Impact of indoor air pollution in nursery and primary schools on childhood asthma

Pedro T.B.S. Branco[1], Maria C.M. Alvim-Ferraz[1], Fernando G. Martins[1], Catarina Ferraz[2], Luísa G. Vaz[2], Sofia I.V. Sousa[1]*

[1] LEPABE – Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal

[2] Departamento de Pediatria (UAG-MC), Centro Hospitalar Universitário de São João (CHUSJ), Alameda Prof. Hernâni Monteiro, 4200-319, Porto, Portugal

*Corresponding author:

Telephone: +351 22 508 2262

Fax: +351 22 508 1449

E-mail address: sofia.sousa@fe.up.pt

Postal address: Rua Dr. Roberto Frias, 4200-465, E221, Porto, Portugal

Citation:

Branco P.T.B.S., Alvim-Ferraz M.C.M., Martins F.G., Ferraz C., Vaz L.G., Sousa S.I.V., 2020. "Impact of indoor air pollution in nursery and primary schools on childhood asthma". Science of The Total Environment. https://doi.org/10.1016/j.scitotenv.2020.140982

Abstract

Poor indoor air quality in scholar environments have been frequently reported, but its impact on respiratory health in schoolchildren has not been sufficiently explored. Thus, this study aimed to evaluate the associations between children's exposure to indoor air pollution (IAP) in nursery and primary schools and childhood asthma. Multivariate models (independent and multipollutant) quantified the associations of children's exposure with asthma-related health outcomes: reported active wheezing, reported and diagnosed asthma, and lung function (reduced FEV_1/FVC and reduced FEV_1). A microenvironmental modelling approach estimated individual inhaled exposure to major indoor air pollutants (CO_2, CO, formaldehyde, NO_2, O_3, TVOC, $PM_{2.5}$ and PM_{10}) in nursery and primary schools from both urban and rural sites in northern Portugal. Questionnaires and medical tests (spirometry pre- and post-bronchodilator) were used to obtain information on health outcomes and to diagnose asthma following the newest international clinical guidelines. After testing children for aeroallergen sensitisation, multinomial models estimated the effect of exposure to particulate matter on asthma in sensitised individuals. The study population were 1530 children attending nursery and primary schools, respectively 648 pre-schoolers (3-5 years old) and 882 primary school children (6-10 years old). This study found no evidence of a significant association between IAP in nursery and primary schools and the prevalence of childhood asthma. However, reported active wheezing was associated with higher NO_2, and reduced FEV_1 was associated with higher O_3 and $PM_{2.5}$, despite NO_2 and O_3 in schools were always below the 200 μg m^{-3} threshold from WHO and National legislation, respectively. Moreover, sensitised children to common aeroallergens were more likely to have asthma during childhood when exposed to particulate matter in schools. These findings support the urgent need for mitigation measures to reduce IAP in schools, reducing its burden to children's health.

Keywords: Children; exposure; inhaled dose; indoor air; school; asthma

1. Introduction

Air pollution has been associated with several adverse human health outcomes, namely respiratory symptoms and chronic diseases like asthma (Goldizen et al., 2016; Götschi et al., 2008; Norbäck et al., 2018; Norback et al., 2019; Thurston et al., 2017). Those associations were extensively documented for ambient air (Day et al., 2017; Khreis et al., 2017; Tsui et al., 2018), nevertheless, people spend most of their time in indoor environments. Due to their physical constitution and breathing pattern, children are more susceptible to the health effects of air pollution than adults, being considered a frail population (Annesi-Maesano et al., 2003). While the impacts of home environment on childhood asthma have been extensively studied (Breysse et al., 2010; Cui et al., 2020; Ferrero et al., 2017; He et al., 2020; Huang et al., 2020), the school was usually less studied although it is the most important indoor environment for children apart from home, as well as their first place for social activity. Besides, children are frequently physically active in school, increasing their ventilation rate and thus the inhaled dose of pollutant concentrations. School building characteristics have a significant contribution to indoor air exposure (Amato et al., 2014; Salonen et al., 2019), and building maintenance is usually challenging in schools (Hauptman and Phipatanakul, 2015; Sá et al., 2017).

Poor indoor air quality (IAQ) in schools has been often reported and related to: i) respiratory disturbances, namely affecting nasal patency (Simoni et al., 2010); ii) increased prevalence of clinical manifestations of asthma and rhinitis, with a higher risk for children with a background of allergies (Annesi-Maesano et al., 2012); and iii) wheezing and lung function abnormality in pre-schoolers, especially related with exposures to particulate matter (PM), TVOC and carbon monoxide (CO) (Rawi et al., 2015). Although poor IAQ in scholar environments have been frequently reported, relationships between IAQ in schools and the allergic and respiratory health of schoolchildren have been insufficiently explored (Annesi-Maesano et al., 2013; Annesi-Maesano et al., 2012; Patelarou et al., 2015). Moreover, published studies regarding the

relationship between IAQ in schools and children's allergies and respiratory health, in particular childhood asthma, usually presented at least one of following gaps: i) focus only on urban areas, neglecting rural sites where both children's time-activity-patterns and outdoor air concentrations are expected to differ; ii) classrooms' concentrations were usually assumed as exposure, not considering children's time-location patterns and neglecting other relevant indoor microenvironments (canteens, bedrooms); iii) inhalation exposure models were commonly used, although they did not strictly take into account the inhaled dose of airborne compounds, but only the presence of air pollutants near the breathing zone of a person; iv) consider single or few pollutants individually, neglecting their combined effects; and v) respiratory health data, especially asthma-related, is usually parent-reported in a survey, instead of measured and confirmed by a physician.

