**Prenatal exposure to metals in the first and third trimester of pregnancy and neuropsychological development at the age of 4 years**

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**ABSTRACT**

**Background:** There is a lack of epidemiological evidence for most of the associations between the current levels of metals and neuropsychological development during childhood. To add something about why is prenatal exposure important since it is when you measured it?

**Objective:** Our goal was to evaluate the potential neurotoxic effect of prenatal exposure to 7 metals (cobalt, cupper, arsenic, cadmium, antimony, thallium and lead) during the first and third trimester of pregnancy on child neuropsychological development at the age of 4 years.

**Methods:** I would start saying that you use data from a prospective birth cohort study, etc. We assessed the neuropsychological development of 553 4-year-olds from the cohort of Sabadell, Spain (INMA-project) using a thorough protocol of assessment. Several aspects of neuropsychological development were evaluated including general cognition, executive function, social competence, as well as attention deficit and hyperactivity disorder (ADHD) symptomatology. Metals were measured in 485 urine samples collected from mothers during the 1st and 3rd trimester of pregnancy. Tertiles of the average of the two creatinine-adjusted metal concentrations were used as exposure variable.

**Results:** We found a negative effect of the highest tertile of cobalt during the 1st trimester on executive function score (Coefficient (Coef) = -4.92; 95% Confidence Interval (CI) = -8.36 to -1.49). The highest levels of lead in the 3rd trimester of pregnancy were negatively associated to general cognitive scale (Coef = -3.87; 95%CI = -7.64 to -0.10). No associations were found between social competence or ADHD symptomatology and prenatal exposure to metals.

C**onclusion:** Prenatal exposure to lead during the third trimester and to cobalt during the first trimester may hamper child cognitive development during preschool period, even at the current low levels of these metals. However, for most of the metals we did not find an effect.

**Key words:** heavy metal poisoning, nervous system; child development; neuropsychology; environmental exposure.

**1. INTRODUCTION**

Lead or arsenic are metals with a recognized neurotoxic effect for developing brain at elevated doses (Grandjean and Landrigan 2006). Several studies have confirmed that pre- and postnatal exposure to lead (Pb) impairs behavioural and cognitive development in children even at low levels (Lanphear et al. 2005). Chronic exposure to arsenic (As) has been associated with reduced neuropsychological development during childhood, independently from neurotoxic effects of other contaminants such as lead (Calderón et al. 2001; Rosado et al. 2007). Over the last decade, a considerable effort has been done to reduce the body burden of metals present in the environment. Currently, population concentrations for the majority of metals have been reduced substantially (Llop et al. 2012; NHANES Report 2009).

Several studies, including systematic reviews, have concluded that there are inconsistencies between studies that have hindered to reach conclusive results about the neurotoxic effect of some metals at current low-level (Bellinger 2008a; Brinkel et al. 2009; Menezes-Filho et al. 2009; Wigle et al. 2008). Furthermore, the majority of these studies have focused on a few number of metals (Methylmercury, Pb and As) and evidence is still lacking for other metals with potential neurotoxicity, such as cadmium (Kim et al. 2012), thallium (Peter and Viraraghavan 2005) and cobalt (Simonsen et al. 2012). In addition, the effects of some trace metals (including copper (Cu) and antimony (Sb)) related to traffic air pollution (from traffic exhaust and also from tire and brake wear) (Steiner et al. 2007) need to be investigated in relation to developmental neurotoxicity.

Why prenatal exposure can be important? I think the justification of why you check this is missing…

This study aimed to investigate whether prenatal exposure to several metals with a potential neurotoxic effect was associated with neuropsychological development of children at the age of 4 in a Spanish birth cohort. Specifically, we measured concentrations of 7 metals presents in human tissues (Co, Cu, As, Cd, Sb, Tl and Pb) in 2 maternal urine samples collected at 12th and 32th weeks of pregnancy. Since the vulnerability of the brain could vary widely over the course of pregnancy (Mendola et al. 2002), this design could allow us to disentangle if the metal exposure may be particularly important during the first or third trimester of pregnancy.

