Foetal exposure to heavy metals and risk of atopic diseases in early childhood

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Running Title : Heavy metals *in utero* and atopic diseases

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I. CONFLICT OF INTERESTS STATEMENT

All authors declare no competing interests.

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III. ABSTRACT AND KEYWORDS

ABSTRACT – words 244 (Max 250)

Background: Accumulating evidence suggests that in utero exposures can influence the development of the immune system and thus contribute to disease development. Studies investigating the association between prenatal exposures to heavy metals and atopic diseases, however, are scarce.

Methods: Children from the EDEN birth-cohort were prospectively followed-up using parental questionnaires with validated questions on asthma, allergic rhinitis, eczema and food allergy symptoms. The questionnaires were administered every 4 months during the children's first year, and then every year until the age of 5, with a final survey at the age of 8. Serum concentrations of lead (Pb), cadmium (Cd) and manganese (Mn) were assessed in maternal blood samples collected during mid-pregnancy and in cord blood of 651 mother-children pairs. Hazard ratios (HR) for the incidence of each atopic disease in relation to the exposure to metals were calculated using Cox proportional-hazard models.

Results: Levels of Cd in cord blood were associated with greater risk of asthma (hazard ratio [95% confidence interval] for upper vs. lower quartile: 1.81 [1.00-3.29]), eczema 1.60 [1.09-2.35]), and food allergy (3.17 [1.36-7.38]), while Mn levels in maternal serum were associated with eczema (1.55 [1.05-2.28]). These associations were similar in males and females and were confirmed using log-concentrations of metals as exposures.

Conclusions: Our results support the hypothesis that foetal exposure to heavy metals may affect the development of asthma, eczema and food allergy in childhood, and suggest that timing of exposure in utero may have a role in these associations.

Keywords: asthma, atopic dermatitis, eczema, in utero exposure, food allergy, allergy, cadmium, lead, manganese

Key message: The effects of prenatal exposures to heavy metals on allergic diseases are largely unknown. Using a prospective data from the EDEN birth cohort, we showed that high cord-blood levels of cadmium are associated with greater risks of developing asthma, eczema and food allergy, whilst high concentrations of manganese in maternal mid-pregnancy blood are associated with a greater risk of eczema.

IV. MAIN TEXT

INTRODUCTION

An increasing body of evidence is underlining the critical role of early life conditions in the etiology of several childhood and adult chronic diseases, substantiating the Developmental Origins of Health and Disease (DOHaD) paradigm.[1]

The fetus is especially vulnerable to the effects of environmental stressors and agents that may disrupt developmental processes, affect fetal growth, and influence different aspects of the children health, including neurodevelopment, childhood growth and obesity, respiratory and immune health.[2]

Recent epidemiological studies have shown that in utero exposures, such as maternal smoking, air pollution, biological infections and antibiotics, alcohol, and even maternal psychological stress, were linked with greater risk of developing asthma and other allergic diseases in childhood,[3-7] possibly by altering the early development of the immune system.[8]

Heavy metals are natural elements that can have both beneficial and adverse effects on health. Some induce toxic effects at low levels of exposures. Main sources of heavy metals include contaminated food and drinking water, and tobacco smoking.[9] Metals absorbed in maternal blood stream can be then differentially transferred through the placenta to the fetus.[10,11]

Early-life exposure to heavy metals has previously been associated to several adverse health outcomes in childhood, including reduced fetal growth and prematurity, as well as neurodevelopmental and metabolic problems.[2] The literature on the effects of heavy metals on atopic diseases in childhood, however, is

extremely scarce, and only few associations between fetal exposure to selected heavy metals and some allergic diseases have been investigated.[12-16]

In this work we used the data collected in the prospective mother-child cohort EDEN to investigate whether there is evidence of associations between the fetal exposure to lead (Pb), cadmium (Cd), and manganese (Mn), collected in maternal blood during mid-pregnancy and in cord blood at delivery, with the risk of developing four of the most common allergic diseases in childhood, namely asthma, allergic rhinitis, eczema and food allergy.

METHODS

Study population and data collection

EDEN is a mother-child prospective birth cohort study set up with the objective of investigating the relations between prenatal and early life exposures and the child health and development. Between 2003 and 2006, the study enrolled 2,002 women in their early pregnancy in two university maternity clinics in the French towns of Nancy and Poitiers. Between the 24th and 28th gestational week, the expecting mothers underwent clinical examinations and interviews on their life style and health. [17] Detailed information on the pregnancy, birth and newborn characteristics were obtained through the use of standardized questionnaires and from obstetric records. The children were followed up for 8 years using standardized parental-administered questionnaires regarding the children's health and their growth environment. The follow-up questionnaires were administered every 4 months during the children's first year, and then every year until the age of 5, with a final survey at the age of 8.

The study was approved by the relevant ethical committees and written consent was obtained from all mothers.

Measurements of Pb, Cd, and Mn in blood samples

Blood samples were drawn from mothers in their mid-pregnancy pregnancy (between the 24th and 28th gestational weeks) and from the umbilical cord at delivery, and stored following standardized procedures. Blood concentrations of lead (Pb), cadmium (Cd) and manganese (Mn) were measured by electrothermal atomic-absorption spectrometry (model 4100 ZL; Perkin-Elmer, Courtaboeuf, France) with Zeeman background correction, as previously described.[18] The instrument had a resolution of 0.1 µg/L and an accuracy within 3%. Only the first half

of mother-child pairs chronologically enrolled in EDEN were selected for the metal dosage protocol.

Outcome definitions

The questions for the identification of cases of asthma, rhinitis, eczema and food allergy were derived by the International Study on Asthma and Allergies in Childhood (ISAAC) questionnaire.[19] Based on the answers reported by the parents on the follow-up questionnaires, the children were considered to have had:

- Asthma, if the child was reported to have had an asthma attack in at least one questionnaire;
- Allergic rhinitis, if the child had sneezing, runny or stuffy nose when he/she had no respiratory infection (no colds, nasopharyngitis, flu, …) AND these problems were accompanied by tearing or itchy eyes;
- Eczema, if the child had an itchy rash (red patches, pimples, …) on the skin which was coming and going and affecting the folds of the elbows, behind the knees, in front of the ankles, under the buttocks, around the neck, or around the eyes or ears;
- Food allergy, if the child had any exaggerated reaction (swelling of the lips, face, gastrointestinal problems) after consuming a food.

Statistical analyses

The characteristics of the children were summarized with percentage, mean ± standard deviation or median with interquartile range, according to the distribution of the variables. Differences between groups were assessed by Pearson's X^2, Student's T or Wilcoxon's rank-sum tests, as appropriate.

To reduce the skewness of their distribution, metal concentrations were log-2 transformed. The correlations between metal levels in mid-pregnancy and cord blood samples were estimated using Pearson's pairwise correlations.

The associations between maternal and cord metal concentrations and the incidence rates of each of the four allergic outcomes (asthma, allergic rhinitis, eczema and food allergy) were estimated by hazard ratios (HR) with 95% confidence intervals (95%CI), using Cox regression models after testing the proportional hazards assumptions; metal exposures were included either as a continuous variable (log2-transformed) or as categorical variables using quartiles of the distribution, adjusting for potential confounders (described in Text E1).

Interaction terms with sex and age of onset were included in the Cox models to test if foetal exposures to heavy metals had different effects in males and in females or at different ages i.e. infancy (0-3 years] vs. early childhood (3-8 years]. Moreover, as Cd levels are strongly correlated with smoking,[**20**] we also tested the interactions between maternal smoking and Cd in maternal/cord blood on the incidence of atopic diseases.

The statistical analyses were performed with STATA 15.

RESULTS

Characteristics of the cohort sample.

The selection of the study population within the EDEN cohort is shown in the flow-chart in Figure 1. Their characteristics are shown in the Table E1. Overall, the sample included 339 males (52%) and 312 females (48%); about 80% of the children included have been surveyed at the age of 5, and nearly 60% were surveyed at the age of 8. About 98% of the mothers were born in Europe, as well as 94% of maternal grandparents.

Concentrations of heavy metals in sera from mid-pregnancy maternal blood and cord blood.

The distribution of Pb, Cd, and Mn concentrations in samples obtained from maternal mid-pregnancy blood and cord blood is shown in Table 1 and in Figure E1. Average concentrations of Pb and Cd are 24% and 37% lower, respectively, in cord blood than in mid-pregnancy maternal blood; the concentrations of Mn, contrariwise, are 3-fold greater in cord blood. There was a weak-to-moderate correlation between the concentrations of metals measured in maternal mid-pregnancy and cord blood samples (Pearson's r: Pb: 0.362, Cd: 0.316, Mn: 0.158; all $p \leq 0.0001$, Figure E2).

Associations of metal concentrations in maternal mid-pregnancy and cord serum with incidence rates of asthma, rhinitis, eczema, and food allergy.

The lifetime prevalence of asthma was 17% in our sample; 22% had rhinitis, 43% had eczema, and 11% suffered from food allergy. Overall, 60% experienced at least one of the outcomes, and 25% reported multiple allergic outcomes (Table E2). The incidence rates of asthma, allergic rhinitis, eczema and food allergy were,

respectively, 29.5 [cases/person-years: 113/3,830], 38.6 [144/3,731], 100.1 [283/2,827] and 18.9 [74/3,915] per 1000 (Table 2).

Cd concentrations were significantly higher in cord blood of children who developed asthma and food allergy by the age of 8 (both $p<0.01$), and had a borderline association with children who had eczema ($p<0.10$). Mn in maternal serum was higher in children who later developed eczema, while Pb in cord blood was lower in children who had allergic rhinitis (Table E3).

The incident rates of asthma, eczema and food allergy were significantly higher in children with high cord-blood Cd levels (4th quartile) than those in the low exposure group (1st quartile); children with high maternal-blood Mn had also greater rates of asthma, while children with high cord-blood Pb levels had lower incidence of rhinitis (Tables E4-E7).These associations were confirmed using the multivariable models; after adjusting for potential confounders including children and family characteristics, pregnancy and birth characteristics and other metal exposures, in fact, children in the high cord-blood Cd exposure group had significantly higher incidence rates of asthma (+81%), eczema (+60%) and food allergy (+217%) than children in the low exposure group (HR for Q4 vs. Q1 [95%CI]: asthma: 1.81 [1.00-3.29]; eczema: 1.60 [1.09-2.35]; ,food allergy: 3.17 [1.36-7.38], Figure 2 and Table 3). Children highly exposed to maternal Mn had also had 55% greater rates of eczema (1.55 [1.05-2.28]) than those in the low-exposure group. An inverse, borderline trend was observed between cord-blood levels of Pb and incidence of rhinitis.