Thus, by following INAIRCHILD project (Sousa et al., 2012a) and its previous results (Branco et al., 2020; Branco et al., 2019) and to fulfil the gaps in the existing literature, this study mainly aimed to evaluate the associations between children's exposure/inhaled dose to indoor air pollutants and childhood asthma in nursery and primary schools. This study goes further on the literature because it: i) considered both urban and rural sites and included children from two different age groups (pre- and primary school children); ii) used a microenvironmental modelling approach to estimate indoor air pollutants' exposures and inhaled doses, considering classrooms, but also other different indoor scholar environments; iii) analysed several major indoor air pollutants, individually and combined; and iv) diagnosed asthma based on medical doctors' physical examinations according to the most recent guidelines. Two complementary hypotheses were tested: i) if exposures/inhaled doses of indoor air pollutants in nursery and primary schools are associated with childhood asthma prevalence, reported respiratory symptoms and/or changes in lung function; and ii) if children's sensitisation (to the most common aeroallergens) influence on that association, i.e., associations between indoor air

pollutants exposures/inhaled doses and childhood asthma differences among sensitised and non-sensitised children.

2. Materials and methods

2.1. Study population and health assessment

This cross-sectional study involved children randomly recruited from the nursery and primary schools (urban and rural) participating in the INAIRCHILD project in the academic year of 2013/2014 (campaign 1) and 2015/2016 (campaign 2, to increase sample size), including pre-schoolers (3-5 years old) and primary school children (6-10 years old) but excluding infants (under 3 years old). Those nursery and primary schools were located in both urban and rural sites in northern Portugal (41ºN, 8ºW), and their governance bodies consented to perform this study. Parents or guardians signed an informed consent according to the Helsinki Declaration developed by the World Medical Association and completed an ISAAC-derived questionnaire. Medical doctors validated all questionnaires. At any stage of the study, the potential children's dissent was always respected. This study was approved by both the Ethics Commission of Universidade do Porto and the Ethics Commission for Health of Centro Hospitalar Universitário de São João, Porto.

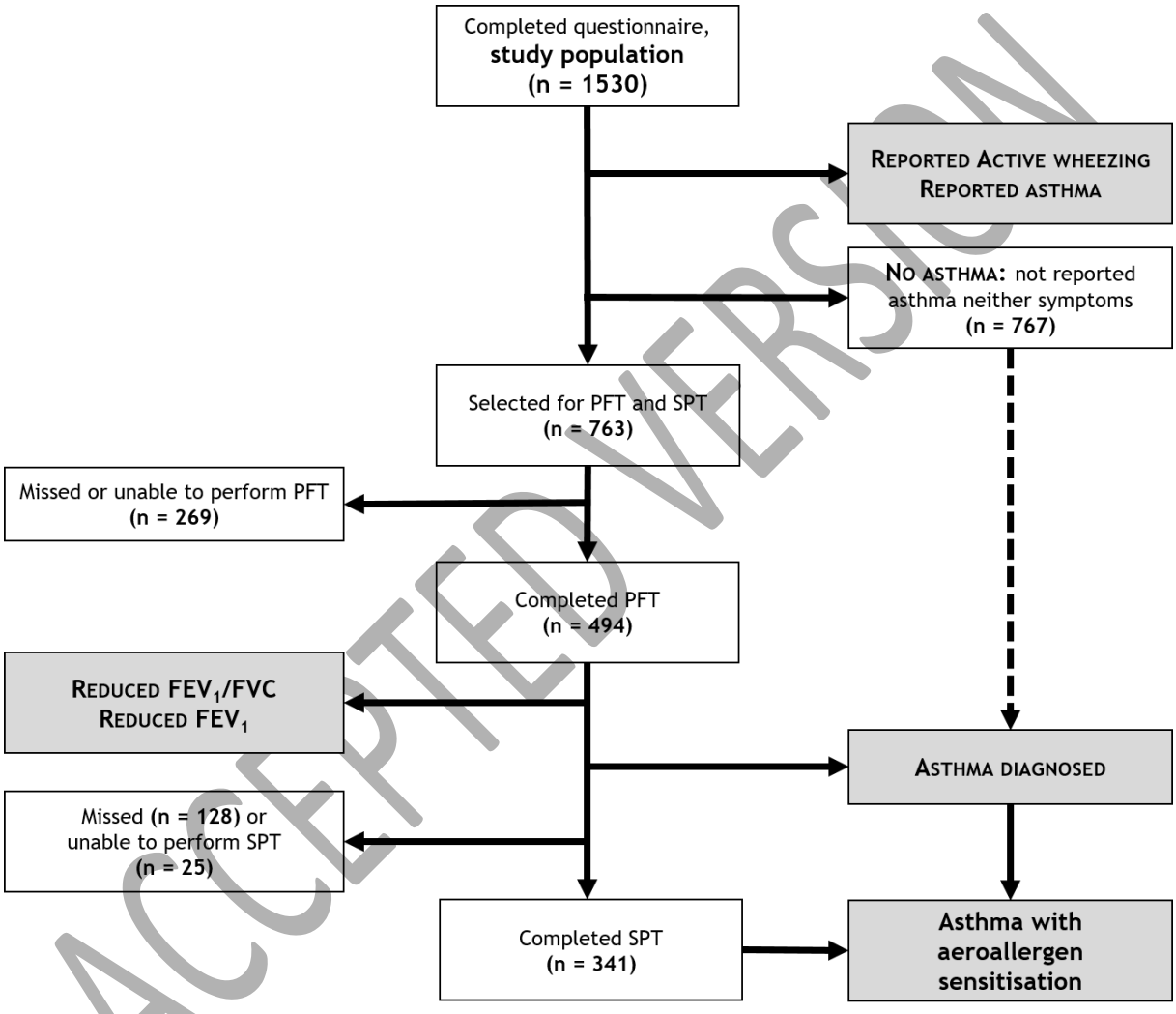
According to the Global Initiative for Asthma (GINA, 2018), asthma diagnosis should be based on the history of characteristic respiratory symptoms and the demonstration of variable expiratory airflow limitation. Thus, children who were reported being asthmatic in the questionnaire and those who reported at least one asthmatic symptom ever in life (wheezing, dyspnoea, or nocturnal cough in the absence of upper respiratory infection) were selected for pulmonary function tests (PFT).

