# Early life house dust mite allergens, childhood mite sensitization and respiratory outcomes.

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**Abstract**

**Background:** Exposure to indoor allergens during early life may play a role in the development of the immune system and inception of asthma.

**Objective:** To describe the house dust mite (HDM) allergen concentrations in bedroom dust during early life and to evaluate its associations with HDM sensitization, wheezing and asthma, from birth to school age, in 5 geographically spread European birth cohorts.

**Methods:** We included 4,334 children from INMA-Menorca (Spain), BAMSE (Sweden), LISAplus and MAS (Germany), and PIAMA-NHS (Netherlands). Dust samples were collected from bedrooms during early life and analyzed for *Dermatophagoides pteronyssinus* (*Der p1*) and *Dermatophagoides farinae* (*Der f1*). HDM concentrations were divided into 4 categories. Sensitization was determined by specific IgE. Wheezing and asthma information up to 8/10 years was collected through questionnaires. We performed mixed-effects logistic regression models and expressed associations as odds ratios with 95% confidence intervals.

**Results:** HDM concentrations varied across cohorts. Mean allergen concentrations were highest in INMA-Menorca (Geometric mean (GM) *Der p1*=3.3µg/g) and LISAplus (GM *Der f1*=2.1 µg/g) and lowest in BAMSE (GM *Der p1*=0.1µg/g, *Der f1*=0.3µg/g). Moderate and high HDM concentrations were significantly (p-values<0.05) associated with 50% to 90% higher prevalence of HDM sensitization. No significant associations were observed with respiratory outcomes.

**Conclusion:** Our study based on geographically spread regions, a large sample size and a wide range of allergen concentration shows that HDM allergen concentrations

vary across regions; and that exposure during early life plays a role in the development of allergic sensitization but not in the development of respiratory outcomes.

## Introduction

Previous studies observed positive associations between HDM allergen levels and HDM sensitization (1–7). However, a direct association of HDM allergen levels with respiratory health has not been confirmed (1,2,8–11). Intervention studies evaluating the effects of the use of mite-impermeable mattress covers showed inconsistent results for asthma symptoms (12–17). A positive family history of allergy (high risk) or health conditions may increase susceptibility to HDM exposure. Currently, no significant associations in high risk children have been shown (1,2,9). Nevertheless, indication of increased morbidity in asthmatic and sensitized children in relation to HDM exposure has been reported (18).

Associations of early life allergen exposure with sensitization or respiratory health may only be evident at exposure to certain HDM concentration (5,19). Geographical variation in HDM concentrations and in the prevalence of allergic and respiratory disorders exist (20–23). However, previous studies covered a limited range of exposure, based its results on different exposure cut-offs and did not include geographically spread regions. The present study includes harmonized data of five geographically spread European birth cohorts involved in the Global Allergy and Asthma European Network (GA2LEN) initiative (24) that followed a large number of children up to the age of 8/10 years. Our study aims to describe the allergen concentrations measured in home dust of children in the first months of life, and to evaluate the associations of early life HDM allergen

concentrations in indoor dust with HDM sensitization, respiratory symptoms and asthma from birth to school age.

## Methods

### Study design and population

We included five birth cohorts involved in the GA2LEN initiative (24) with dust samples collected during early life (2 to 6 months old): INMA (INfancia y Medio Ambiente [Environment and Childhood])-Menorca (25) in Spain, BAMSE (Stockholm Children Children Allergy and Environmental Prospective Birth Cohort Study) (26) in Sweden, LISAplus (influence of life-style factors on the development of the immune system and allergies in East and West Germany plus the influence of traffic emissions and genetics) (27) and MAS (Multicentre Allergy Study) (28) in Germany, and PIAMA-NHS (Prevention and Incidence of Asthma and Mite Allergy–Natural History Study) (29) in the Netherlands. All studies were approved by their local Institutional Ethics Committees and all participants' parents provided written informed consent. We included 4,334 children with measured HDM allergen levels (*Dermatophagoides pteronyssinus* (*Der p1*) or *Dermatophagoides farinae* (*Der f1*)) in home dust samples collected during early life, and with a follow-ups until age 8 years (PIAMA-NHS and BAMSE) or 10 years (INMA-Menorca, LISAplus and MAS.

### Dust collection, extraction and analyses

Bedroom dust samples were collected using vacuum cleaners equipped with ALK filterholders containing paper filters on the child's mattress (INMA-Menorca, LISAplus,

and PIAMA-NHS), bedroom floor (MAS) or parents' mattress (BAMSE), stored at -20°C for up to 6 years and analyzed as described elsewhere (5,27,30–32). In brief, extracts were analysed for *Der p1* and *Der f1* (in INMA only *Der p1*) using reagents for a sandwich enzyme immunoassay, purchased from Indoor Biotechnologies (Cardiff, UK). Levels were measured in µg of allergen per mL of extract and the lower limit of detection (LOD) were 5µg/mL for *Der p1* in INMA-Menorca, 5µg/g for *Der p1* and *Der f1* in BAMSE, 4µg/mL for *Der p1* and *Der f1* in LISAplus and MAS, and 8µg/mL for *Der p1* and 4µg/mL for *Der f1* in PIAMA-NHS. The levels were converted into concentrations in the original dust and expressed as µg/g of dust. To evaluate the effects of total exposure to both mite species (*Der p1* and *Der f1*), we created a new variable that was the sum of the concentrations of *Der p1* and *Der f1* (*Der p1*+*Der f1*).

**HDM sensitization, wheezing and asthma**

Health questionnaires were administered at regular intervals and included standard questions on respiratory health. Children's blood samples were taken once in INMA-Menorca, and twice in the other cohorts. The timing of follow-ups is shown in Table E1 in the Online Repository.

Sensitization to HDM was defined as sIgE (specific Immunoglobulin E) to *Der p*>0.35kU/L in children's blood. Because of the high cross-reactivity between sIgE to *Der p* and sIgE to *Der f* (33), sIgE to *Der f* was not measured. Two variables were created: sensitization up to 6 years and after age 6 years. Information on sIgE was available for 59% and 41% of the study population, respectively.

Information on wheezing from birth to 8/10 years (“In the last 12 months, did your child experience whistling or wheezy sounds in their chest while breathing?”) was pooled in one four-category variable: never wheezing (reference category) included children who never reported wheezing in the first 8/10 years of life; early transient wheezing included children who ever reported wheezing before 6 years old but not thereafter, late-onset wheezing included children who reported wheezing after 6 years old but not earlier; and persistent wheezing included children who reported wheezing in each of the following periods: birth to 2 years, 3 to 6 years, and after 6 years old.

Asthma was defined by the presence of two out of the following three conditions: physician-diagnosed asthma, current parental-reported wheezing (last 12 months) and current asthma medication intake (last 12 months). In the 5 cohorts, the questions for the three conditions were: “in the last 12 months, was your child diagnosed with asthma?”, “In the last 12 months, did your child experience whistling or wheezy sounds in their chest while breathing?” and “In the last 12 months, did your child receive treatment for their asthma symptoms?” The asthma definition was applied to each follow-up and cohort. Based on this, we created two asthma variables: up to 6 years (i.e. ever asthma in follow-ups at ages 1 to 6) and after 6 years (i.e. ever asthma in follow-ups after age 6).