**2. METHODS**

**2.1. Study design**

This analysis used data from the population based cohort established in the city of Sabadell (Barcelona, Catalonia, Spain) as part of the INMA (Environment and Childhood) Project (Guxens et al. 2011). A total of 657 eligible women (≥16 years, intention to deliver at the reference hospital, ability to communicate in Spanish or regional languages, singleton pregnancy, unassisted conception) were recruited during the first prenatal visit (1st trimester of pregnancy). A total of 619 (94.2%) children were enrolled at birth, and 553 (84.2%) were followed-up until their fourth year of life and performed the neuropsychological test. The study was approved by the Clinical Research Ethical Committee of the Municipal Institute of Healthcare (CEIC-IMAS), and all the participating mothers gave informed consent.

**2.2. Neuropsychological testing**

Neuropsychological assessment was conducted in 553 children at the age of 4 (range 4.1- 5.6). A standardized version of the McCarthy Scales of Children's Abilities (MSCA) adapted to the Spanish population (McCarthy 2009) was used to assess the cognitive development. The general cognitive scale (which includes verbal, perceptual-performance and quantitative scales) was examined. In addition, we included a new MSCA score of executive function by the reorganization of the MSCA subtests (Julvez et al. 2011). All testing was done in the health care center by 1 specially trained psychologist. The psychologist was not aware of any exposure information. The psychologist also flagged children difficult to evaluate because of less than optimal cooperation who were classified as having neuropsychological tests of uncertain quality and excluded from data analyses (n= 12). Some other child conditions, such as feeling seek during examination (n = 29), not sleeping well the night before examination (n = 39), mood changes during the last days (n = 17), visiting regularly a psychologist (n = 48) or being diagnosed as having a neuropsychological disorder (n = 27) were reported by the mothers and taken in consideration for statistical analyses as covariates. MSCA raw scores were standardized to a mean of 100 and a standard deviation (SD) of 15 to homogenize all the scales.

Children’s teachers filled-in two different questionnaires to assess social competence development and attention deficit and hyperactivity disorder (ADHD) symptomatology. Social competence was evaluated using the California Preschool Social Competence Scale (CPSCS) (Julvez et al. 2008), a test particularly designed to evaluate of social competence in children aged from 2.5 to 5.5 years. The CPSCS items cover a wide range of behaviors such as response to routine, response to the unfamiliar, following instructions, making explanations, sharing, helping others, initiating activities, giving direction to activities, reaction to frustration, and accepting limits. Raw scores were standardized to a mean of 100 and SD of 15. We assessed the ADHD symptomatology, using the ADHD Criteria of Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (ADHD-DSM-IV) form (American Psychiatric Association 2002). ADHD-DSM-IV consists of a list of 18 symptoms categorized under two separate symptom groups: inattention (nine symptoms) and hyperactivity/impulsivity (nine symptoms). Each ADHD-DSM-IV item is rated on a 4-point scale (0 = never or rarely, 1 = sometimes, 2 = often, or 3 = very often). We analyzed the inattention scale (IA) and the hyperactivity/impulsivity scale (HI) as continuous variables. Higher scores indicate higher symptomatology (worse score).

**2.3. Urinary metals determination**

Urine samples (80 mL) were collected in the first and third trimester of pregnancy from 485 women. The samples were stored in polyethylene tubes at -20ºC until further processing. Samples were analyzed in the Department of Environmental Chemistry, Institute of Environmental Assessment and Water Research (Barcelona).

Urine samples were analyzed by inductively coupled plasma quadruple mass spectrometry (Q-ICP-MS). Prior to instrumental analysis urine samples were digested and diluted as follows: 3 mL of urine were introduced in Teflon vessels together with 3 mL of Instra-Analysed 65% HNO3 (J.T. Baker, Germany) and 1.5 mL of Instra-Analysed 30% H2O2 (Baker) and they were left in an oven at 90ºC overnight. Once evaporated, the resulting solid samples were dissolved with 3 mL of 4% HNO3 dilution, placed in 7 mL glass bottles and subsequently stored in a refrigerator until instrumental analysis. An internal standard of Indium (10 ppb) was introduced and depending on sample density samples were diluted with MilliQ water to 30 mL or 60 mL to avoid spectral interferences. Q-ICP-MS analysis was performed by a X-SERIES II device from Thermo Fisher Scientific. One MilliQ water blank was processed in each batch of samples to control for possible contamination. Instrumental limit of detection (LOD) for all metals was 0.2 ng/mL. Metal urine concentrations were standardized to creatinine content determined at the Echevarne laboratory of Barcelona (Spain) by the 156 Jaffé method (kinetic with target measurement, compensated method) with Beckman 157 Coulter© reactive in AU5400 (IZASA®).