These results were confirmed when using continuous log-concentrations for assessing the exposures to metals (Table E8), and the associations found in the

fully-adjusted models were consistent with the estimates found in the univariate analyses and in those found including different sets of covariates (Tables E9-E12).

Interactions of heavy metals concentrations with sex, age and maternal smoking habits.

There were no significant differences between boys and girls in the associations between heavy metal exposures in utero and atopic outcomes (Table E13), and the estimates for the associations were overall similar in infancy and in early childhood (Table E14).

Cd concentrations were significantly higher in maternal blood of mothers who smoked in pregnancy than those who did not (mean: 1.11 vs. 0.76 μg/L, $p<0.001$), while Cd levels in cord blood were similar (Table E15). However, we found no significant interactions between Cd blood levels and maternal smoking in pregnancy associated with the incidence rates of atopic diseases (Table E16).

DISCUSSION

We used prospective data from 651 children of the EDEN mother-child birth cohort to prospectively estimate the associations between fetal exposure to lead (Pb), cadmium (Cd) and manganese (Mn) and the incidence of asthma, allergic rhinitis, eczema and food allergy by the age of 8. Our main findings are the following:

- high cord-blood Cd was significantly associated with greater rates of asthma, eczema and food allergy in childhood;
- high mid-pregnancy maternal Mn was significantly associated with greater risk of developing eczema;
- no significant differences in the estimates were found overall between males and females, and between infancy and early childhood.

As exposures to Pb and Cd have previously been linked to reduced fetal growth and prematurity,[21] it's worth noting that the associations found between heavy metals and allergic diseases are independent from children's birth characteristics such as low birth weight, preterm and caesarean section delivery, as well as from other potential confounders such as maternal smoking and socio-economic status.

Ours is one of the first studies that has examined the effects of prenatal exposures to Cd, Pb and Mn on childhood allergic diseases, and the only that follow-up the children to a median age of 8 years; thus, there are very few studies with which directly compare our results with.[2] To our knowledge, moreover, this is the first prospective study to investigate the associations of prenatal metal concentrations with asthma, allergic rhinitis and food allergy.

Several studies have showed that Cd may have immunomodulatory effects and may alter the activation of immune cell subpopulations and the production of IgE,

potentially affect the risk of developing atopic diseases.[22,23] In adults, some studies reported an association between high blood concentrations of Cd and greater odds of current asthma,[24,25] in agreement with our results showing increased rates of incident asthma in children exposed to higher Cd concentrations in the cord blood. In line with the findings of the MOCEH study, which reported greater prevalence of atopic dermatitis at 6 months in infants with higher cord-blood levels of Cd,[13] we found that Cd in cord-blood affected the development of eczema throughout early childhood. We also reported - for the first time to our knowledge - evidence for an association of symptoms of food allergy in childhood with cord blood Cd, which could also be due to the effects on developing immune system of early exposures to Cd.[22-24,26,27]

Mn is an essential trace element with anti-oxidant properties, and dietary Mn has been associated with lower asthma in adults.[28] Mn salts, however, are widely used in cosmetics and potentially allergenic.[29] In our study, the maternal blood Mn levels were associated with greater risk of developing eczema; the effects of Mn complex because it is considered both an essential nutrient and a potential toxicant, depending on the amount of exposure.[30] High levels of Mn are thought to contribute to oxidative stress, as this metal can catalyze oxidative cellular reactions.[31] We cannot exclude, however, that high levels of Mn in maternal blood might be a proxy indicator of another noxious exposure.

We found a positive association – although not a statistical significance level - between cord blood Pb levels and rates of eczema. This finding, however, does not match with the results of the ALSPAC study (UK), which reported a negative association between eczema symptoms at 18-30 months and iron levels in umbilical cord, but no associations with Pb or Mn concentrations.[12] Two Asian birth-cohorts

found inconsistent results on fetal exposure to Pb and atopic dermatitis: the above-mentioned MOCEH study found no association between Pb and atopic dermatitis in infants,[13] while Pb levels in cord blood were associated with atopic dermatitis duration in children aged 5, as well as with increased interleukin-13 production from cord blood mononuclear cells, in the COCOA birth-cohort.[14]

Two independent studies on large samples of children living in the US reported no associations of blood Pb levels with asthma [15,32], in agreement with our study, but showed at the same time a positive association with eosinophils and IgE levels and with T-cell dysregulation,[15,33] suggesting a potential effect on the immune system. [26] We unexpectedly found a borderline inverse association between cord blood Pb levels and allergic rhinitis, which would require further confirmation in independent cohorts.

Finally, we found no significant associations between allergic outcomes and Pb or Cd in maternal blood drawn during mid-pregnancy; this finding agrees with a recent Canadian mother-child study that found no alteration of cord blood immune system biomarkers with greater exposure to maternal Pb concentrations.[34]

The mothers and children included in our study came from the general population and from two French urbanized settings,[17] and the metal concentrations detected in maternal and cord blood in our study represent not-extreme exposures. The average concentrations of Pb, Cd and Mn measured in maternal and cord blood in our sample are similar to those found in other birth cohort from different countries [reviewed in 11]. _Concentrations of Pb and Cd were 25 to 40% lower in cord than in maternal blood and, contrariwise, Mn concentrations were 3-fold higher in cord blood. As the placenta establishes an interface between maternal circulation and the fetus by regulating the transport of nutrients and filtering the passage of

potentially toxic substances, our findings support the hypothesis that this may cause a differential distribution of metals across the placenta by filtering the passage of Pb and Cd and, contrariwise, favoring the accumulation of Mn in the cord blood.[10,11] The correlations between maternal blood at 24th-28th week and cord blood concentrations, however, were weak-to-moderate for all metals, indicating that there is a sensible variability in metal concentrations even between the same mother-fetus pair. This would partially explain why the significant associations observed between allergic outcomes and metal concentrations measured in cord-blood were not mirrored by those where the metal was measured in maternal blood. An alternative explanation for the differences in the associations with allergic outcomes observed between metal concentrations measured in mid-pregnancy maternal blood versus cord blood might be that the time-window during which the fetus is exposed plays an important role in the susceptibility to developing allergic diseases.

Strengths and limitations.

The strengths of the study include the prospective mother-birth cohort design, as well as the early inclusion of expectant mothers from the French general-population and the follow-up of the children from foetal life to the age of 8 with frequent data collection, which minimized the issues of recall bias. In the EDEN study, it was not possible to ask for the ethnicity of the participants for legal reasons. However, about 98% of the mothers was born in France or in another European country, and the great majority of them had both parents born in Europe, indicating a predominant Caucasian ancestry, even we cannot exclude among them some not-Caucasian, third-generation immigrants. This may limit the ethnic comparison and the generalizability of our findings to other ethnic groups.

The use of parental-reported symptoms to identify children with asthma, rhinitis, eczema and food allergy might be considered a weakness; nevertheless, this is a common method in epidemiological research on children and validated questions from internationally standardized questionnaires were adopted.[19] The lifetime prevalence of allergic outcomes in our study are higher than those reported by other cross-sectional studies on the French general paediatric population,[35-38] possibly because our definitions are based on parental reported symptoms at multiple time-point, which are more likely to detect minor-severity or intermittent symptoms.

As only one maternal blood sample was taken during the second trimester, we could not consider the potential variations in metal exposure that might occur earlier and later in the pregnancy; evaluation of heavy metals levels collected at multiple points during pregnancy would be helpful to better understand the effects of exposure to different metals throughout pregnancy.[39] The relatively low number of the sample-size (n=651) limited the power of our statistical analyses. Taking this into account, our results are to be interpreted with caution.

Conclusions

High cord-blood levels of cadmium are associated with a greater risk of developing asthma, eczema and food allergy in infancy and early childhood, whilst high mid-pregnancy maternal manganeseis is significantly associated with a greater risk of developing eczema. Our findings suggest that either the organ where metals are accumulated or the timing of exposure may play a role in the associations with atopic diseases. Further studies should be conducted to validate our findings in independent cohorts and to clarify the underlying biological mechanisms linking the prenatal exposure to metals and the allergic phenotypes.

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VII. REFERENCES

1. Gillman MW. (2005) Developmental origins of health and disease. N Engl J Med. 2005;353(17):1848-1850. doi:10.1056/NEJMe058187
2. Vrijheid M, Casas M, Gascon M, Valvi D, Nieuwenhuijsen M. (2016) Environmental pollutants and child health-A review of recent concerns. Int J Hyg Environ Health. 2016;219(4-5):331-342. doi:10.1016/j.ijheh.2016.05.
3. Hehua Z, Qing C, Shanyan G, Qijun W, Yuhong Z. (2017) The impact of prenatal exposure to air pollution on childhood wheezing and asthma: A systematic review. Environ Res. 2017;159:519-530. doi:10.1016/j.envres. 2017.08.038
4. Burke H, Leonardi-Bee J, Hashim A, et al. (2012) Prenatal and passive smoke exposure and incidence of asthma and wheeze: systematic review and meta-analysis. Pediatrics. 2012;129(4):735-744. doi:10.1542/peds.2011-2196
5. Pesce G, Marchetti P, Calciano L, et al. (2017) Fetal exposure to maternal pregnancy complications and respiratory health in childhood. Pediatr Allergy Immunol Pneumol. 2017;218-226 doi.org/10.1089/ped.2017.0786
6. Pesce G, Marcon A, Marchetti P, Girardi P, de Marco R. (2014) Febrile and gynecological infections during pregnancy are associated with a greater risk of childhood eczema. Pediatr Allergy Immunol. 2014;25(2):159-165. doi: 10.1111/pai.12160
7. de Marco R, Pesce G, Girardi P, et al. (2012) Foetal exposure to maternal stressful events increases the risk of having asthma and atopic diseases in childhood. Pediatr Allergy Immunol. 2012;23(8):724-729. doi:10.1111/j. 1399-3038.2012.01346.x

