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## Prenatal mercury exposure, maternal seafood consumption and associations with child language at five years

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### ABSTRACT

**Background:** Methyl mercury (MeHg) is a well-known neurotoxin and evidence suggests that also low level exposure may affect prenatal neurodevelopment. Uncertainty exists as to whether the maternal MeHg burden in Norway might affect child neurodevelopment.

**Objective:** To evaluate the association between prenatal mercury exposure, maternal seafood consumption and child language and communication skills at age five.

**Methods:** The study sample comprised 38,581 mother-child pairs in the Norwegian Mother and Child Cohort Study. Maternal mercury blood concentration in gestational week 17 was analysed in a sub-sample of 2239 women. Prenatal mercury exposure from maternal diet was calculated from a validated FFQ answered in mid-pregnancy. Mothers reported children's language and communications skills at age five by a questionnaire including questions from the Ages and Stages Questionnaire (ASQ), the Speech and Language Assessment Scale (SLAS) and the Twenty Statements about Language-Related Difficulties (language 20). We performed linear regression analyses adjusting for maternal characteristics, nutritional status and socioeconomic factors.

**Results:** Median maternal blood mercury concentration was 1.03 µg/L, dietary mercury exposure was 0.15 µg/kg bw/wk, and seafood intake was 217 g/wk. Blood mercury concentrations were not associated with any language and communication scales. Increased dietary mercury exposure was significantly associated with improved SLAS scores when mothers had a seafood intake below 400 g/wk in the adjusted analysis. Sibling matched analysis showed a small significant adverse association between those above the 90th percentile dietary mercury exposure and the SLAS scores. Maternal seafood intake during pregnancy was positively associated with the language and communication scales.

**Conclusion:** Low levels of prenatal mercury exposure were positively associated with language and communication skills at five years. However, the matched sibling analyses suggested an adverse association between mercury and child language skills in the highest exposure group. This indicates that prenatal low level mercury exposure still needs our attention.

### 1. Introduction

In utero exposure to nutrients and toxins plays a vital role in fetal development and may result in persistent changes that influence health later in life (Barker, 1990; Grandjean and Landrigan, 2014). Dietary fish and seafood is the primary source of both n-3 long-chain

polyunsaturated fatty acids (n-3 LCPUFA) and methyl mercury (MeHg) (Mahaffey et al., 2009). High in utero exposure to MeHg is toxic and leads to irreversible neurodevelopmental damage in children, as seen in the Minamata tragedy (Ekino et al., 2007). The developing nervous system of the fetus appears to be especially vulnerable, and more knowledge is needed to establish whether prenatal MeHg exposure

**Abbreviations:** ASQ, Ages and Stages Communication scale; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FFQ, food frequency questionnaire; Hg, total mercury; KJ, kilojoule; Language 20, twenty statements about language-related difficulties; MBRN, Medical Birth Registry of Norway; MeHg, methylmercury; MoBa, the Norwegian Mother and Child Cohort Study; MoBa Etox, Human environmental biobank project; n-3 LCPUFA, n-3 long-chain polyunsaturated fatty acids; NIPH, Norwegian Institute of Public Health; Se, selenium; SLAS, Speech and Language Assessment Scale; TEQ, Toxic Equivalents per kg bw/day; TWI, tolerable weekly intake

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from regular seafood consumption below the tolerable weekly intake (TWI) of 1.3 µg/kg bw may cause adverse neurodevelopmental effects (EFSA, 2012). Recent reviews of health risks and benefits of fish and seafood consumption, including neurodevelopmental endpoints for sensitive groups, concluded that the beneficial effects of seafood in the maternal diet outweigh the risk associated with prenatal MeHg exposure below the TWI (Gil and Gil, 2015; EFSA, 2015). However, intake of predatory fish species can lead to exceedance of TWI for MeHg and adversely affect fetal development (Grandjean et al., 2012; Davidson et al., 2010). The fact that MeHg concentrations in seafood varies substantially between and within species and regions is a challenge that complicates general dietary advice with regard to fish and seafood consumption across Europe (EFSA, 2015).

In a previous study investigating prenatal MeHg exposure in the Norwegian Mother and Child Cohort Study (MoBa), we found that pregnant mothers had a mean calculated dietary MeHg exposure of 1.30 µg/day (corresponding to 0.14 µg/kg bw/wk). However, an exposure level above the 90th percentile (> 2.6 µg/day, > 0.29 µg/kg bw/wk) was associated with impaired language development at child age three years, indicating that for a subgroup, adverse effects of prenatal MeHg exposure below the TWI could outweigh the beneficial nutritional effect of maternal seafood consumption (Vejrup et al., 2016).

The purpose of this study is to evaluate the associations between total mercury (Hg) concentration in maternal blood during pregnancy as well as calculated MeHg exposure from maternal pregnancy diet, with language and communication development at five years in MoBa.