Spirometry pre and post-bronchodilator administration (200 µg of salbutamol) were used to perform the PFT according to the latest guidelines from ERS/ATS and GINA (Beydon et al.,

2007; GINA, 2018; Thurston et al., 2017); a Vitalograph ALPHA Track (Vitalograph, UK) was used at one specific room of each school to where medical doctors brought the necessary equipment. That room was specifically chosen to avoid confounding effects related to weather and other indoor environmental conditions. Although children, particularly pre-schoolers, present a number of special challenges regarding PFT, technically acceptable spirometry is feasible in those ages if following specific recommendations (Beydon et al., 2007; Branco et al., 2020). In this study, the protocol was similar for all the participants independently of their age, spirometry was performed by experienced operators (medical doctors specialised in paediatric pulmonology) and the specific recommendations for spirometry in the pre-school age were considered, namely: i) children were instructed how to do the manoeuvres, repeating them at least three times until reproducibility was reached; ii) as the majority of children was doing this test for the first time, a training period was considered to familiarise them with the equipment and technician; iii) flow- and volume-driven interactive computerised incentives were used to encourage manoeuvre; iv) the operator observed the child closely to ensure there was no leak, and that the manoeuvre was performed optimally; v) both volume-time and flow-volume curves were visually inspected in real-time; vi) FVC and FEV1 indices were inspected by the operator before the next attempt; and vii) only subjects producing at least three acceptable curves were considered. Children were seated and no nose clip was used. Pulmonary function indexes were measured in each attempt and predicted for each individual using the latest recommendations (Quanjer et al., 2012), namely: i) forced expiratory volume in 1 second (FEV_1) which is the volume exhaled during the first second of a forced expiratory manoeuvre started from the level of total lung capacity; and ii) forced vital capacity (FVC) which is the volume of air that can forcibly be blown out after full inspiration. The highest FEV_1 and FVC were considered, after examining data from all of the usable curves, even if they did not come from the same curve. FEV_1/FVC ratio was calculated.

147 Asthma was diagnosed based on GINA guidelines (GINA, 2018), if at least one asthmatic
148 symptom (wheezing, dyspnoea or nocturnal cough in the absence of upper respiratory infection)
149 was reported simultaneously with spirometry results revealing both airflow limitation
150 (obstruction) and excessive variability in lung function (positive bronchodilator reversibility
151 test with an increase in FEV_1 higher than 12% predicted), with or without reporting a previous
152 diagnosis.
153 Those who completed PFT were also selected to perform medical skin prick tests (SPT) for
154 evaluating allergen sensitisation to common aeroallergens (Migueres et al., 2014), namely: i)
155 house dust mites (*Dermatophagoides pteronyssinus* (Dp), *Dermatophagoides farinae* (Df) and
156 *Lepidoglyphus destructor* (Ld)); ii) pollens (wild grasses composed by a mixture of *Agrostis*,
157 *Anthoxanthum odoratum*, *Dactylis glomerata*, *Festuca pratensis*, *Holcus lanatus*, *Lolium*
158 *perenne*, *Phleum pratense* and *Poa pratensis*, sown grasses composed by a mixture of *Secale*
159 *cereale*, *Hordeum vulgare* and *Triticum*, and tree pollen composed by a mixture of *Fraxinus*
160 *excelsior*, *Populus* and *Salix*); and iii) animal dander – dog (Canis familiaris) and cat (*Felis*
161 *domesticus*). The allergens used were obtained from Bial (Aristegui, Produtos Farmacêuticos
162 S.A., Portugal). The SPT were performed on the anterior face of the child's forearm, using the
163 tip of a metallic lancet. Skin reaction confirmed allergen sensitisation depending on the skin
164 wheal size and flare reaction in comparison with the positive control (histamine solution) and
165 the negative control (saline control). Children were considered sensitised if revealed positive to
166 at least one of the studied aeroallergens.
167 Figure 1 shows the flowchart with the study population for each step of the methodology. For
168 the association with IAQ, this study considered five health outcomes: i) reported active
169 wheezing – if reported wheezing in the last 12 months; ii) reported asthma - if answered "Yes"
170 to the question "Does the child have or ever had asthma?"; iii) diagnosed asthma, when asthma
171 was diagnosed based on GINA guidelines above referred; iv) FEV_1/FVC (< 0.90), which

indicates an airflow limitation (obstruction); v) reduced FEV_1 (< 80% predicted), which indicates abnormal lung function. Moreover, this study also classified children as having asthma with aeroallergen sensitization (if diagnosed both asthma and sensitization), asthma without aeroallergen sensitization (if diagnosed asthma, but not sensitization), or no asthma (if not asthmatic).