8. Dietert RR, Zelikoff JT. (2008) Early-life environment, developmental immunotoxicology, and the risk of pediatric allergic disease including asthma. Birth Defects Res B Dev Reprod Toxicol. 2008;83(6):547-560. doi: 10.1002/bdrb.20170
9. Borchers A, Teuber SS, Keen CL, Gershwin ME. Food safety. Clin Rev Allergy Immunol. 2010;39(2):95-141. doi:10.1007/s12016-009-8176-4
10. Needham LL, Grandjean P, Heinzow B, et al. Partition of environmental chemicals between maternal and fetal blood and tissues. Environ Sci Technol. 2011;45(3):1121-1126. doi:10.1021/es1019614
11. Bocca B, Ruggieri F, Pino A, et al. (2019) Human biomonitoring to evaluate exposure to toxic and essential trace elements during pregnancy. Part A. concentrations in maternal blood, urine and cord blood. Environ Res. 2019;177:108599. doi:10.1016/j.envres.2019.108599
12. Shaheen SO, Newson RB, Henderson AJ, et al. (2004) Umbilical cord trace elements and minerals and risk of early childhood wheezing and eczema. Eur Respir J. 2004;24(2):292-297. doi:10.1183/09031936.04.00117803
13. Kim JH, Jeong KS, Ha EH, et al. (2013) Association between prenatal exposure to cadmium and atopic dermatitis in infancy. J Korean Med Sci. 2013;28(4):516-521. doi:10.3346/jkms.2013.28.4.516
14. Kim J, Kim S, Woo SY, et al. (2019) Prenatal Exposure to Lead and Chromium is Associated with IL-13 Levels in Umbilical Cord Blood and Severity of Atopic Dermatitis: COCOA Study. Immune Netw. 2019;19(6):e42. Published 2019 Dec 2. doi:10.4110/in.2019.19.e42
15. Wells EM, Bonfield TL, Dearborn DG, Jackson LW. (2014) The relationship of blood lead with immunoglobulin E, eosinophils, and asthma among children:

NHANES 2005-2006. Int J Hyg Environ Health. 2014;217(2-3):196-204. doi: 10.1016/j.ijheh.2013.04.010

16. Stelmach I, Grzelewski T, Bobrowska-Korzeniowska M, et al. (2014) The role of zinc, copper, plasma glutathione peroxidase enzyme, and vitamins in the development of allergic diseases in early childhood: The Polish mother and child cohort study. Allergy Asthma Proc. 2014;35(3):227-232. doi:10.2500/aap.2014.35.3748
17. Heude B, Forhan A, Slama R, et al. (2016) Cohort Profile: The EDEN mother-child cohort on the prenatal and early postnatal determinants of child health and development. Int J Epidemiol. 2016;45(2):353-363. doi:10.1093/ije/dyv151
18. Yazbeck C, Thiebaugeorges O, Moreau T, et al. Maternal blood lead levels and the risk of pregnancy-induced hypertension: the EDEN cohort study. Environ Health Perspect. 2009;117(10):1526-1530. doi:10.1289/ehp.0800488
19. Asher MI, Keil U, Anderson HR, et al. International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. Eur Respir J. 1995;8(3):483-491. doi:10.1183/09031936.95.08030483
20. Hashim A, Fathima H, Muhammed RCS, Neevan DRD. Analysis of Lead, Cadmium, and Nickel in Blood Donors in Relation to Smoking-A Comparative Study. J Environ Pathol Toxicol Oncol. 2019;38(2):165-172. doi:10.1615/JEnvironPatholToxicolOncol.2019028792
21. Al-Saleh I, Al-Rouqi R, Obsum CA, et al. (2015) Interaction between cadmium (Cd), selenium (Se) and oxidative stress biomarkers in healthy mothers and

its impact on birth anthropometric measures. Int J Hyg Environ Health. 2015;218(1):66-90. doi:10.1016/j.ijheh.2014.08.001

22. Kindgren E, Guerrero-Bosagna C, Ludvigsson J. (2019) Heavy metals in fish and its association with autoimmunity in the development of juvenile idiopathic arthritis: a prospective birth cohort study. Pediatr Rheumatol Online J. 2019;17(1):33. Published 2019 Jul 2. doi:10.1186/s12969-019-0344-3
23. Nygaard UC, Li Z, Palys T, et al. (2017) Cord blood T cell subpopulations and associations with maternal cadmium and arsenic exposures. PLoS One. 2017;12(6):e0179606. Published 2017 Jun 29. doi:10.1371/journal.pone.0179606
24. Park S, Lee EH, Kho Y. The association of asthma, total IgE, and blood lead and cadmium levels. J Allergy Clin Immunol. 2016;138(6):1701-1703.e6. doi:10.1016/j.jaci.2016.04.030
25. Yang G, Sun T, Han YY, et al. (2019) Serum Cadmium and Lead, Current Wheeze, and Lung Function in a Nationwide Study of Adults in the United States. J Allergy Clin Immunol Pract. 2019;7(8):2653-2660.e3. doi:10.1016/j.jaip.2019.05.029
26. Krocova Z, Macela A, Kroca M, Hernychova L. (2000) The immunomodulatory effect(s) of lead and cadmium on the cells of immune system in vitro. Toxicol In Vitro. 2000;14(1):33–40.
27. Jelovcan S, Gutschi A, Kleinhappl B, Sedlmayr P, Barth S, Marth E. Effects of low concentrations of cadmium on immunoglobulin E production by human B lymphocytes in vitro. Toxicology. 2003;188(1):35-48. doi:10.1016/s0300-483x(03)00044-1

28. Patel BD, Welch AA, Bingham SA, et al. (2006) Dietary antioxidants and asthma in adults. Thorax. 2006;61(5):388-393. doi:10.1136/thx.2004.024935
29. Bocca B, Pino A, Alimonti A, Forte G. (2014) Toxic metals contained in cosmetics: a status report. Regul Toxicol Pharmacol. 2014;68(3):447-467. doi: 10.1016/j.yrtph.2014.02.003
30. Zota AR, Ettinger AS, Bouchard M, et al. Maternal blood and infant birth weight. Epidemiology. 2009;20(3):367-373
31. Hamai D, Bondy SC. Oxidative basis of manganese neurotoxicity. Redox-Active Metals Neurol Disord. 2004;1012:129 –141
32. Joseph CL, Havstad S, Ownby DR, et al. (2005) Blood lead level and risk of asthma. Environ Health Perspect. 2005;113(7):900-904. doi:10.1289/ehp. 7453
33. Hsiao CL, Wu KH, Wan KS. (2011) Effects of environmental lead exposure on T-helper cell-specific cytokines in children. J Immunotoxicol. 2011;8(4):284-287. doi:10.3109/1547691X.2011.592162
34. Ashley-Martin J, Dodds L, Arbuckle TE, et al. Blood metal levels and early childhood anthropometric measures in a cohort of Canadian children. Environ Res. 2019;179(Pt A):108736. doi:10.1016/j.envres.2019.108736
35. Pénard-Morand C, Charpin D, Raherison C, et al. Long-term exposure to background air pollution related to respiratory and allergic health in schoolchildren. Clin Exp Allergy. 2005;35(10):1279-1287. doi:10.1111/j. 1365-2222.2005.02336.x
36. Saadeh D, Salameh P, Caillaud D, et al. High body mass index and allergies in schoolchildren: the French six cities study. BMJ Open Respir Res.

2014;1(1):e000054. Published 2014 Dec 24. doi:10.1136/bmjresp-2014-000054

37. Sasso F, Izard M, Beneteau T, et al. 18-year evolution of asthma and allergic diseases in French urban schoolchildren in relation to indoor air pollutant levels. Respir Med. 2019;148:31-36. doi:10.1016/j.rmed.2019.01.007

38. Rancé F, Grandmottet X, Grandjean H. Prevalence and main characteristics of schoolchildren diagnosed with food allergies in France. Clin Exp Allergy. 2005;35(2):167-172. doi:10.1111/j.1365-2222.2005.02162.x

39. Liang CM, Wu XY, Huang K, et al. Trace element profiles in pregnant women's sera and umbilical cord sera and influencing factors: Repeated measurements. Chemosphere. 2019;218:869-878

VIII. TABLES

Table 1: Distribution of lead (Pb), cadmium (Cd) and manganese (Mn) in maternal blood during mid-pregnancy and in cord blood. †paired sample Student's T-tests.

Exposure		**Maternal blood** (µg/L)	**Cord blood** (µg/L)	**p-value for difference**†
Lead - Pb	mean ± SD	19.1 ± 12.3	14.5 ± 9.7	<0.0001
	median [1st-3rd quartile]	17 [12,22]	12 [8.7,18]	
	range	1-141	1-78	
Cadmium - Cd	mean ± SD	0.8 ± 0.6	0.5 ± 0.4	<0.0001
	median [1st-3rd quartile]	0.8 [0.5,1.1]	0.5 [0.3,0.7]	
	range	0.1-9.6	0.1-4.6	
Manganese - Mn	mean ± SD	10.5 ± 4.7	33.1 ± 14.2	<0.0001
	median [1st-3rd quartile]	10 [8,12]	31 [24,41]	
	range	1-53	1-107	

Table 2: Incidence cases and rates of asthma, rhinitis, eczema, and food allergy in the study cohort.

	Person-years at risk	**Incident cases** (%)	**Rates** per 1000 years	**95% CI**
Asthma	3829.5	113 (17.4%)	29.5	[24.1,35.0]
Rhinitis	3730.5	144 (22.1%)	38.6	[32.3,44.9]
Eczema	2826.7	283 (43.5%)	100.1	[88.5,111.8]
Food allergy	3915.0	74 (11.4%)	18.9	[14.6,23.2]

Table 3: Associations of metal levels in maternal and cohort blood with the incidence of asthma, rhinitis, eczema and food allergy in children. Exposure to metal concentrations were grouped by quartiles as follows: **Q_1**: 1st quartile (lowest exposure); **Q_{2-3}**: 2nd and 3rd quartiles; **Q_4**: 4th quartile (highest exposure). Hazard ratios (HR, with 95% confidence intervals) for Q_{2-3} and Q_4 are estimated with Q_1 as reference, and adjusted for sex, centre, BMI, maternal education, parental smoking, parental history of allergy, maternal smoking in pregnancy, birthweight, gestational age at delivery and type of delivery, and mutually adjusted for other metal exposures. P-for-trends were calculated by including each metal exposure as log2-transformed continuous variable. †: p<0.10; *: p<0.05; **p<0.01

	Asthma	**Rhinitis**	**Eczema**	**Food Allergy**
	HR [95%CI]	**HR [95%CI]**	**HR [95%CI]**	**HR [95%CI]**
Lead (Pb)				
Maternal blood				
Q_{2-3} vs. Q_1	0.97 [0.61-1.56]	0.85 [0.57-1.28]	0.78 [0.57-1.05]	0.62 [0.35-1.09]†
Q_4 vs. Q_1	1.25 [0.71-2.20]	0.86 [0.51-1.43]	1.04 [0.73-1.48]	1.02 [0.51-2.01]
P for trend	0.261	0.915	0.828	0.709
Cord blood				
Q_{2-3} vs. Q_1	0.82 [0.52-1.30]	0.98 [0.65-1.47]	1.42 [1.03-1.96]*	1.21 [0.67-2.21]
Q_4 vs. Q_1	0.74 [0.41-1.33]	0.64 [0.37-1.11]	1.35 [0.92-1.98]	0.57 [0.25-1.34]
P for trend	0.509	0.049*	0.110	0.905
Cadmium (Cd)				
Maternal blood				
Q_{2-3} vs. Q_1	0.87 [0.53-1.44]	0.99 [0.64-1.52]	1.05 [0.77-1.44]	0.65 [0.35-1.2]
Q_4 vs. Q_1	1.16 [0.66-2.01]	0.81 [0.49-1.35]	1.03 [0.72-1.48]	0.81 [0.41-1.6]

