., 1992; Cernichiari et al., 1995) but also rather lower than in previous studies conducted in New Zealand (Kjellström et al., 1989) and in the Spanish INMA study (Llop et al., 2012). Nevertheless, the mean and median THg in maternal hair, maternal blood, and cord blood in our overall cohort and the sub-cohorts from Greece, Italy, Croatia and Slovenia were not very different from those measured in populations of most European countries, US regions and the reference dose (RfD) of 5,5 ng/g for Hg in

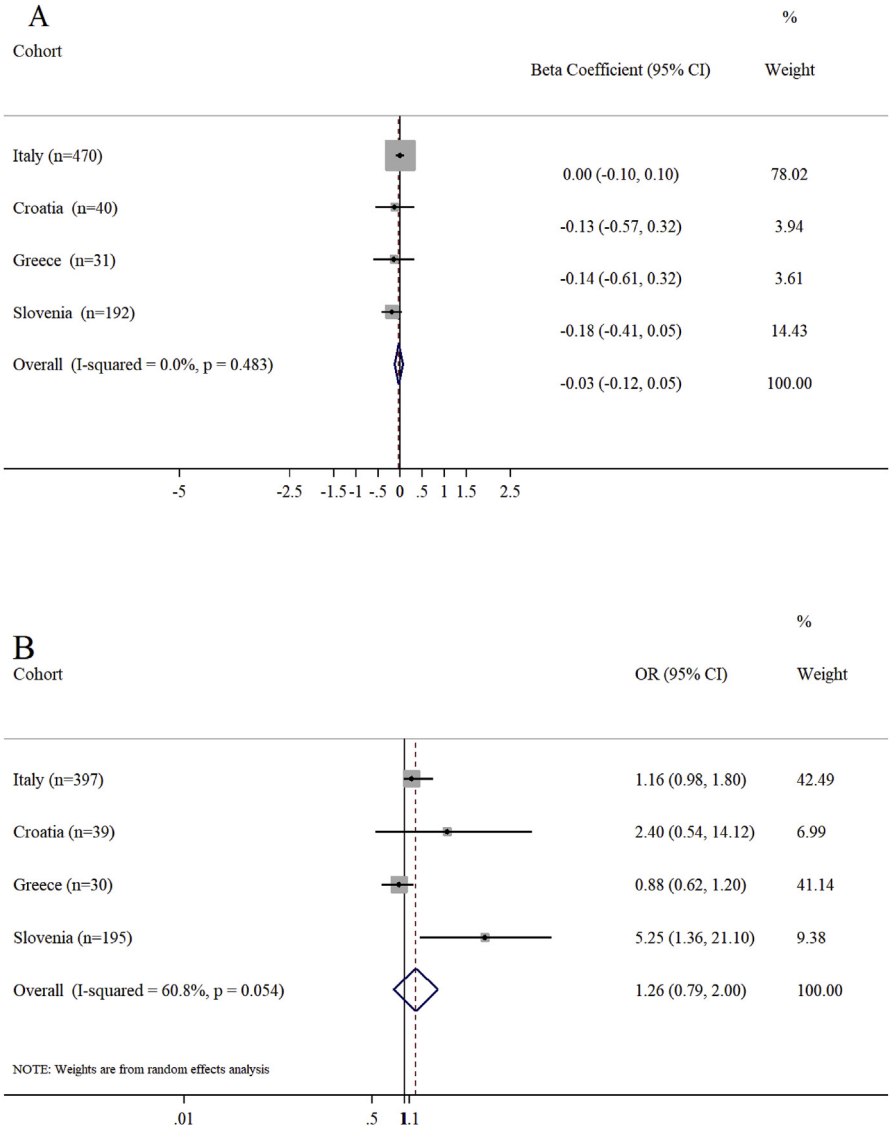

Fig. 3. Meta-analysis of the multiple linear regression (A) and of the multiple logistic regression (B) between FM scaled score and THg concentrations in breast milk.

For multiple linear regression THg concentrations are $\log_2$ transformed.

A. Adjusted for mother's educational level, child's gender, child's birth weight (grams), breastfeeding history, mother's fish consumption during pregnancy.

B. Adjusted for child's gender, mother's smoking habits during pregnancy, mother's weight gain during pregnancy, breastfeeding history, day care attendance at 18 months.

cord blood, calculated by EPA (Gao et al., 2007; Mahaffey et al., 2009). For this reason, we believe that our results may be relevant, especially when extrapolated to most western populations.

In our study, the main results of the meta-analysis do not show overall negative associations with cognitive and language scales. More complex is the interpretation of the results found in relation to motor scales. In addition, although differences exist by country in terms of mother's age at delivery, employment and marital status, educational level, maternal intake of fish during pregnancy, child's intake of fish up to 18 months and mean BSID-III composite and scaled scores, the results of the meta-analysis focused on the effects of THg on the BSID-III do not show a significant heterogeneity by country, except for some THg measurements and LAN and FM.