**2.4. Other variables**

Information on parental education, social class, country of birth (Spain, foreign) and maternal smoking during pregnancy was obtained through questionnaires administered during the 1st and 3rd trimesters of pregnancy. Parental educational level was defined using three categories: primary or less, secondary school, and university. Parental social class based on occupation was derived from the longest-held job reported during pregnancy or, for those mothers not working during their pregnancy, the job most recently held. When social class could not be derived, the last job of the father was used. Nine social class categories were created according to the set of National Occupational Codes-94 and regrouped into three categories: I+II for managers, technicians, and associate professionals (non-manual), III for other non-manual workers, and IV+V for skilled, semi-skilled and unskilled manual workers (Domingo-Salvany et al. 2000). Information related to the child's gestational age, sex and birth weight was obtained from clinical records. In subsequent interviews at 6 and 14 months, data on breastfeeding practices were collected. All questionnaires were administered face-to face by trained interviewers. Information on maternal diet was obtained in the first trimester of pregnancy using a 101-item semiquantitative validated food frequency questionnaire (Vioque et al. 2007). The exposure to NO2 and benzene during pregnancy was also measured (Aguilera et al. 2010). Finally, at the child’s age of 4 years, we assessed parental intelligence and mental health using Similarities subtest of the Weschler Adult Intelligence-Third Edition (WAIS-III)(Weschler 2001) and the Revised Symptom Checklist (SCL-90-R) (Martínez-Azumendi et al. 2001), respectively.

**2.5. Statistical analysis**

Metal urinary concentrations below the LOD were assigned a value of ½ of LOD. Metals concentrations were examined in tertiles, comparing the 3rd vs the 1st tertile. This categorical approach allowed us to explore the effects of metals on neuropsychological development between extreme groups. We used a linear regression models to analyze the MSCA and CPSCS scores. Negative binomial regression models were used for IA and HA scales of ADHD-DSM-IV, to account for over-dispersion.

We fitted multiple linear regression models for each pair outcome-metal adjusting for all the covariates that fulfilled the confounding criteria. Covariates retained in the model were those showing associations with neuropsychological tests with p-values of <0.05 or those whose inclusion resulted in a change in the regression coefficient of the metals ≥ 10%.

In a next step, we performed sensitivity analyses to assess the robustness of our results. A series of models were run to assess the effect of additionally adjusting one by one each of the variables related in the literature to metals distribution such as fish intake, smoking and traffic air pollution (NO2 and benzene) during pregnancy to minimize the likelihood of residual confounding (García-Esquinas et al. 2011; Jain 2013; Martí-Cid et al. 2008; Steiner et al. 2007).

**3. RESULTS**

Our analysis was based on 377 children with complete information on neuropsychological development assessment and metals exposure. A description of the characteristics of the study population is shown in Table 1. The mean age of assessment was 4.4 years. Forty-nine percent of children were females. Forty-three percent of mothers had secondary educational level while 33% had university degree. More than 50% of fathers belonged to a manual social-class. We also studied the differences between participants (n=377) (those with completed data on neuropsychological development and metals exposure) and non-participants (n=192) (those without complete data on neuropsychological development and metals exposure). Non-participants in this study only differed from participants in maternal education. Non-participant mothers had lower educational level than non-participants. No differences were found in variables such as child’s sex, maternal social class or country of origin (data not shown).

In table 2 concentrations of metals in urine samples during first and third trimester of pregnancy are shown. All metals excepting Tl were detected in more than 60% of urine samples during both trimesters. All metals exhibited statistically significant differences between both periods (p<0.001) except Tl, Pb and As. Nevertheless, all of them showed statistically significant correlations between measurements in both stages (p<0.001 for the rest). I would add that correlations go from 0.2 to 0.6. Metals with the lowest median concentrations during both periods were Tl (0.14 ug/g creatinine 1st trimester; 0.13 ug/g creatinine 3rd trimester) and Sb (0.36 ug/g creatinine 1st trimester; 0.28 ug/g creatinine 3rd trimester). The metal with the highest median concentration was As (32 ug/g creatinine 1st trimester; 36 ug/g creatinine 3rd trimester).