P for trend	0.906	0.416	0.622	0.697
Cord blood				
Q_{2-3} vs. Q_1	1.18 [0.66-2.10]	1.36 [0.85-2.18]	1.52 [1.06-2.17]*	2.07 [0.92-4.68]†
Q_4 vs. Q_1	1.81 [1.00-3.29]*	1.20 [0.71-2.04]	1.60 [1.09-2.35]*	3.17 [1.36-7.38]**
P for trend	0.039*	0.412	0.258	0.017*
Manganese (Mn)				
Maternal blood				
Q_{2-3} vs. Q_1	1.52 [0.91-2.54]	1.11 [0.72-1.70]	1.47 [1.05-2.06]*	0.91 [0.49-1.69]
Q_4 vs. Q_1	1.61 [0.88-2.96]	0.83 [0.48-1.41]	1.55 [1.05-2.28]*	1.12 [0.55-2.27]
P for trend	0.279	0.356	0.015*	0.821
Cord blood				
Q_{2-3} vs. Q_1	0.77 [0.49-1.22]	0.88 [0.58-1.33]	1.12 [0.82-1.53]	1.2 [0.64-2.23]
Q_4 vs. Q_1	0.82 [0.48-1.42]	0.98 [0.60-1.60]	1.14 [0.79-1.64]	1.04 [0.49-2.19]
P for trend	0.681	0.536	0.572	0.701

IX. FIGURE LEGENDS

Figure 1: Flow-chart of the study population selection design. †mother-children pairs with measurements of lead (Pb), cadmium (Cd) and manganese (Mn) in both maternal and cord blood.

Figure 2: **Associations of prenatal metal concentrations of lead (Pb), cadmium (Cd) and manganese (Mn) levels in maternal and cord blood with the risk of asthma, rhinitis, eczema and food allergy.** The figure represents the hazard ratios (HR, with 95% confidence intervals) of the risk of developing atopic diseases for highest quartile vs. lowest quartile (Q4 vs.Q1) of each metal level exposure, adjusted for sex, centre, BMI, maternal education, parental smoking, parental history of allergy, maternal smoking in pregnancy, birthweight, gestational age at delivery, type of delivery, and mutually adjusted for other metal exposures.

X. APPENDICES

A supplementary material file is included, containing the following text, figures and tables:

Text E1: Additional methods and description of the covariates.

Figure E1: Box plots for the distribution of lead (Pb), cadmium (Cd) and manganese (Mn) concentrations in maternal blood during mid-pregnancy and in cord blood in the EDEN study sample.

Figure E2: Correlation matrix (with Pearson's correlation coefficients and p-values) of lead (Pb), cadmium (Cd) and manganese (Mn) log-2 concentrations in mid-pregnancy maternal and cord blood.

Table E1: Children characteristics.

Table E2: Frequency of multiple allergic outcomes in the sample.

Table E3: Metal concentrations in maternal and cord blood by status of asthma, allergic rhinitis, eczema and food allergy.

Table E4: Incidence of asthma by quartile of fetal exposure to Pb, Cd and Mn.

Table E5: Incidence of allergic rhinitis by quartile of fetal exposure to Pb, Cd and Mn.

Table E6: Incidence of eczema by quartile of fetal exposure to Pb, Cd and Mn.

Table E7: Incidence of food allergy by quartile of fetal exposure to Pb, Cd and Mn.

Table E8: Associations of metal levels (included as continuous variable using log-2 concentrations) in blood with the risk of asthma, rhinitis, eczema and food allergy in children from the EDEN cohort.

Table E9: Associations (odds ratios for a unit increase in log2 concentrations) of fetal exposure to heavy metals with the incidence of asthma in children.

Table E10: Associations (odds ratios for a unit increase in log2 concentrations) of fetal exposure to heavy metals with the incidence of allergic rhinitis in children.

Table E11: Associations (odds ratios for a unit increase in log2 concentrations) of fetal exposure to heavy metals with the incidence of eczema in children.

Table E12: Associations (odds ratios for a unit increase in log2 concentrations) of fetal exposure to heavy metals with the incidence of food allergy in children.

Table E13: Interactions between heavy metal concentrations and sex in the associations with the incidence of asthma, rhinitis, eczema and food allergy in children.

Table E14: Interactions between heavy metal concentrations and age of disease onset (3 years or less vs. 3 to 8 years) in the associations with the incidence of asthma, rhinitis, eczema and food allergy in children.

Table E15: Maternal and cord blood levels of heavy metals by maternal smoking status in pregnancy.

Table E16: Associations between foetal exposure to maternal and cord blood cadmium and the incidence of atopic diseases in childhood according to maternal smoking status in pregnancy.

Online Supplement

Exposure to heavy metals *in utero* and incidence of atopic diseases in early childhood.

This file contains the following additional material:

Text E1: Additional methods and description of the covariates.

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Table E1: Children characteristics.

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Table E4: Incidence of **asthma** by quartile of fetal exposure to Pb, Cd and Mn.

Table E5: Incidence of **allergic rhinitis** by quartile of fetal exposure to Pb, Cd and Mn.

Table E6: Incidence of **eczema** by quartile of fetal exposure to Pb, Cd and Mn.

Table E7: Incidence of **food allergy** by quartile of fetal exposure to Pb, Cd and Mn.

Table E8: Associations of metal levels (included as continuous variable using log-2 concentrations) in blood with the risk of asthma, rhinitis, eczema and food allergy in children from the EDEN cohort.

Table E9: Associations (odds ratios for a unit increase in log2 concentrations) of fetal exposure to heavy metals with the incidence of **asthma** in children.

Table E10: Associations (odds ratios for a unit increase in log2 concentrations) of fetal exposure to heavy metals with the incidence of **allergic rhinitis** in children.

Table E11: Associations (odds ratios for a unit increase in log2 concentrations) of fetal exposure to heavy metals with the incidence of **eczema** in children.

Table E12: Associations (odds ratios for a unit increase in log2 concentrations) of fetal exposure to heavy metals with the incidence of **food allergy** in children.

Table E13: Interactions between heavy metal concentrations and sex in the associations with the incidence of asthma, rhinitis, eczema and food allergy in children.

Table E14: Interactions between heavy metal concentrations and age of disease onset (3 years or less vs. 3 to 8 years) in the associations with the incidence of asthma, rhinitis, eczema and food allergy in children.

Table E15: Maternal and cord blood levels of heavy metals by maternal smoking status in pregnancy.

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Text E1. Additional methods and description of the covariates.

Additional methods

Incidence rates of asthma, allergic rhinitis, eczema and food allergy were calculated by dividing the number of children who suffered from each of these symptoms by the total time at risk in the years. The time at risk for each allergic outcome was calculated for each child from birth to the age when the child was first reported to have had the outcome, or to the age of the child when the last questionnaire was administered if he/she did not suffer from the disease.

To test the stability of the estimates for the associations between metal concentrations and allergic health outcomes in relation to different covariates, the HR have been adjusted for 4 sets of variables: 1) sex and EDEN centre; 2) variables in "1" plus child BMI, maternal education, parental smoking and parental history of allergy; 3) variables in "2" plus maternal smoking in pregnancy, birth weight, gestational age and type of delivery. 4) variables in "3" plus mutual adjustment for other heavy metal exposures. This last (fully-adjusted) was used as main model throughout the manuscript.

Description of the covariates

The following variables were included in the models as potential confounders of the associations and coded as follows: sex at birth, EDEN center (Poitier or Nancy), child's BMI at last examination (Kg/m^2, continuous), highest maternal educational degree as proxy of socio-economic status (primary or vocational school diploma; secondary school diploma; university degree or higher), parental history of allergy (at least one parent, yes/no), parental smoking habits during the child's childhood as proxy of second-hand smoking exposure, maternal smoking during pregnancy,

birthweight (<2500 g; 2500-4200 g; ≥4200 g), prematurity (if born before the 37th gestational week), type of delivery (vaginal; caesarean section; vacuum extraction or forceps).

Figure E1: Distribution of lead (Pb), cadmium (Cd) and manganese (Mn) concentrations in maternal blood during mid-pregnancy and in cord blood in the EDEN study sample.

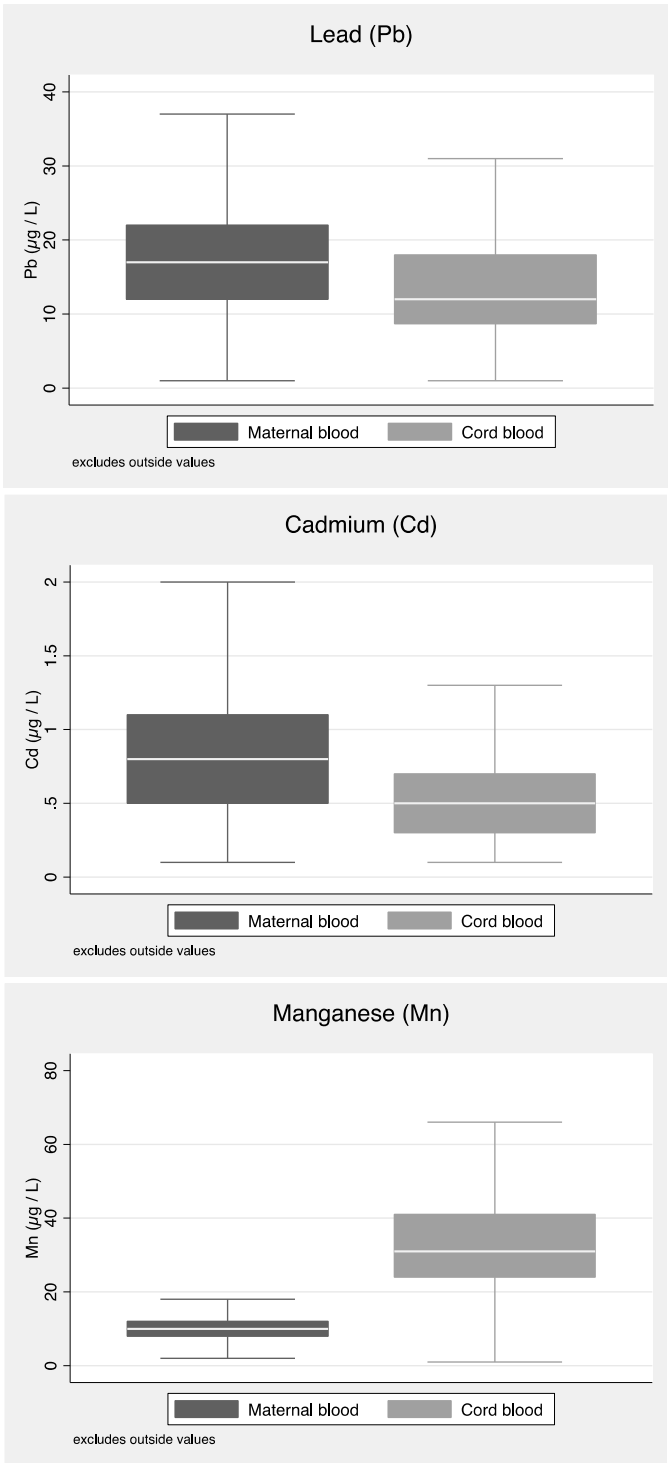


Figure E2: correlation matrix (with Pearson's correlation coefficients and p-values) of lead (Pb), cadmium (Cd) and manganese (Mn) log-2 concentrations in mid-pregnancy maternal and cord blood.