**Potential confounders and effect modifiers**

The administered questionnaires included socio-demographic, health and environmental information (sex, number of siblings at birth, parental education, parental asthma and/or allergic rhinitis, and maternal smoking during pregnancy). Children of parents (at least one) with asthma and/or allergic rhinitis were considered high risk children.

**Statistical analyses**

Samples with HDM allergen concentrations below the LOD were assigned a value of 2/3 of the lowest concentration observed among the samples with detectable allergens. The distributions of allergen concentrations were right-skewed and described using geometric means (GM) and their 95% confidence intervals (CI). To assess the functional relationship of the log-transformed allergen concentrations with HDM sensitization, wheezing and asthma, we used cohort-adjusted Generalized Additive Models (GAM). The results showed deviations from a linear fit (p-values<0.05). Therefore, we computed three four-category variables, one for each exposure (*Der p1*, *Der f1* and *Der p1+Der f1*).

It was not possible to set a common LOD cut-off due to the differences in the LOD across cohorts. Consequently, we defined the cut-off for the lowest exposure category as the highest concentration among all samples classified as <LOD: *Der p1*=0.12µg/g, *Der f*=0.07µg/g and *Der p1+Der f1*=0.19µg/g (reference category). The shape of the GAM plots determined the cut-off for the medium exposure category: 0.4µg/g. The cut-off for the highest exposure category was based on the threshold of 2µg/g suggested by Sporik et al. (7) for *Der p1* and applied to the three exposure variables.

To assess the adjusted associations between the outcomes and the exposure variables, we performed multivariable mixed-effects logistic and multinomial regression models, using cohort as random intercept. Potential confounders were a-priori identified from the literature and selected based on their relationship with the outcome and exposure variables. Each exposure variable was included separately in the models. Associations are reported as odds ratios (OR) with CI.

In sensitivity analyses, we evaluated potential modifications of the HDM effects by high risk children and sex by stratified analyses. Also, we included cohort as a fixed effect to control for differences between cohorts. Moreover, we explored to what extent the associations observed between allergens and health outcomes were driven by only one cohort by excluding one cohort at a time from the models. Furthermore, we restricted the analysis to cohorts with >10% of the samples >LOD or in the reference category of HDM concentration (LISAplus, MAS, PIAMA-NHS).

Data analysis was conducted with STATA SE 10.0 statistical software (Stata Corporation, College Station, TX, USA).

**Results**

Approximately half of the allergen levels were below the LOD and there was geographical variation in allergen concentrations. INMA-Menorca had the lowest number

of samples <LOD for *Der p1*, while more than 95% of the samples in BAMSE were below the LOD for *Der p1* and *Der f1*. The GM of *Der p1* was highest in INMA-Menorca and for *Der f1* and *Der f1+Der p1* in LISAplus. BAMSE and MAS showed the lowest GM of the three exposure variables (Table 1).

Table 2 shows the description of the population and prevalence of health outcomes by cohort. About half of the parents were highly educated in all cohorts except in INMA-Menorca (23%). In INMA-Menorca the percentage of mothers who smoked during pregnancy was highest and the prevalence of parental asthma and/or allergic rhinitis lowest. The prevalence of HDM sensitization at both periods was lowest in BAMSE, the prevalence of persistent wheezing was highest in MAS, and asthma at both periods was most prevalent in BAMSE.

Significant deviations from a linear fit (p-values < 0.05) were observed for associations of *Der f1* with HDM sensitization, and of *Der p1* with persistent wheezing and asthma after 6 years (Figure E1 in the Online Repository). Table 3 shows the adjusted associations between allergen concentrations and health outcomes. High concentrations were associated with increased prevalence of HDM sensitization at both time periods. At allergen concentration ≥0.4 µg/g, the odds of HDM sensitization up to 6 years was 50% to 80% higher (p-values<0.05) compared to the respective reference categories. The odds of HDM sensitization after 6 years was 50% to 90% higher (p-values<0.05) at allergen concentrations ≥0.07 µg/g compared to the reference category. No significant associations with HDM concentrations were found for wheezing or asthma.

Overall, no significant differences were observed between high risk and non-high risk children and between girls and boys for sensitization or wheezing. For asthma after 6 years, a statistically significant inverse association was observed in non-high risk children (Tables E2 and E3 in the Online Repository). The effect estimates when using cohort as a fixed effect (data not shown) were not different from those with cohort as a random effect (Table 3). The results from further sensitivity analyses excluding one cohort at a time (Table E4 in the Online Repository) or including cohorts with >10% of their samples >LOD or in the reference category (data not shown) were not different from those presented in Table 3.

## Discussion

Our study is the largest published to date and the first comprising geographically spread regions with a wide range of HDM allergen concentrations. We show that the concentration of HDM allergens in home dust varied widely across cohorts. The highest mean *Der p1* concentration was observed in the Mediterranean cohort (INMA-Menorca) and the lowest *Der p1* and *Der f1* concentration in the Scandinavian cohort (BAMSE). High concentrations of HDM allergens were associated with a higher prevalence of HDM sensitization up to 6 years old and after; no significant associations with HDM allergens were observed for persistent wheezing or asthma. We found no indication of a modification of the HDM effect by parental atopy or sex.

During the nineties, some on-going (birth) cohort studies, including the ones in the present study, started with the aim to unravel the role of allergens and other environmental factors in the development of atopy and respiratory disorders. The concentrations of HDM in indoor dust shown in this study have been separately reported in previous publications (8,21,27,31,32). Besides these, further studies in other regions have determined HDM allergen concentrations in homes. The mean concentration of *Der p1* that we observe for INMA-Menorca is among the highest reported to date, similar to those reported in other humid and rural regions (34,35). In line with our results in BAMSE, studies performed in dry and cold countries such as the Scandinavian countries, observed extremely low HDM concentrations.(22,36). In addition to geographical variation, differences in the HDM concentration according to the sampling location in the same home have been reported (7,8,20,21,30,37). Overall, mattress samples show higher concentrations compared to floor samples. In our study, MAS samples were collected from floors and LISAplus samples from mattresses. Sampling location rather than region may explain the higher HDM concentrations in LISAplus compared with MAS samples.

Consistent with our results, longitudinal studies evaluating the associations between early life HDM concentrations in home dust and child health observed positive non-linear associations with HDM sensitization (1,5–7,35). Because allergic sensitization is related to asthma, several studies, including previous INMA-Menorca, MAS, and PIAMA-NHS studies at earlier ages (1,8–10), evaluated the potential associations between HDM concentrations and respiratory health. Like our study, most studies did not observe

statistically significant associations between respiratory health outcomes and early life HDM allergen concentration.(1,3,9,10,19,38).

Heterogeneity in the study design, population characteristics, age, and range of HDM allergen concentration make it difficult to compare association estimates obtained in the previous studies. The differences in the HDM concentrations across studies combined with their non-linear relationship with the outcomes resulted in the use of different cut-offs per study. Consequently, different parts of the association curve may have been explored. Moreover, geographical differences in allergen exposure and respiratory health have not been considered. Our study includes indoor dust measurements of 4,334 homes in 5 European birth cohorts and health outcomes up to the age of 10 years. The home dust samples were collected during early life, following similar protocols, and the questionnaires recalled similar information on child and parents' health in all cohorts. The participating cohorts are located in northern, mid, and southern European regions with different climates, HDM concentrations and prevalence of health outcomes which allows evaluating a wider spectrum in the association curve and considering geographical variation. In addition, our study is based on general population and homogeneous population in terms of ethnicity, enabling us to investigate associations in high and low risk children separately. Moreover, the repeated data collection from early life to school age permits the evaluation of associations at two different periods during childhood.