4.1. Cognitive scales

Combined analyses did not find convincing associations between THg and cognitive outcomes.

In our study THg levels in cord blood do not correlate better with BSID-III composite scores compared to THg levels in mother's hair. This observation differs with some evidence of the literature (Barregård, 2005). According to some authors, THg concentration in cord blood is the preferred biomarker for prenatal exposure. Hg concentrations in hair can be affected by several factors, including hair color and variable growth rates, limiting its usefulness as an indicator of Hg concentrations in the body (Budtz-Jørgensen et al., 2004).

Although a large number of covariates were considered to adjust β coefficients and ORs used in the meta-analysis, neurodevelopment might be influenced by variables that are still not known or not well measured. In our study, we did not take into account some pollutants in fish that might affect neurodevelopment and also the genetic susceptibility was not considered. MeHg metabolism differs largely between individuals and may explain differences in MeHg susceptibility, i.e., individuals with a slower MeHg metabolism accumulate MeHg and develop more toxic effects. For the developing child, this means that the slower the metabolism in the mother, the higher the MeHg dose in the fetus; also the elimination half-time of MeHg varies greatly between individuals, ranging from 45 to 70 days, but extreme values, up to almost 190 days have also been reported (Clarkson, 2002). One explanation for this variation might be hereditary differences in MeHg metabolism. The genetic background can result in individual differences in MeHg body retention despite similar fish intake. Some evidence suggests that genetic factors are very important for MeHg toxicity and several key genes for Hg toxicity have already been identified (Custodio et al., 2004; Engström et al., 2013; Gundacker et al., 2009; Schläwicke Engström et al., 2008). For example, there is evidence that variants of genes associated with glutathione (GSH) metabolism are determinants of MeHg retention in the body. Some evidence from two Mediterranean cohorts supports a role of ABC transporter genes in the accumulation of MeHg during early development (Llop et al., 2014). In addition, recent analyses conducted in subjects enrolled in the INMA and in the Italian and Greek components of this PHIME study showed that the BSID mental scale improved with the increase of cord blood Hg concentrations for children carrying a high activity allele of CYP3A, while for children carrying low activity CYP3A alleles the association was near null (Llop et al., 2017). Results from the Croatian and Slovenian cohorts of the PHIME study indicates the importance of the genetic polymorphism apolipoprotein E (APOE). Carriers of the APOE ε4 allele showed a negative association between hair or cord blood THg levels and cognitive scores of the BSID III test, while this was not observed with ε2 or ε3 carriers (Snoj Tratnik et al., 2017).

Despite inconsistent results found in different studies, in an overview Karagas et al. (2012) concluded that the recent evidence suggests that low-level MeHg exposure might affect fetal growth, and evidence exists that low levels of prenatal MeHg exposure may cause neurocognitive effects in early childhood. Our results do not support the conclusions by Karagas and are consistent with those from the INMA cohort (Llop et al., 2012; Ramon et al., 2011).

4.2. Language scales

In the overall Mediterranean PHIME cohort, the meta-analysis carried out using the adjusted β coefficients for the 4 countries showed that higher maternal hair THg levels were associated with better LAN scores. This positive association with maternal hair THg is explained by the RC component, within the LAN scale. The relations between LAN scale and THg measured in cord blood and breast milk do not show a statistically significant positive association. For THg measured in maternal blood heterogeneous results were observed given that only for Croatia a positive, borderline significant, association was seen (Fig. 1). Due to the limited size of the Croatian sub-cohort, the interpretation of these results should be considered with caution. Also, although we adjusted all our analyses for several potential confounders, residual confounding may still be present (Genuis, 2008; Gil and Gil, 2015; Starling et al., 2015).

Although these findings appear to be in contrast with the results conducted in 3 years old children in Taiwan (His et al., 2014), they are consistent with those from ALSPAC (Iles-Caven et al., 2016).

4.3. Motor scales

We found no evidence of adverse associations between THg exposure, measured in all the biological samples, and MOT score. These results are consistent with those from the ALSPAC study (Golding et al., 2016) conducted in England, with the New England Children's Amalgam Trial (Bellinger et al., 2007) and partially with the Spanish INMA study that found that increasing THg levels were associated with significantly lower psychomotor scores only among females (Ramon et al., 2011). On the other hand, in the Japanese Tohoku Study of Child Development, The Psychomotor Development Index (PDI) of BSID-II was significantly correlated with cord-blood THg only in boys, and significance of the association remained unchanged after adjusting for possible confounders (Tatsuta et al., 2017).