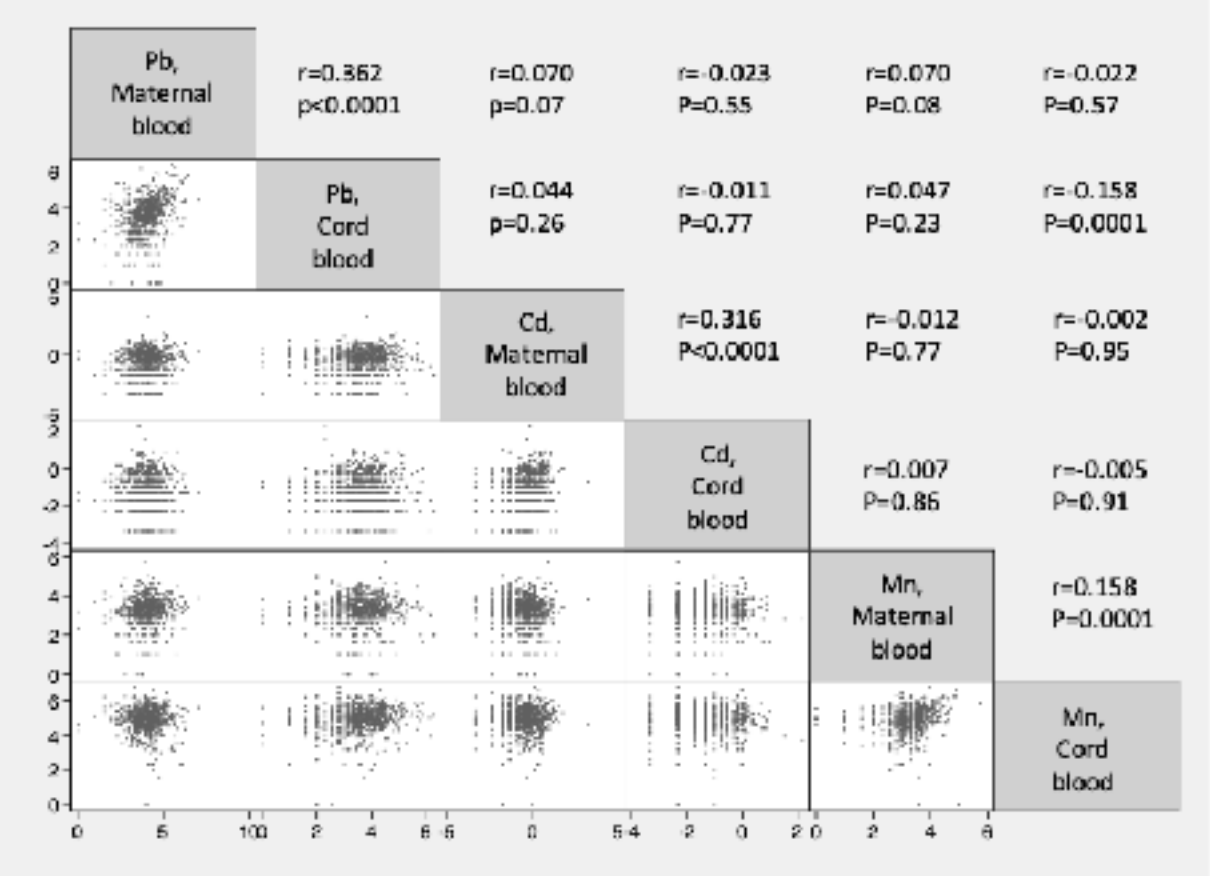


Table E1: Children characteristics.
Data reported as n (%) or mean ± standard deviation.

	EDEN children included in the analyses N=651
Centre	
Poitier	369 (56.7%)
Nancy	282 (43.3%)
Sex at birth	
Boys	339 (52.1%)
Girls	312 (47.9%)
Birthweight	
<2500 g	29 (4.4%)
2500-4200 g	600 (92.2%)
>4200 g	22 (3.4%)
Gestational age at delivery	
< 37 weeks	34 (5.2%)
37-38 weeks	122 (18.8%)
39-42 weeks	495 (76.0%)
Type of delivery	
Vaginal delivery	514 (79.0%)
Vacuum extraction or forceps	61 (9.4%)
Caesarian section	76 (11.7%)
Maternal smoke during pregnancy	
No	491 (75.4%)
Yes	160 (24.6%)
Maternal education	
Vocational school	122 (18.7%)
Secondary school	221 (34.0%)
Higher education	308 (47.3%)
Parental smoking	
No	450 (69.1%)
Yes	201 (30.9%)

Parental history of allergy	
No	412 (63.3%)
Yes	239 (36.7%)
Maternal birthplace	
Europe	635 (97.5%)
Africa	7 (1.4%)
America	3 (0.5%)
Asia	1 (0.2%)
Missing information	3 (0.5%)
Maternal grandparents' birthplace	
Europe, both grandparents	597 (91.7%)
Outside Europe, one grandparent	28 (4.2%)
Outside Europe, both grandparents	18 (2.8%)
Unknown/missing information	8 (1.2%)
Child BMI (Kg/m^2)	
at 3 years old	15.7 ± 1.3
at 4 years old	15.5 ± 1.3
at 5 years old	15.4 ± 1.7
at 8 years old	15.9 ± 1.9
Child age at the last follow-up	
3 years	36 (5.5%)
4 years	86 (18.7%)
5 years	141 (21.7%)
8 years	388 (59.6%)

Table E2: Frequency of multiple allergic outcomes in the sample.

No. of allergic outcomes	N	%
0	262	40.3
1	229	35.2
2	104	16.0
3	47	7.2
4	9	1.4

Table E3: Metal concentrations in maternal and cord blood by status of asthma, allergic rhinitis, eczema and food allergy.
Differences between disease status groups were tested by multiple Wilcoxon's rank-sum tests: †: p<0.1; *: p<0.05; **: p<0.01

	Ashtma		Allergic rhinitis		Eczema		Food allergy	
	No (n=538)	Yes (n=113)	No (n=507)	Yes (n=144)	No (n=368)	Yes (n=283)	No (n=577)	Yes (n=74)
Lead (Pb)								
Maternal blood	19.0 ± 12.0	19.9 ± 13.7	19.2 ± 12.2	18.7 ± 12.7	18.8 ± 12.1	19.5 ± 12.6	19.1 ± 12.0	19.4 ± 14.4
Cord blood	14.6 ± 9.5	13.9 ± 10.6	**14.9 ± 9.8**	**13.0 ± 9.1***	14.2 ± 10.0	14.7 ± 9.3	14.6 ± 9.9	13.5 ± 7.6
Cadmium (Cd)								
Maternal blood	0.84 ± 0.61	0.87 ± 0.53	0.86 ± 0.63	0.81 ± 0.45	0.81 ± 0.48	0.89 ± 0.72	0.82 ± 0.48	1.02 ± 1.16
Cord blood	**0.51 ± 0.36**	**0.61 ± 0.40****	0.53 ± 0.39	0.53 ± 0.31	*0.52 ± 0.41*	*0.54 ± 0.32†*	**0.52 ± 0.37**	**0.61 ± 0.34****
Manganese (Mn)								
Maternal blood	10.5 ± 4.8	10.5 ± 4.2	10.5 ± 4.8	10.7 ± 4.3	**10.1 ± 4.3**	**11.1 ± 5.1***	10.5 ± 4.6	10.9 ± 5.0
Cord blood	32.3 ± 14.3	32.1 ± 14.0	32.3 ± 14.0	32.3 ± 14.3	32.8 ± 14.7	33.5 ± 13.6	33.1 ± 14.3	33.4 ± 14.1

Table E4: Incidence of asthma by quartile of fetal exposure to Pb, Cd and Mn. P-values for differences between exposure quartile groups (with Q1 as refence) were estimated using Cox proportional hazard models.

Exposure	N	Incident cases	Incident proportion (%)	Person-years	Incidence rates/1000	p-value
Lead (Pb)						
Maternal blood						
Q_1 [1-12 µg/L]	155	30	19.4	891	33.7	
Q_{2-3} (12-22 µg/L]	338	54	16.0	2000	27.0	0.349
Q_4 (22-141 µg/L]	158	29	18.4	938.5	30.9	0.776
Cord blood						
Q_1 [1-8.7 µg/L]	164	35	21.3	948.5	36.9	
Q_{2-3} (8.7-18 µg/L]	331	55	16.6	1970.5	27.9	0.220
Q_4 (18-78 µg/L]	156	23	14.7	910.5	25.3	0.148
Cadmium (Cd)						
Maternal blood						
Q_1 [0.1-0.5 µg/L]	161	25	15.5	961.5	26.0	
Q_{2-3} (0.5-1.1 µg/L]	305	52	17.0	1795	29.0	0.669
Q_4 (1.1-9.6 µg/L]	185	36	19.5	1073	33.6	0.347
Cord blood						
Q_1 [0.1-0.3 µg/L]	132	17	12.9	802.5	21.2	
Q_{2-3} (0.3-0.7 µg/L]	326	51	15.6	1958.5	26.0	0.469
Q_4 (0.7-4.6 µg/L]	193	45	23.3	1068.5	42.1	**0.021**
Manganese (Mn)						
Maternal blood						

Q_1 [1-8 µg/L]	139	21	15.1	826.5	25.4	
Q_{2-3} (8-12 µg/L]	355	65	18.3	2081.5	31.2	0.387
Q_4 (12-53 µg/L]	157	27	17.2	821.5	32.9	0.591
Cord blood						
Q_1 [1-24 µg/L]	153	32	20.9	876	36.5	
Q_{2-3} (24-41 µg/L]	333	53	15.9	1955.5	27.1	0.195
Q_4 (41-107 µg/L]	165	28	17.0	998	28.1	0.329

Table E5: Incidence of allergic rhinitis by quartile of fetal exposure to Pb, Cd and Mn. P-values for differences between exposure quartile groups (with Q1 as refence) were estimated using multiple Cox proportional hazard models.