## 2. Material and methods

### 2.1. Study population

The Norwegian Mother and Child Cohort Study (MoBa) is a prospective population based pregnancy cohort conducted by the Norwegian Institute of Public Health (NIPH) (Magnus et al., 2016). The recruitment period was from 1999 to 2008 and included all parts of Norway. Mothers could participate with more than one pregnancy and the cohort includes 95,200 mothers and 114,500 children. Of the invited pregnant women, 41% consented to participate. All participants gave a written informed consent at recruitment. This study is based on data from pregnancy and childhood up to 5 years in version 9 of the quality assured data files released for research in 2015. The dataset is linked to the Medical Birth Registry of Norway (MBRN). The questionnaires used were completed in gestational weeks 15 (Q1) and week 22 (Q2), at 6 months (Q4) after birth and when the child was 5 years (Q5). Q2 is a food frequency questionnaire (FFQ), while Q1, Q4 and Q5 are general questionnaires covering maternal and child health, lifestyle and background factors. We excluded participants with multiple births and mothers reporting unlikely energy intake (< 4.5 or > 20 MJ). The final study population included 38,581 women with singleton births who had completed questionnaires Q1, Q2 and Q5. English translations of the questionnaires are available at the NIPH website (The NIPH, n.d.).

### 2.2. Ethics approval

The data collection in the MoBa study was approved by the Norwegian Data Protection Authority and the Regional Committee for Ethics in Medical Research (S-95113 and S-97045). The Regional Committee for Ethics in Medical Research further approved the current study (2015/1346).

### 2.3. Exposures – Mercury exposure and maternal seafood consumption

The semi-quantitative FFQ was designed to capture the participant's average dietary intake of food since the start of pregnancy (Meltzer

et al., 2008). FoodCalc (Lauritsen, 2005) and the Norwegian food composition table (Norwegian Food Safety Authority, N.D.o.H., Department of Nutrition - University of Oslo, 2003) were used to calculate food and nutrient intakes. A validation study showed that the MoBa FFQ is a valid tool for estimating the intakes of food and nutrients, including different types of fish and seafood, as well as long chain marine fatty acid supplements (Brantsaeter et al., 2010; Brantsaeter et al., 2007; Brantsaeter et al., 2008).

Concentrations of total mercury (Hg) in Norwegian seafood and other food items were compiled in a database (Jenssen et al., 2012). Lower bound concentrations (values < levels of quantification are set to zero) were used to calculate the individual total dietary Hg intake. Methylmercury (MeHg) is the organic and most toxic form of mercury. MeHg constitutes 80–100% of the total Hg in seafood (EFSA, 2015). Seafood contributes 88% of the total Hg among women in MoBa (Vejrup et al., 2013), and we considered the total dietary Hg intake to reflect MeHg exposure (EFSA, 2014).

Total seafood includes intake of all fish, shellfish and crustaceans. We examined seafood intake in grams per week and divided into five categories, with 100 g/wk increase per category to investigate the gradients of seafood intake. To perform stratified analysis we used a cut-off at 400 g of seafood per week. The rationale for this is the recommendations by the Norwegian Directorate of Health of a weekly fish intake of 350–400 g (HDIR, 2017). The FFQ was used to calculate the intake of long chain poly-unsaturated fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) separately from diet and from food supplements. Maternal blood specimens were obtained at the time of enrollment, around 17 weeks of gestation, and shipped by overnight mail to the MoBa biobank at ambient temperature. At the biorepository, whole blood was aliquoted into two polypropylene deep-well plates (930 µl in each, ABgene, Surrey, UK) and stored at –80° (Ronningen et al., 2006).

Maternal whole blood samples from 3000 participants were retrieved from the biobank in 2015 for the Human environmental biobank project (MoBa Etox) at NIPH. Maternal blood concentrations of Hg and selenium (Se) were made available for this study. Se is thought to ameliorate the adverse effects of Hg and is included as a covariate (Rayman, 2000).  $N = 2239$  individuals in the MoBa Etox sample had answered the questionnaire at child age five years and were included in this study. Metal analyses in the blood samples were conducted at the Department of Laboratory Medicine at Lund University (Sweden). Mercury was determined as total mercury in acid-digested samples by cold vapor atomic fluorescence spectrophotometry (Sandborgh-Englund et al., 1998). The detection limit was calculated as three times the standard deviation (SD) of the blank (A1). During the analysis campaign, the laboratory participated in the German External Quality Assessment Scheme (G-EQAS), with good agreement between obtained element concentrations in quality control samples used and expected values. Analysed samples were prepared in duplicate and the method imprecision was calculated as the coefficients of variation in measurements of duplicate preparations. The analytical accuracy was verified towards certified reference material; Seronorm Trace elements whole blood L-1 and L-2 (SERO AS, Billingstad, Norway) (S1). Selenium was analysed by inductive couple plasma mass spectrometry (ICP-MS; iCAP Q, Thermo Fisher Scientific, Bremen, GmbH) equipped with collision cell with kinetic energy discrimination and helium as collision gas. A sample volume of 100 µL were diluted 20 times with an alkaline solution according to Bárány et al. and analysed in peak-jumping mode, rhodium and terbium were used as internal standards (Barany et al., 1997). Detection limits and method imprecision were calculated as for the cold vapor atomic fluorescence spectrophotometry and the same reference material was used to verify the analytical accuracy.

Information on inorganic Hg exposure from maternal amalgam fillings was not included in the analyses. More information on inorganic Hg exposure from amalgam in MoBa can be found in the study by Lygre et al. (Lygre et al., 2016).

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