The interpretation of our study is more complex when the two motor components (FM and GM) of BSID-III are analyzed separately. For the whole PHIME cohort, results of the meta-analysis using adjusted ORs show that THg is associated with a slightly increased risk of a suboptimal GM score only in the cord blood. Heterogeneity by country was found for the association between THg cord blood and FM score, with a borderline statistically significant negative association for the Croatian and Slovenian sub-cohorts (Fig. 2A). These results confirm what was recently published restricted to the Croatian PHIME sub-cohort (Prpić et al., 2017). Furthermore, in the Slovenian sub-cohorts (Fig. 3B) THg concentrations in breast milk appeared to be a risk factor for an FM performance within the lowest quintile. Some evidence has emerged on the interaction effect between gene polymorphisms of APOE and Hg levels on fine motor scores, as in a Taiwanese cohort examined at two years with Hg measured in the cord blood (His et al., 2014).

On the other hand, Hg (with and without eating fish) was associated neither with fine nor with gross motor skills in the ALSPAC study (Iles-Caven et al., 2016). In the Seychelles Child Development Study, there were no adverse associations between prenatal MeHg and any of the measured endpoints, including fine motor skills (Van Wijngaarden et al., 2017).

Finally, a recent review related to the causes of impairment in fine motor development conclude that there is conflicting evidence regarding prenatal and early child effects of Hg. However, the evidence from primate studies and the Minimata accident raises the possibility of an adverse effect many years after exposure (Golding et al., 2014). Therefore, special effort should be devoted to the long-term follow-up of mother-child cohorts especially as it refers to measuring

psychomotor outcomes.

5. Methodological issues

Results from our study must be evaluated bearing in mind the reasons that lead to conflicting evidence concerning prenatal and early child effects of mercury (Starling et al., 2015). Main differences by study protocol may include: type of biological samples used to measure Hg levels (maternal hair, blood and urine; cord blood; breast milk; child hair and urine; etc.), timing of sampling (first, second or third trimester of pregnancy, birth, postnatal, etc.); laboratory methods in Hg measurements; proportion of Hg species; comparability of neurodevelopment testing (Bayley I, II, III, others); child age at follow-up; confounding factors (e.g., maternal age and intelligence, maternal smoke or use of alcohol, breastfeeding, family socioeconomic status, co-exposure to other toxic elements or to beneficial nutrients) and effect modifiers such as child's gender, ethnicity and genetic variations. On this respect, our study was able to measure Hg from several biological samples, but the timing of data collection varied by country. The same lab (Institute Josef Stefan, Ljubljana, Slovenia) measured Hg for all samples but not all measurements included a complete Hg speciation, i.e., THg values from this study were assumed to be representative of a correct MeHg measure. Unmeasured or poorly measured determinants of neurodevelopmental outcomes may have left residual confounding in the measures of association. Additionally, a thorough assessment of interaction was beyond the scope of this general paper. Finally, this article focuses on a neurodevelopment assessment conducted at 18 months of age. Therefore, the full effects of prenatal Hg exposure may not be measurable yet.

Additionally, it cannot be excluded that mother's and child's fish intake could be misreported on the questionnaires and the estimates of weekly servings could be affected by errors leading to residual confounding of Hg by fish intake. Maternal IQ during pregnancy was tested only in the Italian and Croatian sub-cohorts, and measurements of PUFAs were available only for Italy. Finally, our study may not have had sufficient statistical power to detect the effects of Hg if they were very subtle.

Even if we were aware that by dichotomizing the Bayley scores we would have lost valuable information, we ran logistic regression models anyway for the purpose of sensitivity analyses, as also done in the ALSPAC study (Golding et al., 2016). With the odds ratio could be in some conditions (for some outcomes and some cohorts) an overestimate of the relative risk because the outcome (the cut-off was the lowest 20% in Italian subcohort) is not a rare event and in these cases log-binomial regression could have been the most appropriate model.

6. Conclusions

We found some evidence that THg was associated with decreased developmental motor scores at 18 months. However, these results, which describe both fine and gross motor development and were measured in different biological samples and in four Mediterranean populations, have only partial internal and external consistency and should be confirmed by further testing conducted at an older age. No detrimental effects of THg were demonstrated for cognitive and language outcomes so far at these low levels. Follow-up activities within this Mediterranean PHIME cohort are ongoing to elucidate further the long-term neurological effects of prenatal mercury exposure.

Acknowledgments

This work was supported by the EU through its Sixth Framework Programme for RTD project PHIME (contract no FOOD-CT-2006-016253), by Slovenian Agency for Research ARRS under the Programme P1-0143 and by RC 12/12 of Institute for Maternal and Child Health -IRCCS “Burlo Garofolo” – Trieste – Italy funded by Italian Ministry of Health. The paper reflects only the authors views; the European Union is not liable for any use that may be made of the information. The authors declare they have no competing interests. We are grateful to the women who participated in our study as well as the people who helped in the recruitment in the different countries.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ijheh.2018.07.011.

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