Exposure	N	Incident cases	Incident proportion (%)	Person-years	Incidence rates/1000	p-value
Lead (Pb)						
Maternal blood						
Q_1 [1-12 µg/L]	155	40	25.8	854	46.8	
Q_{2-3} (12-22 µg/L]	338	73	21.6	1947	37.5	0.256
Q_4 (22-141 µg/L]	158	31	19.6	929.5	33.4	0.167
Cord blood						
Q_1 [1-8.7 µg/L]	164	42	25.6	954.5	44.0	
Q_{2-3} (8.7-18 µg/L]	331	77	23.3	1859.5	41.4	0.733
Q_4 (18-78 µg/L]	156	25	16.0	916.5	27.3	**0.049**
Cadmium (Cd)						
Maternal blood						
Q_1 [0.1-0.5 µg/L]	161	34	21.1	933	36.4	
Q_{2-3} (0.5-1.1 µg/L]	305	73	23.9	1727.5	42.3	0.526
Q_4 (1.1-9.6 µg/L]	185	37	20.0	1070	34.6	0.794
Cord blood						
Q_1 [0.1-0.3 µg/L]	132	25	18.9	776.5	32.2	
Q_{2-3} (0.3-0.7 µg/L]	326	78	23.9	1858	42.0	0.262
Q_4 (0.7-4.6 µg/L]	193	41	21.2	1096	37.4	0.596

Manganese (Mn)						
Maternal blood						
Q_1 [1-8 µg/L]	139	31	22.3	789.5	39.3	
$Q_{2\text{-}3}$ (8-12 µg/L]	355	84	23.7	2021.5	41.6	0.707
Q_4 (12-53 µg/L]	157	29	18.5	919.5	31.5	0.464
Cord blood						
Q_1 [1-24 µg/L]	153	38	24.8	883	43.0	
$Q_{2\text{-}3}$ (24-41 µg/L]	333	70	21.0	1875	37.3	0.483
Q_4 (41-107 µg/L]	165	36	21.8	972.5	37.0	0.525

Table E6: Incidence of eczema by quartile of fetal exposure to Pb, Cd and Mn. P-values for differences between exposure quartile groups (with Q1 as refence) were estimated using multiple Cox proportional hazard models.

Exposure	N	Incident cases	Incident proportion (%)	Person-years	Incidence rates/1000	p-value
Lead (Pb)						
Maternal blood						
Q_1 [1-12 µg/L]	155	73	47.1	658	110.9	
Q_{2-3} (12-22 µg/L]	338	133	39.3	1528.5	87.0	0.155
Q_4 (22-141 µg/L]	158	77	48.7	640.5	120.2	0.717
Cord blood						
Q_1 [1-8.7 µg/L]	164	59	36.0	788.5	74.8	
Q_{2-3} (8.7-18 µg/L]	331	153	46.2	1410.4	108.5	0.051
Q_4 (18-78 µg/L]	156	71	45.5	627.8	113.1	0.077
Cadmium (Cd)						
Maternal blood						
Q_1 [0.1-0.5 µg/L]	161	60	37.3	732.1	82.0	
Q_{2-3} (0.5-1.1 µg/L]	305	139	45.6	1312.4	105.9	0.162
Q_4 (1.1-9.6 µg/L]	185	84	45.4	782.2	107.4	0.186
Cord blood						
Q_1 [0.1-0.3 µg/L]	132	43	32.6	637.5	67.5	
Q_{2-3} (0.3-0.7 µg/L]	326	147	45.1	1414.9	103.9	**0.026**
Q_4 (0.7-4.6 µg/L]	193	93	48.2	774.3	120.1	**0.009**

Manganese (Mn)						
Maternal blood						
Q_1 [1-8 µg/L]	139	46	33.1	664.4	69.2	
$Q_{2\text{-}3}$ (8-12 µg/L]	355	165	46.5	1508	109.4	**0.014**
Q_4 (12-53 µg/L]	157	72	45.9	654.3	110.0	**0.026**
Cord blood						
Q_1 [1-24 µg/L]	153	59	38.6	724.2	81.5	
$Q_{2\text{-}3}$ (24-41 µg/L]	333	150	45.0	1404.2	106.8	0.174
Q_4 (41-107 µg/L]	165	74	44.8	698.3	106.0	0.202

Table E7: Incidence of food allergy by quartile of fetal exposure to Pb, Cd and Mn. P-values for differences between exposure quartile groups (with Q1 as refence) were estimated using multiple Cox proportional hazard models.

Exposure	N	Incident cases	Incident proportion (%)	Person-years	Incidence rates/1000	p-value
Lead (Pb)						
Maternal blood						
Q_1 [1-12 µg/L]	155	24	15.5	892.6	26.9	
Q_{2-3} (12-22 µg/L]	338	32	9.5	2096.9	15.3	**0.048**
Q_4 (22-141 µg/L]	158	18	11.4	952.5	18.9	0.285
Cord blood						
Q_1 [1-8.7 µg/L]	164	18	11.0	1024.8	17.6	
Q_{2-3} (8.7-18 µg/L]	331	45	13.6	1946.6	23.1	0.379
Q_4 (18-78 µg/L]	156	11	7.1	943.6	11.7	0.241
Cadmium (Cd)						
Maternal blood						
Q_1 [0.1-0.5 µg/L]	161	18	11.2	979.8	18.4	
Q_{2-3} (0.5-1.1 µg/L]	305	31	10.2	1846.1	16.8	0.738
Q_4 (1.1-9.6 µg/L]	185	25	13.5	1089.1	23.0	0.502
Cord blood						
Q_1 [0.1-0.3 µg/L]	132	8	6.1	834.3	9.6	
Q_{2-3} (0.3-0.7 µg/L]	326	34	10.4	1992.6	17.1	0.165
Q_4 (0.7-4.6 µg/L]	193	32	16.6	1088.1	29.4	**0.008**

Manganese (Mn)						
Maternal blood						
Q_1 [1-8 µg/L]	139	16	11.5	831.3	19.2	
Q_{2-3} (8-12 µg/L]	355	38	10.7	2143.9	17.7	0.833
Q_4 (12-53 µg/L]	157	20	12.7	939.8	21.3	0.766
Cord blood						
Q_1 [1-24 µg/L]	153	15	9.8	949	15.8	
Q_{2-3} (24-41 µg/L]	333	41	12.3	1946.4	21.1	0.381
Q_4 (41-107 µg/L]	165	18	10.9	1019.6	17.7	0.743

Table E8: Associations of metal levels in blood with the risk of asthma, rhinitis, eczema and food allergy in children from the EDEN cohort. Hazard ratios (HR, with 95% confidence intervals) are estimated for a unit increase in log2[metal concentrations], and are adjusted for sex, centre, BMI, maternal education, parental smoking, parental history of allergy, maternal smoking in pregnancy, birthweight, gestational age at delivery and type of delivery, and mutually adjusted for other metal exposures. *: p<0.05

	Asthma	**Rhinitis**	**Eczema**	**Food Allergy**
	HR [95%CI]	**HR [95%CI]**	**HR [95%CI]**	**HR [95%CI]**
Lead (Pb)				
Maternal blood	1.15 [0.90-1.48]	1.01 [0.82-1.25]	0.98 [0.84-1.15]	0.94 [0.69-1.28]
Cord blood	0.93 [0.76-1.15]	**0.84 [0.70-1.00]***	1.12 [0.97-1.28]	0.98 [0.76-1.28]
Cadmium (Cd)				
Maternal blood	0.99 [0806-1.22]	0.93 [0.78-1.11]	1.03 [0.95-1.22]	1.05 [0.81-1.38]
Cord blood	**1.25 [1.01-1.54]***	1.08 [0.90 -1.28]	1.08 [0.95-1.22]	**1.37 [1.06-1.77]***
Manganese (Mn)				
Maternal blood	1.18 [0.87-1.59]	1.13 [0.87-1.45]	**1.27 [1.05-1.53]***	0.96 [0.68-1.36]
Cord blood	0.95 [0.74-1.21]	0.93 [0.75-1.16]	1.05 [0.89-1.25]	1.07 [0.75-1.52]

Table E9: Associations (hazard ratios for a unit increase in log2 concentrations) of heavy metals in blood samples with the incidence of asthma in children from the EDEN cohort.

	HR⁰ [95%CI]	HR¹ [95%CI]	HR² [95%CI]	HR³ [95%CI]
Lead (Pb)				
Maternal blood	1.06 [0.84-1.33]	1.09 [0.87-1.38]	1.13 [0.90-1.42]	1.13 [0.89-1.42]
Cord blood	0.92 [0.76-1.10]	0.94 [0.78-1.13]	0.95 [0.79-1.15]	0.97 [0.80-1.18]
Cadmium (Cd)				
Maternal blood	1.06 [0.88-1.29]	1.06 [0,87-1.29]	1.07 [0.87-1.31]	1.06 [0.86-1.29]
Cord blood	1.25 [1.03-1.52]*	1.24 [1.02-1.52]*	1.25 [1.03-1.53]*	1.25 [1.02-1.52]*
Manganese (Mn)				
Maternal blood	1.05 [0.80-1.38]	1.18 [0.89-1.56]	1.17 [0.88-1.55]	1.18 [0.88-1.57]
Cord blood	0.89 [0.71-1.12]	0.91 [0.72-1.15]	0.91 [0.72-1.17]	0.95 [0.74-1.21]

0: unadjusted hazard ratios;
1: hazard ratios adjusted for sex and centre;
2: hazard ratios adjusted for sex, centre, BMI, maternal education, parental smoking, parental history of allergy;
3: hazard ratios adjusted for sex, centre, BMI, maternal education, parental smoking, parental history of allergy, maternal smoking in pregnancy, birthweight, gestational age at delivery and type of delivery.
*: $p<0.05$

Table E10: Associations (hazard ratios for a unit increase in log2 concentrations) of heavy metals in blood samples with the incidence of rhinitis in children from the EDEN cohort.