However, some limitations need to be considered when interpreting our results. Differences across cohorts in dust sampling and analyses and HDM concentrations exist. Samples from different origins (floor dust vs mattress dust) may be the cause for differences in concentrations in the German cohorts. In addition, the analyses of the dust samples were conducted after different storage time and in different laboratories and resulted in slightly different LOD. Nevertheless, the sampling protocols and laboratory techniques were similar across cohorts. In addition, HDM allergen concentrations in stored samples have shown to be stable across time (39). Moreover, the coefficients resulting from models including cohort as a fixed effect were not different from those including cohort as a random effect, indicating that the small differences across cohorts do not influence our results.

In addition, we set common cut-offs for HDM allergen concentrations to assess associations with health outcomes. However, due to the differences in the range of exposure across cohorts, the percentage of children included in each allergen category varied per cohort. Nevertheless, sensitivity analyses excluding one cohort at a time or cohorts with extreme HDM concentrations (INMA-Menorca and BAMSE) did not show significant differences in the results.

Furthermore, other issues regarding allergen measurements and health outcomes need to be considered. Dust samples were collected at different seasons. Nevertheless, sensitivity analyses including season in the statistical models did not change our results. In addition, only Der p1 was measured in the INMA-Menorca dust samples. Results from

the ECRHS study suggested that *Der p1* levels, rather than *Der f1*, are increased by high winter temperatures (40). Therefore, the contribution of *Der p1* to the overall HDM exposure may be bigger in Menorca than in the other cohorts. In addition, the effect estimates obtained for Der p1 were not different from those of Der f1, also when excluding INMA-Menorca. This suggests that the inclusion of *Der f1* measurements in INMA-Menorca would not have modified our conclusions.

Moreover, information on serum sIgE was available for less than 60% of the children. In MAS, children with serum sIgE at the age of 5 years reported more early transient wheezing compared with those without information at this age. In LISAplus, the percentage of children with information on sIgE after 6 years was very low (19%) and, among these children, the prevalence of parental asthma and/or atopy, persistent wheezing and asthma was significantly higher. Asthma was also more prevalent among children with serum sIgE information in BAMSE. No differences were observed between children with and without sIgE measurements in PIAMA and INMA-Menorca. The differences observed among MAS, LISAplus and BAMSE indicate a potential for selection bias. However, the association estimates for sensitization did not change after excluding these 3 cohorts from the analyses. Regarding asthma, the definitions used do not consider later or previous asthma reports. Following the definition we used for wheezing, we created a more detailed 4-category asthma variable that takes temporality into account. The results were not different when using the 4-category asthma variable, but statistical power was limited due to the low number of cases included in some categories.

Potential explanations for the non-significant associations reported for asthma and wheezing include the concomitant exposure to other environmental factors and changes over time in the concentrations of allergens or other exposures. The quantity of microbial markers (endotoxin, extracellular polysaccharides or β-glucan) (41,42) and, more importantly, the diversity of microbes (43) in indoor dust have an impact in the development of allergies and asthma. Lynch et al. (11) evaluated the health effects of early life concomitant exposure to high bacterial richness and allergen concentrations and reported an inverse association with respiratory symptoms and/or atopy at the age of 3 years. However, their analyses for concomitant exposures did not include HDM allergens because they did not observe significant associations between HDM allergens and bacterial richness. In addition, the long term implications of these results are not clear. The association between early life exposure to microbial markers (endotoxin, extracellular polysaccharides (EPS) or β-glucan) and asthma up to the age of 10 years has been investigated in INMA, a sub-sample of LISAplus participants and in the PIAMA intervention study participants (44). Like for the exposure to allergens reported in our study, no significant associations were observed between early life exposure to high concentrations of microbial markers and asthma.

Furthermore, allergen exposure may change over time (45), and current rather than early life exposure may determine the prevalence of respiratory symptoms after the age of 6 years. In addition, parents of allergic and/or asthmatic children may have adopted avoidance measures in the home to reduce the occurrence of symptoms. In our study,

we did not have information on the concentrations of allergens at school-age, therefore we cannot exclude the possibility that the lack of association with respiratory outcomes is related to changes in the exposure over time.

In conclusion, our study confirms the findings reported in previous smaller studies. We observed geographical variation in the concentrations of HDM allergens in home dust, with lower levels in colder and drier areas such as Scandinavian regions and higher in more humid and warm areas, like the Mediterranean regions. In addition, we show a positive association of HDM allergen levels with sensitization at pre-school and school ages, and a lack of association with wheezing or asthma up to the age of 8/10 years. For the first time, these results are confirmed in a large longitudinal study including 5 geographically spread regions with different meteorological conditions, taking advantage of a wide range of HDM allergen concentration, and using common cut-offs in the 5 regions to define exposure.

**Author contributions:** L Casas was involved in the study design, performed the statistical analyses and wrote the manuscript, J Sunyer and M Torrent were involved in the study design and revision of the manuscript, R García-Esteban contributed to the statistical analyses and revised the manuscript, all other authors carefully revised the manuscript.

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## TABLES

Table 1. Description of the allergen concentrations (µg/g of dust) per cohort.

| | **INMA-Menorca n=468** | **BAMSE n=508** | **LISAplus n=1617** | **MAS n=838** | **PIAMA-NHS n=903** |
|---|---|---|---|---|---|
| ***Der p1* (µg/g)** | | | | | |
| **<LOD: n (%)** | 12 (2.6) | 487 (95.9) | 992 (61.4) | 274 (32.8) | 589 (65.3) |
| **GM (95%CI)[a]** | 3.26 (2.83-3.75) | 0.13 (0.09-0.19) | 1.7 (1.5-1.93) | 0.21 (0.18-0.25) | 1.49 (1.32-1.68) |
| **GM (95%CI)[b]** | 2.91 (2.5-3.38) | 0.04 (0.04-0.04) | 0.12 (0.11-0.14) | 0.05 (0.04-0.06) | 0.28 (0.26-0.31) |
| **Categories: n (%)** | | | | | |
| **<0.12 µg/g** | 15 (3.2) | 500 (98.4) | 995 (61.5) | 524 (62.7) | 589 (65.4) |
| **≥0.12 to <0.4 µg/g** | 32 (6.8) | 5 (1.0) | 114 (7.1) | 123 (14.7) | 24 (2.7) |
| **≥0.4 to <2 µg/g** | 145 (31.0) | 3 (0.6) | 272 (16.8) | 100 (12.0) | 185 (20.6) |
| **≥2 µg/g** | 276 (59) | 0 (0) | 236 (14.6) | 89 (10.7) | 102 (11.3) |
| ***Der f1* (µg/g)** | | | | | |
| **<LOD: n (%)** | - | 485 (95.5) | 574 (35.5) | 261 (31.2) | 474 (52.9) |
| **GM (95%CI)[a]** | - | 0.32 (0.17-0.6) | 2.08 (1.88-2.3) | 0.18 (0.16-0.2) | 1.1 (0.97-1.25) |
| **GM (95%CI)[b]** | - | 0.02 (0.02-0.02) | 0.54 (0.48-0.6) | 0.06 (0.05-0.07) | 0.25 (0.23-0.28) |
| **Categories: n (%)** | | | | | |
| **<0.07 µg/g** | - | 488 (96.1) | 575 (35.6) | 472 (56.3) | 474 (53.5) |
| **≥0.07 to <0.4 µg/g** | - | 10 (2.0) | 139 (8.6) | 196 (23.4) | 85 (9.6) |
| **≥0.4 to <2 µg/g** | - | 8 (1.6) | 462 (28.6) | 111 (13.3) | 221 (24.9) |
| **≥2 µg/g** | - | 2 (0.4) | 441 (27.3) | 59 (7.0) | 106 (12.0) |
| ***Der p1* + *Der f1* (µg/g)** | | | | | |
| **GM (95%CI)[b]** | - | 0.06 (0.06-0.06) | 0.97 (0.87-1.08) | 0.21 (0.18-0.24) | 0.71 (0.64-0.78) |
| **Categories: n (%)** | | | | | |
| **<0.19 µg/g** | - | 492 (96.9) | 500 (30.9) | 413 (49.4) | 384 (43.5) |
| **≥0.19 to <0.4 µg/g** | - | 4 (0.8) | 92 (5.7) | 105 (12.6) | 23 (2.6) |
| **≥0.4 to <2 µg/g** | - | 9 (1.8) | 444 (27.5) | 168 (20.1) | 264 (29.9) |
| **≥2 µg/g** | - | 3 (0.6) | 581 (35.9) | 150 (17.9) | 212 (24.0) |