PFT – Pulmonary Function Test; SPT – Skin Prick Test

Figure 1 – Flow chart including the study population in the different steps of the methodology. Grey boxes represent the health outcomes considered.

2.2. Exposure and inhaled dose assessment

Children's daily exposure to indoor air pollutants in nursery or primary school (E_i) was estimated based on a microenvironmental modelling approach (Branco et al., 2014b), as the sum of the product of time (t_{ij}) spent by the child *i* in different indoor school microenvironments *j* (ME) and the corresponding time-averaged air pollution concentrations (C_{ij}) (equation 1).

$$E_i = \sum_{j=1}^{J} C_{ij} t_{ij} \quad (1)$$

This study considered the main indoor microenvironments (classrooms, canteens and bedrooms used for naps after lunch when applicable) from 17 nursery schools for pre-schoolers (children usually aged 3-6) and 8 primary schools (children usually aged 6-10), all located in both urban and rural areas from northern Portugal (Branco et al., 2019). Canteen was here defined as the place where children had lunch and sometimes the snack, which had an attached kitchen with gas stoves.

Indoor concentrations of CO_2, CO, formaldehyde, NO_2, O_3, TVOC, $PM_{2.5}$ and PM_{10} were continuously monitored from at least 24 hours to 9 consecutive days (not simultaneously) in each studied room, and were already reported in Branco et al. (2019). Sampling methods and main characteristics of each sensor were previously described in detail (Branco et al., 2015a; Branco et al., 2014a; Branco et al., 2015b). Indoor air pollutants' samplings occurred in 69 classrooms and 15 canteens, one or more representative classrooms and canteens in each nursery and primary school building. Although samplings occurred twice in some rooms, namely during cold season (October to March) and warm season (April to September), they cannot be considered repeated measurements as they occurred in distinct academic years (from 2013 to 2016), corresponding to the two recruitment campaigns, thus with distinct occupants, occupancy and activities' conditions. This study assumed that each participant had lunch at the school canteen. For exposure estimates, when one of the indoor microenvironments of the

participating child were not sampled, indoor air pollutants' concentrations were obtained from the most similar room (similar room characteristics, occupancy and activity patterns patterns). Time spent by each class in different indoor school microenvironment and the correspondent activity were initially obtained from a parent-reported daily diary (a typical 24-hour weekday divided into log periods of 30-min), then complemented with information from the class timetable, and subsequently validated by the educator/teacher of the class. A total of 507 complete daily diaries from all the classes evaluated were considered (174 from pre-schoolers and 333 from primary school children).

Exposure does not strictly take into account the inhaled dose of indoor air pollutants, but only the presence of them near the breathing zone of a person. Thus, for each child *i*, daily inhaled dose (D_i) in school indoor microenvironments was estimated based on the time-averaged exposure (E_i), inhalation rate (IR_k) adopted for each activity *k* from the US EPA approach (U.S. Environmental Protection Agency (EPA), 2011), and child's body weight (BW_i) obtained from the questionnaire, by using the Equation (2).

$$D_i = \sum_{k=1}^{K}(E_{ik} . IR_k)/BW_i \quad (2)$$

2.3. Data analysis

For each participating child (N = 1530), daily exposures to indoor air pollutants in school, and correspondent inhaled doses were estimated. Prevalence rates were calculated as the ratio between the number of cases and the total number of individuals considered. Descriptive statistics were used to express the characteristics of both health outcomes, exposures and inhaled doses. Phi coefficient (mean square contingency coefficient) was used as a measure of association between the studied binary outcomes.

As all the respiratory health outcomes considered were binary variables, multivariate logistic regression models were used to assess the association between exposure/inhaled dose and each outcome considered.

Firstly, independent models were built for each indoor air pollutant (unipollutant models) to understand the individual influence of each pollutant, by considering continuous exposure/inhaled dose scaled by the interquartile range (IQR) – scaled odds ratios (OR) were obtained representing outcome change relative to an interquartile change in each exposure/inhaled dose metric. The same models were also applied to different types of transformation in the exposure variables, namely: i) dichotomised into 'high' and 'low' by using median as cutoff; ii) dichotomised into 'high' and 'low' by using Portuguese legislation or World Health Organization (WHO) limit values as cutoff; and iii) dichotomised into 'at risk' and 'not at risk' by considering 'at risk' children attending rooms where concentrations exceeded the limit values. As there were no reference values for inhaled doses, these variables were only factorised into 'high' and 'low' by using median as cutoff. The limit values (thresholds) considered were: i) from the Portuguese legislation (Portaria nº 353-A/2013) for CO_2 (2250 mg m^{-3}, plus 30% of margin of tolerance (MT) if no mechanical ventilation system was working in the room), CO (10000 μg m^{-3}), formaldehyde (100 μg m^{-3}), TVOC (600 μg m^{-3}, plus 100% of MT if no mechanical ventilation system was working in the room), and $PM_{2.5}$ and PM_{10} (25 μg m^{-3} and 50 μg m^{-3} respectively, plus 100% of MT if no mechanical ventilation system was working in the room); ii) from the Portuguese legislation (Decreto-Lei nº 79/2006) for O_3 (200 μg m^{-3}); and iii) from the WHO guidelines (WHO, 2010) for NO2 (200 μg m^{-3}).