We found a negative effect for the highest levels of cobalt at the 1st trimester on the executive function score (Coefficient (Coef) = -4.92; 95% Confidence Interval (CI) = -8.36 to -1.49) but not for the levels of cobalt at the 3rd trimester (Coef = -3.08; 95%CI = -6.69 to 0.54) (figure 1). There is a negative trend for the effects of cobalt on global cognitive score, but these effects were not statistically significant. It has also been observed a negative association between the highest levels of lead on general cognitive scale at the 3rd trimester (Coef = -3.87; 95%CI = -7.64 to -0.10). This negative association was not found for the levels of lead at the 1st trimester. We found positive coefficients for Sb on general cognitive scale and executive function score during the two periods, although these coefficients were not statistically significant. We observe positive coefficients for the highest levels of As on both general cognitive and executive function scores, but not statistically significant.

The results for the CPSCS were inconclusive at all. None of the metals were associated with this scale (data not shown). The associations between metals and ADHD-DSM-IV scales were presented in figure 2. We only found a greater risk to increase the HI scale in those children exposed to the highest levels of As, although the association was marginally significant (Incidence Rate Ratio = 1.45; 95%CI = 1.00 to 2.11). No more associations were found for the rest of metals and ADHD-DSM-IV scales. The inclusion of some variables in the final multivariable models such as fish intake during pregnancy, smoking during pregnancy and traffic air pollution (NO2 and benzene) did not materially change the results (data not shown).

**4. DISCUSSION**

To our knowledge, this is the first study to estimate the effects of a complete set of metals measured in two different time-periods of pregnancy on child neuropsychological development during preschool period. The results of the present study suggest that prenatal exposure to high levels of lead and cobalt may negatively affect the cognitive development of the child during preschool period. The negative effects of cobalt seem to be more important during the 1st trimester of pregnancy, whereas the negative effects of lead were greater in the 3rd trimester of pregnancy. No more associations were found between the rest of metals analyzed (cupper, arsenic, cadmium, antimony and thallium) and the child neuropsychological development at the age of 4 years.

In this longitudinal birth cohort study, we measured concentrations of a large set of heavy metals on maternal urine samples collected at 12th and 32th weeks of pregnancy: cobalt, cupper, arsenic, cadmium, antimony, thallium and lead. Concentrations of most of metals were statistically different between both stages. Nevertheless, correlations of all metals between first and third trimester are significant, likely reflecting the absence of major changes in metal exposure along pregnancy. Most of the metals measured showed concentrations which are similar to those reported in previous and current studies worldwide, especially from non-contaminated sites (Benes et al. 2002; Komaromy-Hiller et al. 2000). However, it is important to remark that the effect of lead withdrawal from gasoline was clearly observed when comparing our concentrations with those reported for Italian population during the last eighties (Minoia et al. 1990).

The neurotoxic effects of high levels of lead exposure on the developing brain have been extensively reported in the past decades (Grandjean and Landrigan 2006). Since lead is a ubiquitous pollutant in the ecosystem, efforts to reduce exposure have been applied. Despite of this reduction in the levels, lead is still considered a primary environmental hazard on child health (Costa et al. 2004). Some authors have stated that “no level of lead exposure appears to be 'safe' and even the current 'low' levels of exposure in children are associated with neurodevelopmental deficits” (Bellinger 2008b). There are recent evidences about the negative effect of prenatal exposure to very low-levels of lead on cognitive development during childhood (Jedrychowski et al. 2009; Schnaas et al. 2006). In the present study, we observed a negative effect on general cognitive scale for those children prenatally exposed to high levels of lead. These deleterious effects seem to be particularly important during the third trimester of pregnancy exposure. These results are not in accordance with a previous study in that the authors reported a greater negative effect for the levels of lead in the first trimester than second or third trimester levels (Hu et al. 2006). They hypothesized that lead could affect the neural differentiation process which is primarily a first-trimester event. However, our findings seem to be pointed out that lead exposure could specially affects other critical processes of brain development which are markedly important during the second and third trimesters such as myelination and synaptogenesis (Johnston and Goldstein 1998; Mendola et al. 2002). Our results support the results of a cohort study in Mexico in which the authors found that exposure to lead during the third trimester of pregnancy may produce “lasting and possibly permanent effects” because this period may constitute a critical period for subsequent intellectual child development (Schnaas et al. 2006).