LOD: limit of detection. The LOD is 5 µg/mL for *Der p1* in INMA-Menorca, 5 µg/g for *Der p1* and *Der f1* in BAMSE, 4 µg/mL for *Der p1* and *Der f1* in LISAplus and MAS, and 8 µg/mL for *Der p1* and 4 µg/mL for *Der f1* in PIAMA-NHS. [a] Excludes samples with values <LOD. [b] Includes samples with values <LOD. These samples were given a value equal to 2/3 of the minimum concentration detected in each cohort.

Table 2. Description (n and %) of the study population characteristics and health outcomes (HDM sensitization, wheezing and asthma) in INMA-Menorca, BAMSE, LISAplus, MAS, PIAMA-NHS.

| | INMA-Menorca n=468 | BAMSE n=508 | LISAplus n=1617 | MAS n=838 | PIAMA-NHS n=903 |
|---|---|---|---|---|---|
| **Population characteristics** | | | | | |
| **Sex (female)** | 230 (49.2) | 239 (47.1) | 800 (49.5) | 404 (48.2) | 421 (48.9) |
| **Siblings at birth** | 236 (50.4) | 269 (53.0) | 685 (42.4) | 337 (40.2) | 417 (48.4) |
| **Parental education:** | | | | | |
| **High** | 111 (23.9) | 243 (47.8) | 1073 (67) | 474 (57.1) | 430 (50.2) |
| **Medium** | 132 (28.5) | 151 (29.7) | 476 (29.7) | 185 (22.3) | 325 (37.9) |
| **Low** | 221 (47.6) | 114 (22.4) | 52 (3.3) | 171 (20.6) | 102 (11.9) |
| **Maternal smoking during pregnancy** | 175 (37.4) | 83 (16.3) | 218 (13.6) | 165 (21) | 157 (18.4) |
| **Parental asthma and/or allergic rhinitis*** | 178 (38.3) | 264 (52.2) | 831 (54.9) | 393 (47.4) | 410 (48.2) |
| **Health outcomes** | | | | | |
| **Sensitization to HDM** | | | | | |
| **Up to 6 years old** | 41 (11.8) | 6 (1.6) | 107 (13.0) | 78 (11.9) | 51 (13.6) |
| **After 6 years old** | - | 16 (4.5) | 177 (56.9) | 88 (15.2) | 116 (22.6) |
| **Wheezing** | | | | | |
| **Never** | 206 (49.5) | 217 (50.4) | 701 (61.2) | 324 (54.4) | 446 (57.3) |
| **Early transient** | 146 (35.1) | 165 (38.3) | 262 (22.9) | 109 (18.3) | 249 (32.0) |
| **Late** | 10 (2.4) | 3 (0.7) | 64 (5.6) | 63 (10.6) | 15 (1.9) |
| **Persistent** | 54 (13.0) | 46 (10.7) | 118 (10.3) | 100 (16.8) | 68 (8.7) |
| **Asthma** | | | | | |
| **Up to 6 years old** | 36 (7.9) | 154 (31.0) | 45 (2.9) | 59 (7.5) | 173 (19.5) |
| **After 6 years old** | 26 (6.2) | 71 (16.0) | 78 (6.7) | 59 (11.3) | 86 (10.6) |

Table 3. Adjusted* associations (odds ratios (OR) and 95% confidence intervals (CI)) between early life allergen concentrations (µg/g) and HDM sensitization up to 6 years old and after, persistent wheezing, and asthma up to 6 years old and after in all the study population of INMA-Menorca, BAMSE, LISAplus, MAS and PIAMA-NHS (n=4,334).

| | ***Der p1*** | ***Der f1*[b]** | ***Der p1 + Der f1*[b]** |
|---|---|---|---|
| **Sensitization to HDM** | | | |
| **≤6 years old** | | | |
| **<Low[a]** | 1 | 1 | 1 |
| **≥Low[a] to <0.4 µg/g** | 1.4 (0.8-2.2) | 1.4 (0.9-2.2) | 1.0 (0.5-1.9) |
| **0.4 to <2 µg/g** | **1.5 (1.0-2.1)** | **1.6 (1.1-2.3)** | **1.5 (1.0-2.2)** |
| **≥2 µg/g** | **1.5 (1.1-2.3)** | **1.5 (1.0-2.3)** | **1.8 (1.2-2.6)** |
| **>6 years old[b]** | | | |
| **<Low[a]** | 1 | 1 | 1 |
| **≥Low[a] to <0.4 µg/g** | 0.9 (0.5-1.4) | **1.9 (1.3-2.8)** | 1.2 (0.7-2.2) |
| **0.4 to <2 µg/g** | **1.5 (1.1-2.2)** | **1.5 (1.1-2.1)** | **1.5 (1.1-2.1)** |
| **≥2 µg/g** | 1.3 (0.9-1.9) | **1.8 (1.2-2.7)** | **1.7 (1.2-2.3)** |
| **Persistent wheezing** | | | |
| **<Low[a]** | 1 | 1 | 1 |
| **≥Low[a] to <0.4 µg/g** | 1.1 (0.7-1.8) | 1.2 (0.8-1.8) | 1.3 (0.8-2.2) |
| **0.4 to <2 µg/g** | 0.9 (0.7-1.3) | 1.1 (0.8-1.5) | 1.1 (0.8-1.5) |
| **≥2 µg/g** | 0.8 (0.5-1.1) | 1.1 (0.8-1.6) | 0.9 (0.7-1.3) |
| **Asthma** | | | |
| **≤6 years old** | | | |
| **<Low[a]** | 1 | 1 | 1 |
| **≥Low[a] to <0.4 µg/g** | 1.4 (0.9-2.3) | 1.2 (0.8-1.8) | 1.5 (0.9-2.6) |
| **0.4 to <2 µg/g** | 1.1 (0.8-1.6) | 1.2 (0.8-1.6) | 1.4 (1.0-1.9) |
| **≥2 µg/g** | 1.0 (0.7-1.5) | 1.2 (0.8-1.8) | 1.1 (0.8-1.6) |
| **>6 years old** | | | |
| **<Low[a]** | 1 | 1 | 1 |
| **≥Low[a] to <0.4 µg/g** | 1.1 (0.6-1.8) | 1.0 (0.7-1.6) | 1.3 (0.8-2.3) |
| **0.4 to <2 µg/g** | 1.1 (0.8-1.6) | 1.0 (0.7-1.4) | 1.1 (0.8-1.6) |
| **≥2 µg/g** | 0.7 (0.4-1.0) | 1.1 (0.7-1.6) | 1.0 (0.7-1.4) |

*Adjusted for sex, nº of siblings at birth, parental education, maternal smoking during pregnancy and parental asthma or atopy. Cohort was included as random intercept. [a] For *Der p1* "Low" was equal to 0.12 µg/g, for *Der f1* "Low" was 0.07 µg/g and for *Der p1 + Der f1* "Low" was 0.19 µg/g. [b] The INMA-Menorca cohort was not included (included: n=3,866). Bold indicates p-value<0.05.