Secondly, to understand the combined influence of exposure/inhaled dose of all the studied gaseous indoor air pollutants and $PM_{2.5}$, multipollutant logistic regression models were built, also by considering continuous exposure/inhaled dose to all the studied indoor air pollutants

scaled by IQR. The same models were also applied to the different types of transformations in the exposure variables considered in unipollutant models.

Finally, multinomial logistic regression models were used to estimate the effect of indoor air pollutants' exposure/inhaled dose on the probability that the outcome (asthma diagnosed) is: no asthma, asthma with aeroallergen sensitization (AS) or asthma without aeroallergen sensitization (AS). No asthma was chosen as the comparison level, and 2 regression coefficients, corresponding to each other outcome levels, were estimated for each exposure variable in these regression models. These models were built by considering the same exposure/inhaled dose transformations as in the previous analyses.

Previous knowledge was considered to define potential adjustment for confounders (Branco et al., 2019; Branco et al., 2016). Thus, all models were adjusted for site location (if urban or rural), campaign (1 or 2, to account for potential differences in time and season), sex, age group (pre- or primary school children), body mass index (BMI) and parental history of asthma. As home indoor exposures were not quantified, although they might have contributed to the studied health outcomes, all models were also adjusted for covariates that represented indirect measures of relevant home indoor exposures, namely mother education as a measure of the family socioeconomic status, and exposure to tobacco smoke at home (living with a smoker). Multinomial logistic regression models were also adjusted for child's contact with farm animals in the first year of life, and with pets (cat or dog) at home in the previous year and/or in the first year of life, which might also indirectly represent relevant home exposures.

Statistical computations were performed with R software version 3.4.3. The level of statistical significance was set at 0.05, except when stated otherwise.

3. Results

3.1. Characterization of the study population and health outcomes' prevalence

With a participation rate of approximately 39%, this study involved 1530 children attending nursery (648 pre-schoolers) and primary schools (882 primary school children), both from urban (59.8%) and rural areas (40.2%). Children were randomly recruited from nursery and primary schools (both urban and rural), and no inclusion/exclusion criteria were used, to avoid potential selection bias. Mean age (SD) of this study population was 6.0 (2.1) years old, with 4.0 (0.9) years old in pre-schoolers and 7.5 (2.5) in primary school children. Females were 51.0% of the study population. Study population had a mean (SD) BMI of 17.0 (3.0), being the majority (59.5%) of them classified with normal BMI, although 33.2% were overweight or obese. Main personal characteristics and prevalence of respiratory health outcomes considered are detailed in Table 1.

Wheezing on the previous 12 months (here considered as active wheezing) was higher in pre-school age and urban sites, while reported being previously diagnosed as asthmatic (reported asthma) was also higher in urban sites but for older children (primary school age). Half of the study population (49.9%) reported being asthmatic in the questionnaire or reported at least one asthmatic symptom ever in life (wheezing, dyspnoea, or nocturnal cough in the absence of upper respiratory infection), being selected for PFT and SPT to confirm asthma diagnosis and to obtain information on lung function, as well as to evaluate sensitization to common aeroallergens. The number of symptomatic children was higher among the youngest (pre-schoolers) and those from urban sites. From those who completed PFT, 36.4% were found to have a reduced FEV_1/FVC (airway obstruction), while 23.1% of them presented a reduced FEV_1. Moreover, 64.0% of those having reduced FEV_1 were also diagnosed with reduced FEV_1/FVC, which might indicate reduced lung function growth or restriction. Asthma was diagnosed in 5.5% of the study population, being higher in primary school children (6.2%) than

299 in pre-schoolers (4.5%), and higher in urban (6.0%) than in rural sites (4.8%), although neither
300 statistically significant (*p*-value = 0.23 and 0.41, respectively) (Branco et al., 2020).
301 To understand if there was an association between the studied health outcomes, phi coefficients
302 were used showing weak or negligible positive associations in most cases (0.01 < phi < 0.38),
303 except between reported and diagnosed asthma (phi = 0.87). Still, all outcomes were considered
304 independently for the following analyses.
305 From those who were selected for PFT and SPT, 67.0% completed SPT (of those, 57.1% were
306 pre-schoolers and 73.7% primary school children, 57.6% were from urban sites and 85.8% from
307 rural ones). Sensitization to aeroallergens was higher in older children and urban sites. From
308 this study population, 2.5% had asthma with aeroallergen sensitization, while 2.9% had asthma
309 without aeroallergen sensitization. In primary school children, there were more asthmatics with
310 aeroallergen sensitization than asthmatics without it, while with the youngest (pre-schoolers)
311 occurred the opposite. Results from aeroallergen sensitization are detailed in Supplementary
312 Material (Table S1). Sensitizations to dust mites were the most commonly found (25%),
313 followed by animal dander (15%) and pollens (11%). Sensitizations to dust mites were higher
314 in primary school children than in younger ones, while sensitizations to pollens were the
315 opposite. Sensitizations to dust mites and pollens were both higher in children from urban sites,
316 while sensitizations to animal dander were higher in rural individuals.
317