Our results also provided evidence of an adverse effect of prenatal exposure to cobalt during the first trimester on neuropsychological development of preschoolers, particularly on executive function. Cobalt is a relatively rare magnetic element which is an essential oligoelement which enters in the composition of vitamin B12 (Barceloux 1999; Lauwerys and Lison 1994). From general population, diet is the main source of exposure to cobalt. Although cobalt is an essential element, at high concentrations it is toxic (Kubrak et al. 2011). Increases in cobalt ions can directly induce DNA damage, interfere with DNA repair, and lead to DNA–protein cross linking and sister chromatid exchange (Calderón-Garcidueñas et al. 2012; Hengstler et al. 2003; Leonard et al. 1998). In animal studies, it has been found that excessive exposure to cobalt during embryonic period causes oxidative stress in brain and other tissues (Cai et al. 2012; Kubrak et al. 2011). The detrimental effects of cobalt are higher during the 1st trimester of pregnancy. This might indicate that exposure to cobalt could affect critical processes of brain development that occur during the 1st trimester such as neural migration and differentiation (Mendola et al. 2002). To date, the possible developmental neurotoxic effect of cobalt in humans has not observed. Further research in this birth cohort study is needed to disentangle if this finding is maintained in the future.

It is worth noting that in a large population-based sample of children, we collected biological samples at two different points in time during pregnancy (at 12th and 32th week of pregnancy) and analyzed a large number of metals. We also have standardized neuropsychological measurements of cognitive development at age 4, and collected data on a variety of potential socio-demographical factors including parental education, social class, intelligence quotient or mental health. For the MSCA assessment, several quality controls were introduced and the psychologist received extensive training to this end. This study, however, was limited by a number of factors. Probably, the main limitation is related to the type of sample analyzed, particularly for lead. We analyzed the concentrations of metals in urine samples. This could hinder the comparability of our results to the previous studies since most of the articles analyzing the effects of lead and other metals on neuropsychological development are based on blood samples. Urine is the preferred non-invasive matrix in heavy metals biomonitoring (Esteban and Castaño 2009). The variability of urine volume and chemical concentration are the main drawbacks of urine measurements that can be corrected by using creatinine concentration (Barr et al. 2005), as performed in the present study. In addition, we only could analyze 377 subjects with information on metals and neuropsychological development. This could introduce selection bias. There were no differences between participants and non-participants in terms of socio-demographical variables, which suggest that selection bias was minimal.

Our results suggest that prenatal exposure to higher levels of lead and cobalt may hamper the optimal cognitive development in preschoolers from general population. The negative effects of lead exposure during the third trimester of pregnancy are particularly important, whereas the negative effects of cobalt are major in relation to the first trimester exposure. Persistence of these effects should be further assessed at later stages of development of this birth cohort study, as well as the quantification of these metals in blood samples from children is needed.

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MARATÓ

IDAEA

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**Table 1. Neuropsychological scores and sociodemographic characteristics of participants (n=377):**

|  |  |  |  |
| --- | --- | --- | --- |
|  | **P50** | **(p25-p75)** | |
| **Neuropsychological tests** |  |  |  |
| Age at examination (yrs) | 4.43 | (4.33 - | 4.53) |
| General cognitive scale (MSCA) | 100.90 | (91.51 - | 110.29) |
| Executive function score (MSCA) | 100.21 | (91.19 - | 110.52) |
| Social competence (CPSCS) | 102.37 | (91.05 - | 110.85) |
| Inattention scale (DSM-IV-ADHD) | 2 | (0 - | 5) |
| Hyperactivity/Impulsivity scale (DSM-IV-ADHD) | 2 | (0 - | 5) |
|  |  |  |  |
| **Sociodemographic characteristics** |  |  |  |
| Sex, female (%) | 49.09 |  |  |
| Birthweight (gr) | 3290 | (2980 - | 3520) |
| Gestational age (weeks) | 39.86 | (38.86 - | 40.71) |
| Maternal social class (%) |  |  |  |
| CSI+II | 22.6 |  |  |
| CSIII | 32.5 |  |  |
| CSIV+V | 44.9 |  |  |
| Maternal education (%) |  |  |  |
| Primary | 24.3 |  |  |
| Secondary | 42.8 |  |  |
| University | 32.9 |  |  |
| Paternal social class (%) |  |  |  |
| CSI+II | 24.4 |  |  |
| CSIII | 18.4 |  |  |
| CSIV+V | 57.2 |  |  |
| Paternal education (%) |  |  |  |
| Primary | 33.8 |  |  |
| Secondary | 42.9 |  |  |
| University | 23.3 |  |  |
| Maternal country of origin (%) |  |  |  |
| Spain | 91.6 |  |  |
| Others | 8.4 |  |  |
| Paternal country of origin (%) |  |  |  |
| Spain | 89.8 |  |  |
| Others | 10.2 |  |  |
| Duration of any breastfeeding (months) | 25.86 | (13.00 - | 43.43) |
| Maternal smoking during pregnancy, yes (%) | 27.4 |  |  |