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Table E1. Follow-up periods per cohort (questionnaire administration and blood samples).

| | Up to 6 years old | | | | | | After 6 years old | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Age (years)** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** |
| **Questionnaires (respiratory health)** | | | | | | | | | | |
| **INMA-Menorca** | X | X | X | X | | X | | | | X |
| **BAMSE** | X | X | | X | | | | X | | |
| **LISAplus** | X | X | | X | | X | | | | X |
| **MAS** | X | X | X | X | X | X | X | X | X | X |
| **PIAMA-NHS** | X | X | X | X | X | X | X | X | | |
| **Blood samples (HDM sensitization)** | | | | | | | | | | |
| **INMA-Menorca** | | | | X | | | | | | |
| **BAMSE** | | | | X | | | | X | | |
| **LISAplus** | | | | | | X | | | | X |
| **MAS** | | | | | X | | X | | | |
| **PIAMA-NHS** | | | | X | | | | X | | |

HDM: house dust mite

Table E2. Adjusted* associations (odds ratios (OR) and 95% confidence intervals (CI)) between early life allergen concentrations (µg/g) and HDM sensitization up to 6 years old and after, persistent wheezing, and asthma up to 6 years old and after in all the study population of INMA-Menorca, BAMSE, LISAplus, MAS and PIAMA-NHS, stratified by high risk children.**

| | **High risk children (n=2,076)** | | | **Non-high risk children (n=2,258)** | | |
|---|---|---|---|---|---|---|
| | ***Der p1*** | ***Der f1*[b]** | ***Der p1 + Der f1*[b]** | ***Der p1*** | ***Der f1*[b]** | ***Der p1 + Der f1*[b]** |
| **Sensitization to HDM** | | | | | | |
| **≤6 years old** | | | | | | |
| **≥Low[a] to <0.4 µg/g** | 0.9 (0.4-1.8) | 1.6 (0.9-2.8) | 0.9 (0.4-2.1) | **3.0 (1.6-5.7)** | 1.4 (0.7-2.9) | 1.2 (0.4-3.7) |
| **0.4 to <2 µg/g** | 1.5 (1.0-2.3) | **1.7 (1.1-2.7)** | **1.8 (1.1-2.9)** | **1.9 (1.1-3.4)** | **1.5 (0.7-2.9)** | 1.3 (0.6-2.8) |
| **≥2 µg/g** | 1.4 (0.8-2.3) | 1.1 (0.6-2.0) | 1.5 (0.9-2.5) | **2.6 (1.6-4.3)** | 2.4 (1.3-4.8) | **2.6 (1.3-5.0)** |
| **>6 years old** | | | | | | |
| **≥Low[a] to <0.4 ng/g** | 0.8 (0.4-1.6) | **2.3 (1.3-3.9)** | 1.7 (0.8-3.6) | 1.0 (0.5-2.1) | **1.5 (0.8-2.7)** | 0.8 (0.3-1.9) |
| **0.4 to <2 µg/g** | 1.4 (0.9-2.3) | **2.1 (1.3-3.3)** | **1.9 (1.2-2.9)** | **1.7 (1.0-2.9)** | **1.0 (0.6-1.7)** | **1.2 (0.7-1.9)** |
| **≥2 µg/g** | 1.5 (0.9-2.7) | **2.2 (1.3-3.8)** | **2.0 (1.3-3.3)** | 1.1 (0.6-2.1) | **1.5 (0.9-2.8)** | **1.3 (0.8-2.2)** |
| **Persistent wheezing** | | | | | | |
| **≥Low[a] to <0.4 ng/g** | 1.1 (0.6-2.0) | 1.1 (0.7-1.9) | 1.6 (0.8-3.1) | 1.3 (0.7-2.6) | 1.4 (0.7-2.8) | 1.1 (0.5-2.6) |
| **0.4 to <2 µg/g** | 1.0 (0.7-1.5) | 1.1 (0.7-1.7) | 1.2 (0.8-1.8) | 1.0 (0.6-1.6) | 1.0 (0.6-1.7) | 0.9 (0.5-1.6) |
| **≥2 µg/g** | 0.6 (0.4-1.0) | 0.9 (0.5-1.4) | 0.8 (0.5-1.3) | 1.1 (0.7-1.8) | **1.8 (1.0-3.1)** | 1.2 (0.7-2.1) |
| **Asthma** | | | | | | |
| **≤6 years old** | | | | | | |
| **≥Low[a] to <0.4 µg/g** | 1.3 (0.7-2.4) | 1.3 (0.7-2.1) | 1.9 (0.9-3.7) | 1.5 (0.8-3.1) | 1.1 (0.6-2.1) | 1.0 (0.4-2.5) |
| **0.4 to <2 µg/g** | 1.4 (0.9-2.2) | 1.4 (0.9-2.2) | 1.5 (1.0-2.3) | 0.8 (0.5-1.4) | 0.8 (0.5-1.4) | 1.1 (0.7-1.9) |
| **≥2 µg/g** | 1.2 (0.8-2.0) | 1.4 (0.9-2.4) | 1.5 (1.0-2.3) | 0.7 (0.4-1.3) | 0.9 (0.5-1.8) | 0.7 (0.4-1.3) |
| **>6 years old** | | | | | | |
| **≥Low[a] to <0.4 µg/g** | 1.1 (0.6-2.1) | 1.3 (0.8-2.3) | 1.8 (0.9-3.5) | 0.9 (0.4-2.0) | 0.7 (0.3-1.6) | 0.8 (0.3-2.2) |
| **0.4 to <2 µg/g** | 1.1 (0.7-1.8) | 1.4 (0.9-2.2) | 1.5 (1.0-2.3) | 1.1 (0.6-1.8) | **0.4 (0.2-0.8)** | 0.6 (0.4-1.2) |
| **≥2 µg/g** | 0.7 (0.4-1.2) | 1.1 (0.6-1.9) | 1.0 (0.6-1.6) | 0.6 (0.3-1.1) | 1.0 (0.6-1.9) | 0.9 (0.5-1.5) |

*Adjusted for sex, nº of siblings at birth, parental education,and maternal smoking during pregnancy. Cohort was included as random intercept. The reference category was below the "Low" cut-off. ** High risk children: children with report of parental asthma and/or allergic rhinitis. [a] For Der p1 "Low" was equal to 0.12 µg/g, for Der f1 "Low" was 0.07 µg/g and for Der p1 + Der f1 "Low" was 0.19 µg/g. [b]The INMA-Menorca cohort was not included (included: n=3,866). Bold indicates p-value<0.05.