Table 1 – Characterization of the study population and prevalence of respiratory health outcomes considered (with 95% confidence intervals), in the whole population and divided by age and by location

Characteristics and health outcomes	Population (n=1530)		by children's age				by location			
			Pre-schoolers (n = 648)		Primary school children (n=882)		Urban (n=915)		Rural (n=615)	
	%	95% CI	%	95% CI	%	95% CI	%	95% CI	%	95% CI
Sex										
Female	51.0	(48.5-53.5)	49.7	(45.8-52.2)	51.9	(48.6-55.2)	50.1	(46.8-52.6)	52.4	(48.4-56.3)
Male	49.0	(46.5-51.5)	50.3	(46.5-52.8)	48.1	(44.8-51.4)	49.9	(46.7-52.5)	47.6	(43.7-51.6)
Age group										
Pre-schooler	42.4	(39.9-44.8)	-	-	-	-	42.4	(39.2-44.9)	42.3	(38.4-46.2)
Primary school children	57.6	(55.2-60.1)	-	-	-	-	57.6	(54.4-60.1)	57.7	(53.8-61.6)
Location										
Rural	40.2	(37.7-42.7)	40.1	(36.3-42.6)	40.2	(37.0-43.5)	-	-	-	-
Urban	59.8	(57.3-62.3)	59.9	(56.1-62.3)	59.8	(56.5-63.0)	-	-	-	-
BMI classification										
Normal	59.5	(56.7-62.4)	56.9	(52.4-59.8)	61.5	(57.7-65.3)	59.6	(56.0-62.5)	59.5	(54.7-64.3)
Underweight	7.2	(5.7-8.8)	10.0	(7.3-11.8)	5.2	(3.5-6.9)	5.5	(3.9-6.9)	10.2	(7.2-13.2)
Overweight	15.8	(13.7-18.0)	14.9	(11.7-17.0)	16.5	(13.6-19.4)	16.9	(14.2-19.1)	13.9	(10.5-17.3)
Obese	17.4	(15.1-19.6)	18.1	(14.6-20.4)	16.8	(13.9-19.7)	17.9	(15.1-20.2)	16.4	(12.8-20)
Mother education										
Medium	31.9	(29.5-34.3)	31.2	(27.6-33.5)	32.4	(29.3-35.6)	28.2	(25.3-30.5)	37.6	(33.7-41.5)
Low	28.5	(26.2-30.8)	24.3	(21.0-26.5)	31.6	(28.5-34.7)	22.7	(20.0-24.8)	37.5	(33.6-41.4)
High	39.6	(37.1-42.1)	44.5	(40.7-47.0)	35.9	(32.7-39.1)	49.1	(45.9-51.7)	24.9	(21.4-28.4)
Born in Portugal, no	4.5	(3.5-5.6)	3.9	(2.4-4.8)	5.0	(3.6-6.5)	2.1	(1.2-2.8)	8.2	(6.0-10.4)
Living with a smoker, yes	41.1	(38.6-43.6)	41.0	(37.2-43.4)	41.2	(38-44.5)	39.2	(36.0-41.7)	43.9	(40.0-47.9)
Asthmatic parent, yes	15.1	(13.3-16.9)	14.4	(11.7-16.2)	15.7	(13.2-18.1)	19.5	(16.9-21.5)	8.7	(6.4-10.9)
Reported asthma	5.9	(4.7-7.0)	4.0	(2.5-5.5)	7.2	(5.5-8.9)	6.9	(5.3-8.6)	4.3	(2.7-5.9)
Active wheezing	13.6	(11.9-15.3)	16.3	(13.4-19.1)	11.7	(9.5-13.8)	16.0	(13.6-18.4)	10.0	(7.6-12.4)
Selected for PFT and SPT	49.9	(47.4-52.4)	53.1	(49.2-55.6)	47.5	(44.2-50.8)	52.2	(49.0-54.7)	46.3	(42.4-50.3)
Reduced FEV_1/FVC [a]	36.4	(32.2-40.7)	27.4	(21.4-33.4)	43.3	(37.5-49.0)	36.9	(31.3-42.6)	35.8	(29.4-42.2)
Reduced FEV_1 [a]	23.1	(19.4-26.8)	17.0	(11.9-22.0)	27.7	(22.4-32.9)	15.1	(10.9-19.2)	33.5	(27.2-39.8)
Reduced FEV_1 degree [a]										
Normal	76.9	(73.2-80.6)	83.0	(78.0-88.1)	72.3	(67.1-77.6)	84.9	(80.8-89.1)	66.5	(60.2-72.8)
Mild	18.0	(14.6-21.4)	16.0	(11.1-21.0)	19.5	(14.9-24.1)	14.7	(10.5-18.8)	22.3	(16.8-27.9)
Moderate	4.9	(3.0-6.8)	0.9	(0.0-2.2)	7.8	(4.7-10.9)	0.4	(-0.3-1.1)	10.7	(6.6-14.8)
Severe	0.2	(0.0-0.6)	0.0	(0.0-0.0)	0.4	(0.0-1.0)	0.0	(0-0)	0.5	(0.0-1.4)
Asthma diagnosed	5.5	(4.2-6.7)	4.5	(2.7-6.2)	6.2	(4.4-7.9)	6.0	(4.3-7.7)	4.8	(3.0-6.6)
Sensitised to aeroallergens [b]	35.2	(30.1-40.3)	25.6	(19.5-30.3)	40.2	(33.3-45.4)	40.3	(33.4-45.5)	28.3	(20.9-35.6)
Allergy and asthma										
Asthma with AS	2.5	(1.4-3.5)	0.7	(0.0-1.2)	3.5	(1.9-4.7)	3.0	(1.5-4.1)	1.7	(0.4-3.1)
Asthma without AS	2.9	(1.8-4.1)	2.3	(1.0-3.3)	3.3	(1.7-4.5)	3.2	(1.6-4.3)	2.6	(0.9-4.3)
No asthma	94.6	(93.1-96.1)	97.1	(95.6-98.2)	93.2	(91-94.9)	93.9	(91.8-95.5)	95.7	(93.5-97.8)