MSCA: McCarthy Scales of Children Abilities; CPSCS: California Preschool Social Competence Scale; DSM-IV-ADHD: ADHD Criteria of Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition.

Fish, traffic, and all the other variables that you use at the end are missing in this table!!

**Table 2. Concentrations of metals in urine samples (ųg/g creatinine) during first and third trimester of pregnancy:**

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **1st trimester** | | |  | **3rd trimester** | | |  | |  | |
|  | **% >LOD** | **Median** | **(p25-p-75)** |  | **% >LOD** | **Median** | **(p25-p-75)** |  | **difference p†** |  | **Spearman rho** |
| Co | 73.6 | 0.43 | (0.19-0.86) |  | 84.3 | 1.28 | (0.69-1.94) |  | <0.001 |  | 0.38\*\*\* |
| Cu | 100 | 11.59 | (8.14-16.79) |  | 100.0 | 14.38 | (9.53-20.27) |  | <0.001 |  | 0.21\*\*\* |
| As | 99.8 | 32.98 | (16.86-71.62) |  | 99.8 | 36.84 | (19.47-76.68) |  | 0.731 |  | 0.24\*\*\* |
| Cd | 90.1 | 0.58 | (0.41-0.89) |  | 87.5 | 0.55 | (0.36-0.83) |  | <0.001 |  | 0.56\*\*\* |
| Sb | 73.7 | 0.34 | (0.19-0.59) |  | 64.8 | 0.28 | (0.16-0.53) |  | <0.001 |  | 0.40\*\*\* |
| Tl | 19.7 | 0.14 | (0.09-0.20) |  | 17.2 | 0.13 | (0.09-0.19) |  | <0.1 |  | 0.21\*\*\* |
| Pb | 98.9 | 3.83 | (2.56-5-56) |  | 100.0 | 3.81 | (2.67-6.24) |  | 0.255 |  | 0.46\*\*\* |

LOD: limit of detection;

**†**Difference p represents the p-value difference between the concentrations of 1st trimester vs 3rd trimester

**Figure 1. Adjusted associations (Coefficient (95% Confidence Interval)) between metals during pregnancy (3st vs 1st tertile) and general cognitive scale and executive function score of MSCA at the age of 4:**

|  |  |
| --- | --- |
| **1st trimester** | **3rd trimester** |
|  |  |

Models were adjusted for child’s age at cognitive assessment, quality of the cognitive test, child’s sex, maternal intelligence quotient, maternal social class, maternal country of birth, child’s mood changes during the days before the cognitive assessment, and child’s being diagnosed of as having a neuropsychological disorder.

MCSA: McCarthy Scales of Children Abilities

Co: Cobalt; Cu: Copper; As: Arsenic; Mo: Molybdenum; Cd: Cadmium; Sb: Antimony; Tl: Thallium; Pb: Lead.

**I think it would be clearer and more comparable to make 2 figures, one for cognitive function and one for executive function, and put them for each metal, the trimesters together, first 1st trim and then 3rd trim**

**Figure 2. Adjusted associations (Incidence Rate Ratio (95% Confidence Interval)) between metals during pregnancy (3st vs 1st tertile) and Inattention and Hyperactivity/Impulsivity Scales of DSM-IV-ADHD at the age of 4:**

|  |  |
| --- | --- |
| **1st trimester** | **3rd trimester** |
|  |  |

Models were adjusted for child’s age, child’s sex, maternal IQ, maternal social class, maternal country of origin and paternal mental health.

DSM-IV-ADHD: ADHD Criteria of Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition.

Co: Cobalt; Cu: Copper; As: Arsenic; Mo: Molybdenum; Cd: Cadmium; Sb: Antimony; Tl: Thallium; Pb: Lead.

I also think it would be clearer to make 1 figure for inattention scale and another for hyperactivity scale, like I suggested before for cognitive function and EF