Table E3. Adjusted* associations (odds ratios (OR) and 95% confidence intervals (CI)) between early life allergen concentrations (µg/g) and HDM sensitization up to 6 years old and after, persistent wheezing, and asthma up to 6 years old and after in all the study population of INMA-Menorca, BAMSE, LISAplus, MAS and PIAMA-NHS, stratified by sex.

| | **Girls (n=2,094)** | | | **Boys (n=2,198)** | | |
|---|---|---|---|---|---|---|
| | ***Der p1*** | ***Der f1*[b]** | ***Der p1 + Der f1*[b]** | ***Der p1*** | ***Der f1*[b]** | ***Der p1 + Der f1*[b]** |
| **Sensitization to HDM** | | | | | | |
| **≤6 years old** | | | | | | |
| **≥Low[a] to <0.4 µg/g** | **2.6 (1.2-5.4)** | 1.5 (0.7-3.2) | 0.7 (0.2-3.3) | 1.0 (0.5-1.8) | 1.5 (0.9-2.6) | 1.1 (0.5-2.3) |
| **0.4 to <2 µg/g** | **2.6 (1.5-4.5)** | **1.8 (1.0-3.3)** | **2.5 (1.3-4.7)** | 1.1 (0.6-1.7) | 1.6 (1.0-2.6) | 1.3 (0.8-2.1) |
| **≥2 ng/g** | **2.1 (1.2-3.9)** | 1.7 (0.9-3.4) | **2.8 (1.5-5.3)** | 1.3 (0.8-2.1) | 1.4 (0.8-2.5) | 1.5 (0.9-2.4) |
| **>6 years old** | | | | | | |
| **≥Low[a] to <0.4 µg/g** | 1.1 (0.5-2.6) | **2.0 (1.0-3.7)** | 2.0 (0.8-4.9) | 0.7 (0.4-1.4) | **1.8 (1.1-3.1)** | 1.0 (0.5-1.9) |
| **0.4 to <2 µg/g** | **2.5 (1.5-4.2)** | **2.0 (1.2-3.4)** | **1.9 (1.1-3.3)** | 1.1 (0.7-1.7) | 1.3 (0.9-2.1) | 1.3 (0.8-2.0) |
| **≥2 µg/g** | 1.7 (0.9-3.2) | **2.0 (1.1-3.7)** | **2.3 (1.3-4.0)** | 1.1 (0.7-1.9) | 1.7 (1.0-2.9) | 1.3 (0.9-2.1) |
| **Persistent wheezing** | | | | | | |
| **≥Low[a] to <0.4 µg/g** | 1.5 (0.8-2.9) | 0.6 (0.3-1.3) | 1.1 (0.4-2.8) | 1.0 (0.5-1.8) | **1.7 (1.0-2.7)** | 1.5 (0.8-2.8) |
| **0.4 to <2 µg/g** | 1.0 (0.6-1.6) | 1.2 (0.7-1.9) | 1.1 (0.7-1.7) | 0.9 (0.6-1.3) | 1.0 (0.6-1.5) | 1.1 (0.7-1.7) |
| **≥2 µg/g** | 0.6 (0.3-1.0) | 1.2 (0.7-2.1) | 0.9 (0.5-1.5) | 0.9 (0.6-1.5) | 1.0 (0.6-1.7) | 0.9 (0.6-1.4) |
| **Asthma** | | | | | | |
| **≤6 years old** | | | | | | |
| **≥Low[a] to <0.4 µg/g** | 1.2 (0.5-2.6) | 0.8 (0.4-1.7) | 1.0 (0.4-2.8) | 1.5 (0.8-2.7) | 1.5 (0.9-2.4) | 1.7 (0.9-3.4) |
| **0.4 to <2 µg/g** | 1.2 (0.7-2.0) | 1.0 (0.6-1.7) | 0.9 (0.6-1.6) | 1.1 (0.7-1.7) | 1.3 (0.8-1.9) | **1.7 (1.1-2.5)** |
| **≥2 µg/g** | 0.8 (0.5-1.6) | 1.4 (0.7-2.5) | 1.1 (0.7-2.0) | 1.1 (0.7-1.8) | 1.1 (0.6-1.8) | 1.1 (0.7-1.8) |
| **>6 years old** | | | | | | |
| **≥Low[a] to <0.4 µg/g** | 1.2 (0.6-2.8) | 0.5 (0.2-1.3) | 0.8 (0.3-2.3) | 0.9 (0.5-1.8) | 1.3 (0.8-2.3) | 1.6 (0.8-3.1) |
| **0.4 to <2 µg/g** | 1.4 (0.8-2.3) | 0.8 (0.5-1.4) | 1.0 (0.6-1.8) | 0.9 (0.6-1.4) | 1.1 (0.7-1.7) | 1.1 (0.7-1.7) |
| **≥2 µg/g** | **0.4 (0.2-0.9)** | 0.9 (0.4-1.7) | 0.8 (0.4-1.4) | 0.8 (0.5-1.2) | 1.1 (0.6-1.9) | 1.0 (0.6-1.6) |

*Adjusted for n$^{o}$ of siblings at birth, parental education, maternal smoking during pregnancy and parental asthma or atopy. Cohort was included as random intercept. The reference category was below the "Low" cut-off. [a]For Der p1 "Low" was equal to 0.12 µg/g, for Der f1 "Low" was 0.07 µg/g and for Der p1 + Der f1 "Low" was 0.19 µg/g. [b]The INMA-Menorca cohort was not included (included: n=3,866). Bold indicates p-value<0.05.

Table E4. Adjusted* associations (odds ratios (OR) and 95% confidence intervals (CI)) between early life *Der p1, Der f1* and *Der p1 + Der f1* concentrations (µg/g) and HDM sensitization up to 6 years old and after, persistent wheezing, and asthma up to 6 years old and after in INMA-Menorca, BAMSE, LISAplus, MAS and PIAMA-NHS, excluding one cohort per model.