[a] these outcomes represent the prevalence in symptomatic children who completed spirometry for pulmonary function test (N = 494); [b] these outcomes represent the prevalence in children who completed spirometry and skin prick tests for aeroallergen sensitization assessment (N = 341); AS – aeroallergen sensitization; CI – confidence interval; BMI – body mass index; PFT – pulmonary function test; SPT – skin prick test

3.2. Time-location-activity patterns, exposure and inhaled dose estimation

Data collected from the parent-reported daily diaries allowed estimating daily patterns for locations in a typical weekday (24-hour) for both pre- and primary school children, from urban and rural sites, considering the major ME: home indoor, home outdoor, school indoor, school outdoor, in transport and others. Time spent in these MEs are summarised in Figure S1 (Supplementary Material), and proportions of time in a typical weekday (24 hours) are detailed in Figure S2 (Supplementary Material). More than half of a weekday was usually spent inside home. Outdoors (home and school) represented less than 10% of the day, and less than 1 hour of the day was usually spent in transport (commuting). These data confirmed that children spent most of their time indoors being a significant portion inside the school (more than 6 hours on average, representing 24-28% of the day). That portion was higher in rural than in urban sites, and higher for primary school children than for pre-schoolers in urban sites and the opposite in rural sites.

School timetable in each class allowed to obtain more detailed information on the time spent in each specific microenvironment inside the schools. Although the classroom was the major indoor school microenvironment, children usually spent 1-2 hours in the canteen, and in some cases, the youngest also spent 1-3 hours in the bedroom after lunch (nap). For exposure estimation in each child, canteens and bedrooms were also considered whenever indoor air pollutants' concentrations there were available.

Parent-reported daily diaries also allowed obtaining information on the specific activities to build time-activity patterns for both pre- and primary school children, from both urban and rural sites, complemented with information from the class timetables and validated by the educators/ teachers. Time-activity patterns are represented in Figure S3 (Supplementary Material), and proportions are detailed in Figure S4 (Supplementary Material). Light activities dominated the period of indoor school. Although some moderate and heavy activities also occurred during

periods of indoor school, mainly associated with playing activities, they usually occurred associated with extracurricular activities. Those moderate and heavy activities were more common in children from urban sites. For each individual, short-term inhalation rates (IR) were obtained from the literature (U.S. Environmental Protection Agency (EPA), 2011), depending on the child's age and the type of activity. Then a mean IR was calculated for each age group of children in each site. Those IR were then used to estimate daily dose inhaled by each child, and they are represented in Table S2 (Supplementary Material).

Indoor air pollutants' concentrations of the several microenvironments studied were previously described in detail (Branco et al., 2019). Children's exposure to indoor air pollutants and inhaled doses in the studied nursery and primary schools were estimated and summarised in Table 2, allowing to evidence important results. Correlation coefficients (ρ, Spearman) between exposure and inhaled dose were detailed in Table 3. Those coefficients varied from 0.711 (CO_2) to 0.992 (NO_2), indicating moderate to strong correlations between exposure and inhaled dose. Usually, pre-schoolers were exposed to higher CO_2 levels and with higher variability, and inhaled higher doses of this gas, when compared to children from primary schools. Results from both formaldehyde and TVOC also revealed a higher variability of these pollutants' exposures and inhaled doses among the studied pre-schoolers. Regarding indoor air pollutants predominantly from outdoor sources (CO and O_3), both exposures and inhaled doses were higher at urban sites. Moreover, for NO_2 the age group seemed to have a greater influence than the location in both exposures and inhaled doses, being usually higher in pre-schoolers. Regarding particulate matter ($PM_{2.5}$ and PM_{10}), at urban sites, daily exposures were usually higher at nursery schools (pre-schoolers), while at rural sites daily exposures were usually higher at primary school. However, at both site locations, pre-schoolers inhaled higher $PM_{2.5}$ and PM_{10} doses when compared to the studied primary school children.

375 **Table 2 –** Descriptive statistics (median and interquartile range) of daily children's (n = 1530) exposure to indoor
376 air pollutants' and inhaled dose in the studied nursery and primary schools, from both urban and rural sites