	HR⁰ [95%CI]	HR¹ [95%CI]	HR² [95%CI]	HR³ [95%CI]
Lead (Pb)				
Maternal blood	0.94 [0.76-1.12]	0.93 [0.76-1.13]	0.94 [0.77-1.14]	0.94 [0.77-1.15]
Cord blood	0.84 [0.72-0.99]*	0.84 [0.72-0.99]*	0.85 [0.73-0.99]*	0.84 [0.71-0.98]*
Cadmium (Cd)				
Maternal blood	0.98 [0.83-1.15]	0.98 [0.83-1.15]	0.96 [0.81-1.13]	0.95 [0.80-1.12]
Cord blood	1.05 [0.89 -1.24]	1.05 [0.89 -1.24]	1.05 [0.89 -1.24]	1.06 [0.89 -1.25]
Manganese (Mn)				
Maternal blood	1.10 [0.86-1.40]	1.11 [0.87-1.42]	1.10 [0.86-1.41]	1.10 [0.86-1.42]
Cord blood	0.92 [0.75-1.13]	0.92 [0.75-1.13]	0.92 [0.74-1.14]	0.91 [0.74-1.14]

0: unadjusted hazard ratios;
1: hazard ratios adjusted for sex and centre;

2: hazard ratios adjusted for sex, centre, BMI, maternal education, parental smoking, parental history of allergy;
3: hazard ratios adjusted for sex, centre, BMI, maternal education, parental smoking, parental history of allergy, maternal smoking in pregnancy, birthweight, gestational age at delivery and type of delivery.
*: $p<0.05$

Table E11: Associations (hazard ratios for a unit increase in log2 concentrations) of heavy metals in blood samples with the incidence of eczema in children from the EDEN cohort.

	HR⁰ [95%CI]	HR¹ [95%CI]	HR² [95%CI]	HR³ [95%CI]
Lead (Pb)				
Maternal blood	1.01 [0.88-1.17]	1.02 [0.88-1.18]	1.03 [0.89-1.19]	1.04 [0.90-1.10]
Cord blood	1.11 [0.98-1.26]†	1.11 [0.98-1.26]†	1.12 [0.99-1.27]†	1.12 [0.99-1.27]†
Cadmium (Cd)				
Maternal blood	1.10 [0.97-1.24]	1.10 [0.97-1.24]	1.08 [0.95-1.22]	1.06 [0.94-1.20]
Cord blood	1.09 [0.97 -1.22]	1.09 [0.96 -1.22]	1.08 [0.96 -1.22]	1.08 [0.96-1.22]
Manganese (Mn)				
Maternal blood	1.26 [1.05 -1.51]*	1.28 [1.06 -1.54]**	1.27 [1.05 -1.53]*	1.28 [1.06 -1.54]**
Cord blood	1.11 [0.94-1.30]	1.11 [0.94-1.31]	1.11 [0.94-1.31]	1.11 [0.94-1.32]

0: unadjusted hazard ratios;
1: hazard ratios adjusted for sex and centre;
2: hazard ratios adjusted for sex, centre, BMI, maternal education, parental smoking, parental history of allergy;
3: hazard ratios adjusted for sex, centre, BMI, maternal education, parental smoking, parental history of allergy, maternal smoking in pregnancy, birthweight, gestational age at delivery and type of delivery.
†: $p<0.10$; *: $p<0.05$; **$p<0.01$

Table E12: Associations (hazard ratios for a unit increase in log2 concentrations) of heavy metals in blood samples with the incidence of food allergy in children from the EDEN cohort.

	HR⁰ [95%CI]	HR¹ [95%CI]	HR² [95%CI]	HR³ [95%CI]
Lead (Pb)				
Maternal blood	1.06 [0.84-1.33]	1.09 [0.87-1.38]	1.13 [0.90-1.42]	1.13 [0.89-1.42]
Cord blood	0.98 [0.77-1.23]	0.97 [0.77-1.22]	0.99 [0.79-1.25]	0.97 [0.77-1.23]
Cadmium (Cd)				
Maternal blood	1.20 [0.90-1.50]	1.20 [0.84-1.53]	1.19 [0.93-1.53]	1.16 [0.90-1.50]
Cord blood	1.38 [1.08-1.76]**	1.39 [1.09-1.77]**	1.41 [1.10-1.80]**	1.39 [1.09-1.77]**
Manganese (Mn)				
Maternal blood	1.02 [0.73-1.42]	0.98 [0.70-1.37]	0.96 [0.69-1.34]	0.98 [0.70-1.36]

Cord blood	1.06 [0.77-1.46]	1.05 [0.76-1.45]	1.05 [0.75-1.46]	1.05 [0.75-1.46]

0: unadjusted hazard ratios;
1: hazard ratios adjusted for sex and centre;
2: hazard ratios adjusted for sex, centre, BMI, maternal education, parental smoking, parental history of allergy;
3: hazard ratios adjusted for sex, centre, BMI, maternal education, parental smoking, parental history of allergy, maternal smoking in pregnancy, birthweight, gestational age at delivery and type of delivery.
†: $p<0.10$; *: $p<0.05$; **$p<0.01$

Table E13: Interaction between sex and heavy metal concentrations in the associations with the incidence of asthma, rhinitis, eczema and food allergy in children. The associations are shown as hazard ratios for a unit increase in log2 concentrations and adjusted for sex, centre, BMI, maternal education, parental smoking, parental history of allergy, maternal smoking in pregnancy, birthweight, gestational age at delivery and type of delivery, and mutually adjusted for other metal exposures. The p-value is obtained using a likelihood ratio test between the model where the 6 metal exposures were included without sex interactions (base model) vs. the model where an interaction term between sex and each of the 6 metal exposures was included.

	Asthma	**Rhinitis**	**Eczema**	**Food Allergy**
	HR [95%CI]	**HR [95%CI]**	**HR [95%CI]**	**HR [95%CI]**
Lead (Pb)				
Maternal blood				
males	1.20 [0.84-1.71]	0.97 [0.70-1.35]	1.04 [0.82-1.33]	0.90 [0.56-1.44]
females	1.11 [0.77-1.60]	1.04 [0.78-1.41]	0.94 [0.77-1.17]	1.03 [0.67-1.58]
Cord blood				
males	0.87 [0.65-1.16]	0.82 [0.62-1.07]	1.06 [0.86-1.31]	0.93 [0.62-1.39]
females	1.01 [0.74-1.37]	0.85 [0.68-1.07]	1.15 [0.96-1.39]	1.03 [0.72-1.47]
Cadmium (Cd)				
Maternal blood				
males	1.07 [0.80-1.43]	0.99 [0.77-1.28]	1.08 [0.90-1.31]	1.03 [0.70-1.51]
females	0.89 [0.65-1.23]	0.88 [0.69-1.12]	0.99 [0.82-1.19]	1.13 [0.78-1.64]
Cord blood				
males	1.23 [0.94-1.60]	1.00 [0.78-1.28]	0.94 [0.79-1.12]	1.20 [0.83-1.73]
females	1.27 [0.89-1.80]	1.16 [0.90-1.50]	1.25 [1.03-1.51]	1.56 [1.06-2.30]
Manganese (Mn)				
Maternal blood				
males	1.16 [0.81-1.66]	1.10 [0.80-1.50]	1.21 [0.95-1.54]	1.07 [0.66-1.72]
females	1.22 [0.72-2.08]	1.21 [0.79-1.84]	1.38 [1.02-1.87]	0.84 [0.51-1.40]
Cord blood				
males	1.07 [0.77-1.47]	0.95 [0.70-1.25]	1.16 [0.91-1.54]	1.36 [0.80-2.33]
females	0.79 [0.54-1.16]	0.90 [0.64-1.28]	0.95 [0.74-1.22]	0.80 [0.48-1.32]
P for sex-metals interaction	0.900	0.976	0.344	0.555

Table E14: Interactions between age of disease onset (3 years or less vs. 3 to 8 years) and fetal exposure to heavy metals in the associations with the incidence of asthma, rhinitis, eczema and food allergy in children. The associations are shown as hazard ratios for a unit increase in log2 concentrations and adjusted for sex, centre, BMI, maternal education, parental smoking, parental history of allergy, maternal smoking in pregnancy, birthweight, gestational age at delivery and type of delivery. The p-value is obtained using a likelihood ratio test between the model where the 6 metal exposures were included without age interactions (base model) vs. the model where an interaction term between age and each of the 6 metal exposures was included.