| | ***Der p1*** | | | | |
|---|---|---|---|---|---|
| **Excluded cohort** | **INMA-Menorca** | **BAMSE** | **LISAplus** | **MAS** | **PIAMA-NHS** |
| **Sensitization to HDM** | | | | | |
| **≤6 years old** | | | | | |
| **≥Low[a] to <0.4 µg/g** | 1.2 (0.7-2.1) | 1.3 (0.8-2.1) | 1.2 (0.6-2.1) | **2.0 (1.1-3.7)** | 1.3 (0.8-2.2) |
| **0.4 to <2 µg/g** | **1.6 (1.1-2.3)** | 1.4 (1.0-1.9) | 1.4 (0.9-2.3) | 1.4 (0.9-2.2) | 1.4 (0.9-2.1) |
| **≥2 µg/g** | **1.6 (1.0-2.4)** | 1.4 (1.0-2.0) | 1.5 (0.9-2.5) | 1.7 (1.1-2.6) | 1.4 (0.9-2.2) |
| **>6 years old** | | | | | |
| **≥Low[a] to <0.4 µg/g** | 0.9 (0.5-1.4) | 0.9 (0.5-1.4) | 1.0 (0.6-1.9) | 0.7 (0.3-1.4) | 0.9 (0.5-1.5) |
| **0.4 to <2 µg/g** | **1.5 (1.1-2.2)** | 1.5 (1.1-2.2) | **1.9 (1.3-2.9)** | 1.5 (1.0-2.2) | 1.3 (0.8-2.0) |
| **≥2 µg/g** | 1.3 (0.9-1.9) | 1.3 (0.9-2.0) | 1.3 (0.8-2.2) | 1.4 (0.9-2.3) | 1.1 (0.7-1.9) |
| **Persistent wheezing** | | | | | |
| **≥Low[a] to <0.4 µg/g** | 1.1 (0.7-1.7) | 1.0 (0.7-1.7) | 0.9 (0.5-1.6) | 1.6 (0.9-2.8) | 1.1 (0.7-1.8) |
| **0.4 to <2 µg/g** | 1.1 (0.8-1.6) | 0.9 (0.7-1.2) | 0.8 (0.6-1.2) | 1.0 (0.7-1.4) | 0.9 (0.6-1.3) |
| **≥2 µg/g** | **0.6 (0.4-0.9)** | 0.7 (0.5-1.1) | 0.8 (0.5-1.2) | 0.9 (0.6-1.3) | 0.8 (0.5-1.2) |
| **Asthma** | | | | | |
| **≤6 years old** | | | | | |
| **≥Low[a] to <0.4 µg/g** | 1.4 (0.9-2.4) | 1.5 (0.9-2.4) | 1.6 (0.9-2.6) | 1.7 (0.9-3.0) | 0.9 (0.5-1.7) |
| **0.4 to <2 µg/g** | 1.3 (0.9-1.8) | 1.1 (0.8-1.6) | 1.1 (0.8-1.6) | 1.2 (0.9-1.8) | 0.9 (0.6-1.5) |
| **≥2 µg/g** | 1.0 (0.6-1.5) | 1.0 (0.7-1.5) | 1.1 (0.7-1.6) | 1.2 (0.8-1.8) | 0.8 (0.5-1.3) |
| **>6 years old** | | | | | |
| **≥Low[a] to <0.4 µg/g** | 1.0 (0.6-1.7) | 1.1 (0.6-1.8) | 1.3 (0.7-2.3) | 1.1 (0.6-2.2) | 0.9 (0.5-1.6) |
| **0.4 to <2 µg/g** | 1.2 (0.8-1.7) | 1.2 (0.8-1.6) | 1.2 (0.7-1.9) | 1.2 (0.8-1.7) | 1.0 (0.6-1.5) |
| **≥2 µg/g** | 0.8 (0.5-1.2) | 0.7 (0.4-1.1) | 0.6 (0.3-1.1) | 0.7 (0.5-1.2) | **0.6 (0.3-0.9)** |

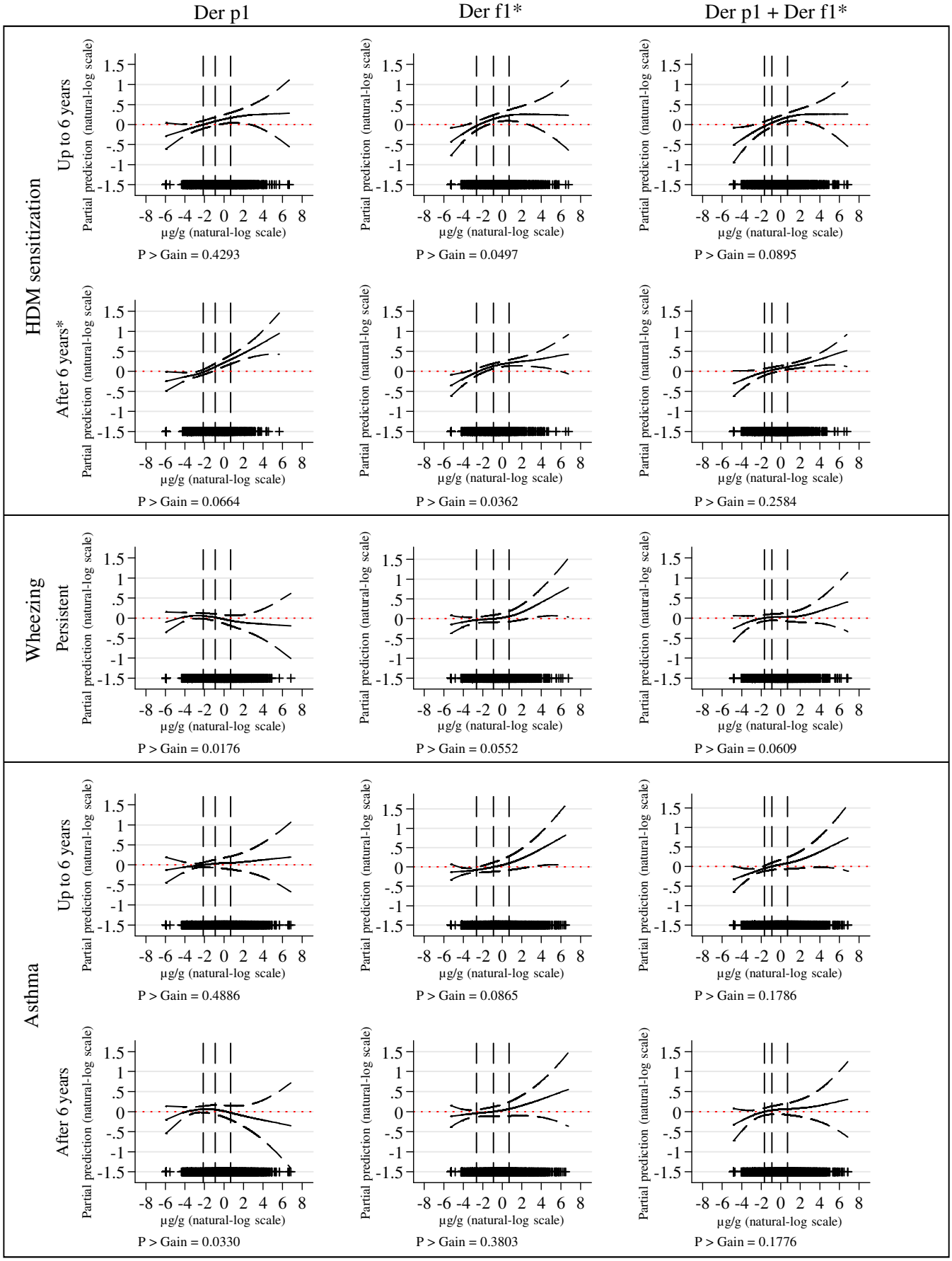
*Adjusted for sex, nº of siblings at birth, parental education, maternal smoking during pregnancy and parental asthma or atopy. Cohort was included as random intercept. The reference category was below the "Low" cut-off. [a] For *Der p1* "Low" was equal to 0.12 µg/g, for *Der f1* "Low" was 0.07 µg/g and for *Der p1 + Der f1* "Low" was 0.19 µg/g. Bold indicates p-value<0.05.

Table E4. (continued) Adjusted* associations (odds ratios (OR) and 95% confidence intervals (CI)) between early life *Der p1, Der f1* and *Der p1 + Der f1* concentrations (µg/g) and HDM sensitization up to 6 years old and after, persistent wheezing, and asthma up to 6 years old and after in INMA-Menorca, BAMSE, LISAplus, MAS and PIAMA-NHS, excluding one cohort per model.

| | ***Der f1*** | | | | ***Der p1 + Der f1*** | | | |
|---|---|---|---|---|---|---|---|---|
| **Excluded cohort** | **BAMSE** | **LISAplus** | **MAS** | **PIAMA-NHS** | **BAMSE** | **LISAplus** | **MAS** | **PIAMA-NHS** |
| **Sensitization to HDM** | | | | | | | | |
| **≤6 years old** | | | | | | | | |
| **≥Low[a] to <0.4 µg/g** | 1.4 (0.9-2.2) | **1.9 (1.1-3.1)** | 0.8 (0.4-1.7) | 1.5 (0.9-2.4) | 0.9 (0.5-1.8) | 1.2 (0.5-2.5) | 0.6 (0.2-2.0) | 1.0 (0.5-1.9) |
| **0.4 to <2 µg/g** | 1.5 (1.0-2.2) | 1.6 (0.9-2.6) | 1.4 (0.9-2.2) | **1.7 (1.1-2.6)** | 1.4 (1.0-2.1) | **1.8 (1.1-2.9)** | 1.5 (0.9-2.4) | 1.4 (0.9-2.2) |
| **≥2 µg/g** | 1.4 (0.9-2.1) | 1.9 (1.0-3.6) | 1.5 (0.9-2.5) | 1.3 (0.8-2.1) | 1.7 (1.2-2.4) | **1.8 (1.1-3.0)** | **2.1 (1.3-3.3)** | 1.5 (1.0-2.3) |
| **>6 years old** | | | | | | | | |
| **≥Low[a] to <0.4 µg/g** | 1.9 (1.3-2.8) | **1.9 (1.2-2.9)** | **1.8 (1.0-3.1)** | **2.1 (1.3-3.3)** | 1.2 (0.7-2.1) | 1.2 (0.6-2.3) | 1.8 (0.8-4.3) | 1.0 (0.5-1.9) |
| **0.4 to <2 µg/g** | 1.5 (1.1-2.1) | **1.7 (1.1-2.6)** | 1.2 (0.8-1.8) | **1.8 (1.2-2.8)** | 1.5 (1.0-2.0) | **1.8 (1.2-2.7)** | 1.5 (1.0-2.2) | 1.2 (0.8-1.9) |
| **≥2 µg/g** | 1.9 (1.3-2.8) | **1.8 (1.1-3.0)** | **1.7 (1.1-2.7)** | **2.0 (1.2-3.3)** | 1.7 (1.2-2.3) | **1.6 (1.0-2.4)** | **1.9 (1.3-2.9)** | 1.5 (1.0-2.3) |
| **Persistent wheezing** | | | | | | | | |
| **≥Low[a] to <0.4 µg/g** | 1.1 (0.8-1.7) | 1.2 (0.8-2.0) | 1.2 (0.7-2.0) | 1.2 (0.7-1.9) | 1.3 (0.8-2.2) | 1.3 (0.7-2.5) | 1.2 (0.6-2.5) | 1.4 (0.8-2.4) |
| **0.4 to <2 µg/g** | 1.1 (0.8-1.5) | 1.0 (0.7-1.6) | 1.0 (0.7-1.4) | 1.2 (0.8-1.8) | 1.1 (0.8-1.5) | 1.1 (0.7-1.6) | 1.1 (0.8-1.6) | 1.1 (0.8-1.6) |
| **≥2 µg/g** | 1.1 (0.7-1.6) | 1.1 (0.6-2.0) | 1.1 (0.7-1.6) | 1.2 (0.8-1.8) | 0.9 (0.6-1.2) | 0.8 (0.5-1.3) | 0.9 (0.6-1.3) | 1.0 (0.7-1.5) |
| **Asthma** | | | | | | | | |
| **≤6 years old** | | | | | | | | |
| **≥Low[a] to <0.4 µg/g** | 1.2 (0.8-1.8) | 1.3 (0.9-2.0) | 1.4 (0.9-2.3) | 0.9 (0.5-1.5) | 1.5 (0.9-2.6) | 1.8 (1.0-3.1) | 1.2 (0.5-2.7) | 1.3 (0.7-2.4) |
| **0.4 to <2 µg/g** | 1.1 (0.8-1.5) | 1.2 (0.8-1.7) | 1.3 (0.9-1.9) | 1.0 (0.6-1.7) | 1.3 (0.9-1.8) | 1.4 (1.0-2.0) | 1.5 (1.0-2.2) | 1.2 (0.8-2.0) |
| **≥2 µg/g** | 1.2 (0.8-1.8) | 1.4 (0.9-2.2) | 1.5 (0.9-2.3) | 0.8 (0.4-1.5) | 1.1 (0.8-1.5) | 1.2 (0.8-1.7) | 1.4 (0.9-2.0) | 0.8 (0.5-1.4) |
| **>6 years old** | | | | | | | | |
| **≥Low[a] to <0.4 µg/g** | 1.0 (0.6-1.6) | 1.1 (0.7-1.8) | 1.2 (0.7-2.1) | 1.0 (0.6-1.6) | 1.2 (0.5-2.5) | 1.5 (0.8-2.9) | 1.2 (0.5-2.7) | 1.2 (0.6-2.3) |
| **0.4 to <2 µg/g** | 1.0 (0.7-1.4) | 0.7 (0.4-1.2) | **1.1 (0.7-1.6)** | 1.2 (0.8-1.9) | 1.8 (1.1-2.9) | 0.9 (0.5-1.4) | 1.3 (0.8-1.9) | 1.1 (0.7-1.8) |
| **≥2 µg/g** | 1.0 (0.7-1.6) | 1.6 (0.9-2.7) | 1.1 (0.7-1.7) | 0.8 (0.5-1.4) | **1.8 (1.1-3.0)** | 1.0 (0.6-1.7) | 1.0 (0.7-1.6) | 0.8 (0.5-1.3) |

*Adjusted for sex, nº of siblings at birth, parental education, maternal smoking during pregnancy and parental asthma or atopy. Cohort was included as random intercept. The reference category was below the "Low" cut-off. [a] For *Der p1* "Low" was equal to 0.12 µg/g, for *Der f1* "Low" was 0.07 µg/g and for *Der p1 + Der f1* "Low" was 0.19 µg/g. Bold indicates p-value<0.05.

Figure E1. Partial predictions (natural-log scale) of HDM sensitization, wheezing and asthma according to log-transformed *Der p1*, *Der f1* and *Der p1 + Der f1* concentrations (µg/g) in INMA-Menorca, BAMSE, LISAplus, MAS and PIAMA-NHS (n=4,334).




Values below the LOD were assigned a value equal to 2/3 the lowest allergen concentration observed among the samples with detectable allergens in each cohort.The vertical dashed lines indicate the log transformed cut-off points used in the multivariate analyses (0.12 µg/g, 0.4 µg/g and 2 µg/g for *Der p1*, 0.07 µg/g, 0.4 µg/g and 2 µg/g for *Der f1* and 0.19 µg/g, 0.4 µg/g and 2 µg/g for *Der p1 + Der f1*). The GAM models are adjusted for sex, nº of siblings at birth, parental education, maternal smoking during pregnancy, parental asthma and/or allergic rhinitis and cohort. *INMA-Menorca not included (n=3,866).