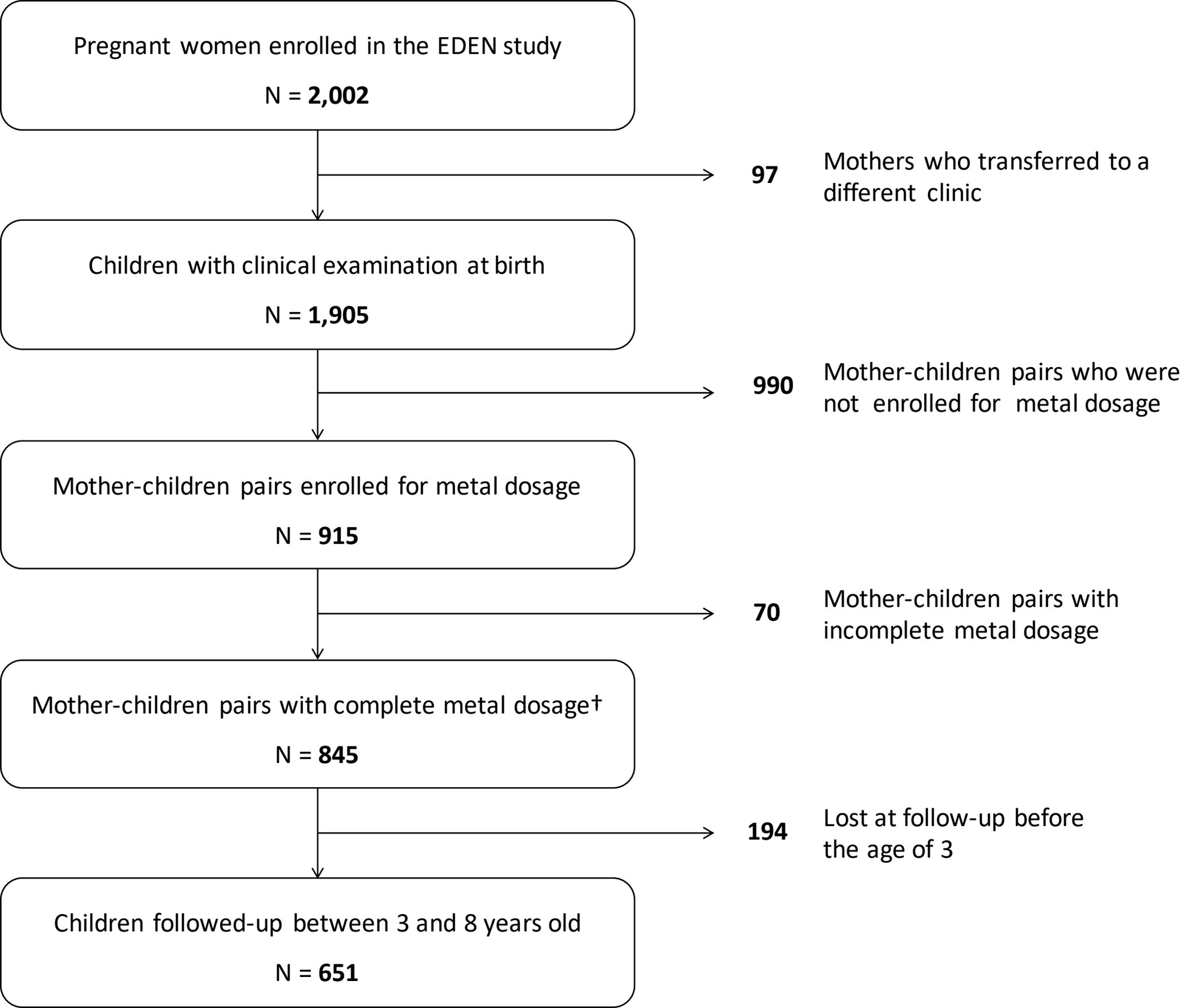
	Asthma	**Rhinitis**	**Eczema**	**Food Allergy**
	HR [95%CI]	**HR [95%CI]**	**HR [95%CI]**	**HR [95%CI]**
Lead (Pb)				
Maternal blood				
0-3 years	1.34 [0.96-1.88]	0.87 [0.66-1.15]	0.98 [0.83-1.15]	0.94 [0.66-1.35]
3-8 years	0.96 [0.67-1.37]	1.28 [0.91-1.81]	1.01 [0.68-1.50]	0.94 [0.52-1.70]
Cord blood				
0-3 years	0.90 [0.68-1.18]	0.88 [0.69-1.11]	1.13 [0.97-1.31]	1.03 [0.75-1.41]
3-8 years	0.98 [0.72-1.33]	0.77 [0.59-1.00]	1.09 [0.79-1.51]	0.90 [0.57-1.42]
Cadmium (Cd)				
Maternal blood				
0-3 years	1.13 [0.84-1.51]	0.96 [0.76-1.20]	1.06 [0.91-1.22]	1.32 [0.96-1.83]
3-8 years	0.83 [0.61-1.13]	0.89 [0.68-1.16]	0.93 [0.69-1.27]	0.61 [0.39-0.84]
Cord blood				
0-3 years	1.13 [0.86-1.49]	1.00 [0.79-1.26]	1.07 [0.93-1.23]	1.24 [0.93-1.67]
3-8 years	1.45 [1.04-2.02]	1.21 [0.92-1.58]	1.11 [0.81-1.51]	1.86 [1.11-3.13]
Manganese (Mn)				
Maternal blood				
0-3 years	1.26 [0.85-1.88]	1.19 [0.85-1.66]	1.19 [0.97-1.46]	0.80 [0.55-1.16]
3-8 years	1.06 [0.69-1.65]	1.03 [0.71-1.50]	1.83[1.10 -3.04]	1.89 [0.87-4.11]
Cord blood				
0-3 years	0.81 [0.60-1.07]	1.08 [0.78-1.50]	1.03 [0.86-1.25]	1.01 [0.68-1.51]
3-8 years	1.26 [0.82-1.96]	0.81 [0.61-1.07]	1.11 [0.75-1.65]	1.22 [0.61-2.42]
P for time-metals interaction	0.204	0.355	0.722	0.059

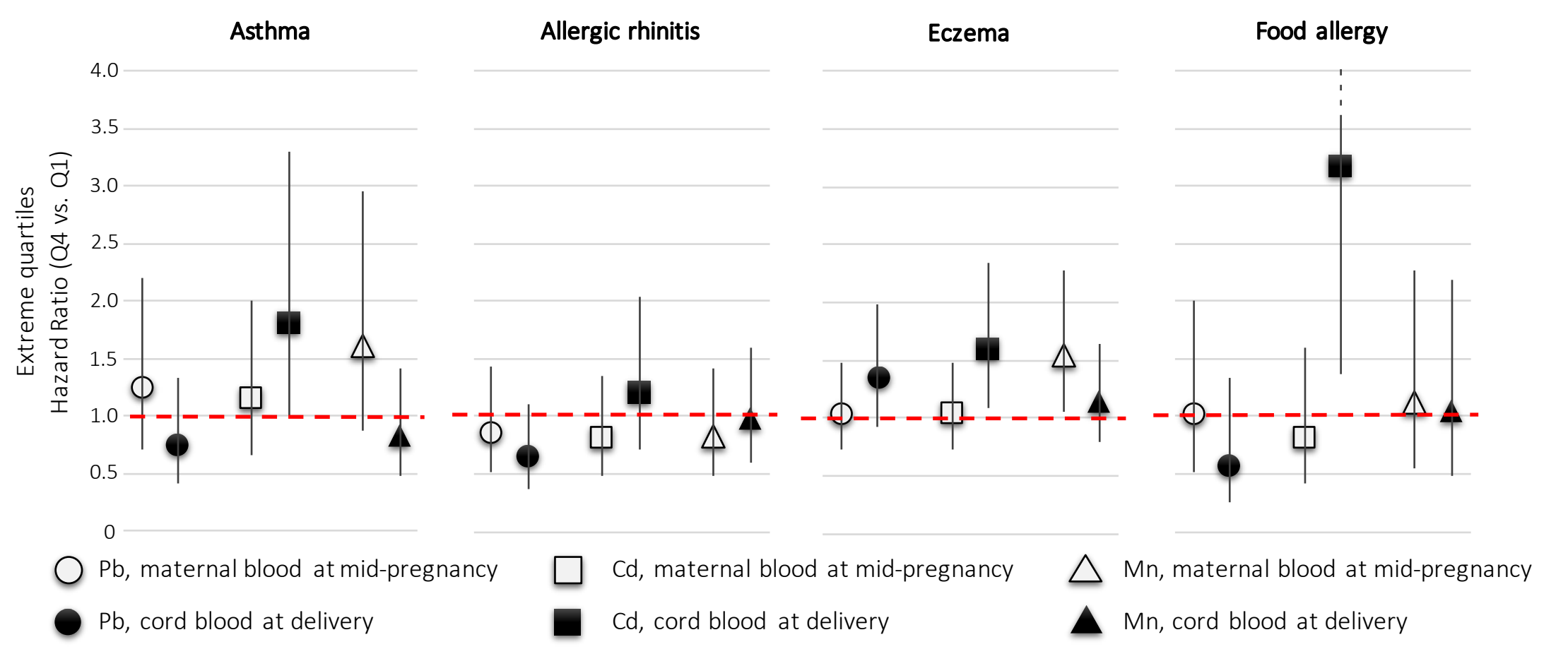
Table E15: Maternal and cord blood levels of heavy metals by maternal smoking status in pregnancy.

		Maternal blood		Cord blood	
		Non-smokers	**Smokers**	**Non-smokers**	**Smokers**
Pb (µg/L)	mean ± SD	18.8 ± 12.2	20.0 ± 12.6	14.1 ± 9.9	15.5 ± 8.9
	median [IQR]	17 [11.5,22]	17 [13,22.8]	12 [8,17]	14 [10,20]
	range	1-141	1-79	1-78	1-52
	Wilcoxon test (p-value)	0.205		**0.011**	
Cd (µg/L)	mean ± SD	0.76 ± 0.43	1.11 ± 0.89	0.52 ± 0.33	0.56 ± 0.47
	median [IQR]	0.7 [0.4,1.0]	1.0 [0.7,0.1.35]	0.5 [0.3,0.7]	0.5 [0.3,0.7]
	range	0.1-2.3	0.1-9.6	0.1-2.9	0.1-4.6
	Wilcoxon test (p-value)	**<0.0001**		0.539	
Mn (µg/L)	mean ± SD	10.6 ± 4.4	10.2 ± 5.3	32.8 ± 14.3	32.9 ± 13.9
	median [IQR]	10 [8,13]	9.9 [8,11.5]	31 [24,40]	31 [24,43]
	range	1-32	1-53	1-107	6-79
	Wilcoxon test (p-value)	0.140		0.586	

Table E16: Associations between foetal exposure to maternal and cord blood cadmium and the incidence of atopic diseases in childhood according to maternal smoking status in pregnancy. Hazard ratios (HR, with 95% confidence interval) are for doubling Cd concentrations and adjusted for sex, centre, BMI, maternal education, parental smoking, parental history of allergy, birthweight, gestational age at delivery, type of delivery and foetal exposures to other metals.

	Asthma	**Rhinitis**	**Eczema**	**Food Allergy**
	HR [95%CI]	**HR [95%CI]**	**HR [95%CI]**	**HR [95%CI]**
Maternal blood Cd				
Non-smokers	0.94 [0.73-1.22]	0.95 [0.77-1.18]	1.02 [0.87-1.20]	1.03 [0.74-1.42]
Smokers	1.08 [0.73-1.61]	0.87 [0.72-1.18]	1.06 [0.83-1.35]	1.09 [0.67-1.75]
Cord blood Cd				
Non-smokers	1.38 [1.06-1.80]	1.15 [0.92-1.44]	1.07 [0.91-1.25]	1.46 [1.04-2.05]
Smokers	1.04 [0.74-1.48]	0.96 [0.72-1.28]	1.09 [0.89-1.35]	1.24 [0.83-1.85]
p-value for Cd-maternal smoking interaction	0.448	0.451	0.934	0.832


Pregnant women enrolled in the EDEN study
N = 2,002
97
Mothers who transferred to a different clinic
Children with clinical examination at birth
N = 1,905
990
Mother-children pairs who were not enrolled for metal dosage
Mother-children pairs enrolled for metal dosage
N = 915
70
Mother-children pairs with incomplete metal dosage
Mother-children pairs with complete metal dosage†
N = 845
194
Lost at follow-up before the age of 3
Children followed-up between 3 and 8 years old
N = 651


Asthma
Allergic rhinitis
Eczema
Food allergy
Extreme quartiles
Hazard Ratio (Q4 vs. Q1)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
Pb, maternal blood at mid-pregnancy
Cd, maternal blood at mid-pregnancy
Mn, maternal blood at mid-pregnancy
Pb, cord blood at delivery
Cd, cord blood at delivery
Mn, cord blood at delivery