Exposure	**CO_2 (mg m^{-3})**	**CO (µg m^{-3})**	**Formaldehyde (µg m^{-3})**	**NO_2 (µg m^{-3})**	**O_3 (µg m^{-3})**	**TVOC (µg m^{-3})**	**$PM_{2.5}$ (µg m^{-3})**	**PM_{10} (µg m^{-3})**
Population								
Mean	2315	2351	35.3	28.1	10.1	104.5	51.3	80.5
SD	851	1660	43.1	42.6	8.0	146.5	25.4	37.4
Pre-schoolers from urban sites								
Mean	1949	2257	39.8	51.2	13.6	78.6	54.7	88.0
SD	721	1610	52.5	55.4	8.8	122.7	23.4	43.3
Pre-schoolers from rural sites								
Mean	2335	1887	37.5	54.2	8.6	149.8	49.0	70.8
SD	1092	1460	52.6	52.3	4.7	189.8	29.9	37.0
Primary school children from urban sites								
Mean	2614	2766	27.9	8.3	12.3	84.5	42.8	66.9
SD	771	1484	34.5	16.0	8.1	80.3	13.1	19.2
Primary school children from rural sites								
Mean	2263	2179	39.5	15.1	4.6	128.2	57.0	91.6
SD	747	1916	34.5	21.1	4.7	189.2	29.7	39.0
Inhaled dose	**CO_2 (mg kg^{-1} d^{-1})**	**CO (µg kg^{-1} d^{-1})**	**Formaldehyde (µg kg^{-1} d^{-1})**	**NO_2 (µg m^{-3} d^{-1})**	**O_3 (µg m^{-3} d^{-1})**	**TVOC (µg kg^{-1} d^{-1})**	**$PM_{2.5}$ (µg kg^{-1} d^{-1})**	**PM_{10} (µg kg^{-1} d^{-1})**
Population								
Mean	71.9	73.6	1.1	1.0	0.3	3.2	1.7	2.6
SD	34.4	56.5	1.7	1.7	0.3	4.8	1.1	1.6
Pre-schoolers from urban sites								
Mean	76.8	91.9	1.6	2.1	0.5	3.2	2.2	3.5
SD	33.0	66.0	2.4	2.3	0.4	5.2	1.1	2.0
Pre-schoolers from rural sites								
Mean	94.2	76.0	1.5	2.0	0.3	5.4	2.0	2.9
SD	49.9	57.7	2.3	1.9	0.2	7.1	1.4	1.7
Primary school children from urban sites								
Mean	66.0	71.5	0.7	0.2	0.3	2.3	1.1	1.7
SD	23.9	44.6	0.8	0.5	0.2	2.3	0.5	0.8
Primary school children from rural sites								
Mean	60.2	54.7	1.0	0.4	0.1	3.1	1.5	2.4
SD	27.5	53.2	0.9	0.6	0.1	4.7	0.9	1.2

377 SD – standard deviation; TVOC – total volatile organic compounds

378

Table 3– Spearman's correlation coefficients (ρ) and their respective 95% confidence intervals (95%CI) between exposure and inhaled dose

Indoor air pollutant	ρ
CO_2	0.711
CO	0.909
Formaldehyde	0.977
NO_2	0.992
O_3	0.942
TVOC	0.985
$PM_{2.5}$	0.825
PM_{10}	0.781

3.3. Associations between indoor air pollutants and childhood asthma

Summary of the odds ratio (OR) and respective 95% confidence interval (CI) for each indoor air pollutant exposure and inhaled dose for each model were summarised in Table S3 (Supplementary Material). The same models were applied to other different types of transformation in the exposure variables (dichotomised by the median, dichotomised by the threshold, dichotomised by risk), being summarised in Tables S4 to S6 (Supplementary Material).

Results did not show statistically significant associations between exposure to any of the specific indoor air pollutant and diagnosed asthma. However, results showed that each IQR increase in the NO_2 and O_3 exposure was associated with an odds increase of reduced FEV_1/FVC in studied pre- and primary school children (OR = 1.33 (1.01, 1.75), and OR = 1.46 (0.98, 2.19), respectively), although those indoor air pollutants never exceeded the reference threshold of 200 µg m^{-3} (from the Portuguese legislation (Portaria nº 353-A/2013) and the World Health Organization (WHO, 2010) limit values) in the studied sites. Each IQR increase in O_3 inhaled dose was also associated with an odds increase of reduced FEV_1/FVC (OR = 1.38 (0.96, 1.99)). Children exposed to high NO_2 concentrations (higher than the median, 4.6 µg m^{-3}), had significantly increased odds of an active wheezing (OR = 1.62 (1.09, 2.43)). Children

exposed to high formaldehyde concentrations (higher than the median, 22.5 μg m^{-3}) had also significantly increased odds of a reduced FEV_1/FVC (OR = 1.87 (1.07, 3.26)), although that was not found when children were exposed to formaldehyde levels higher than the threshold, or when they were exposed at risk (in this study defined as occupying rooms where that threshold was exceeded). On the other hand, occupying rooms exceeding both $PM_{2.5}$ and PM_{10} thresholds significantly increased the odds of having reduced FEV_1 (respectively OR = 2.08 (1.04, 4.14), and OR = 3.19 (1.74, 5.87)). Analyses for exposures and inhaled doses led to similar results.

Except for $PM_{2.5}$ and PM_{10}, all other studied pollutants were weakly correlated (Figure S5), thus multipollutant multivariate logistic regression models were built to quantify the combined effects of exposure/ inhaled dose of all the studied gaseous indoor air pollutants and $PM_{2.5}$. OR and respective 95% CI are represented in Figure 2, by considering continuous inhaled dose of all the studied indoor air pollutants scaled by IQR. Corresponding results from exposure models were summarised in Figure S6 (Supplementary Material), and results from the same models applied to the other transformations (dichotomised by the median, dichotomised by the threshold, dichotomised by risk) in the exposure variables were summarised in Figures S7 and S8 (Supplementary Material).

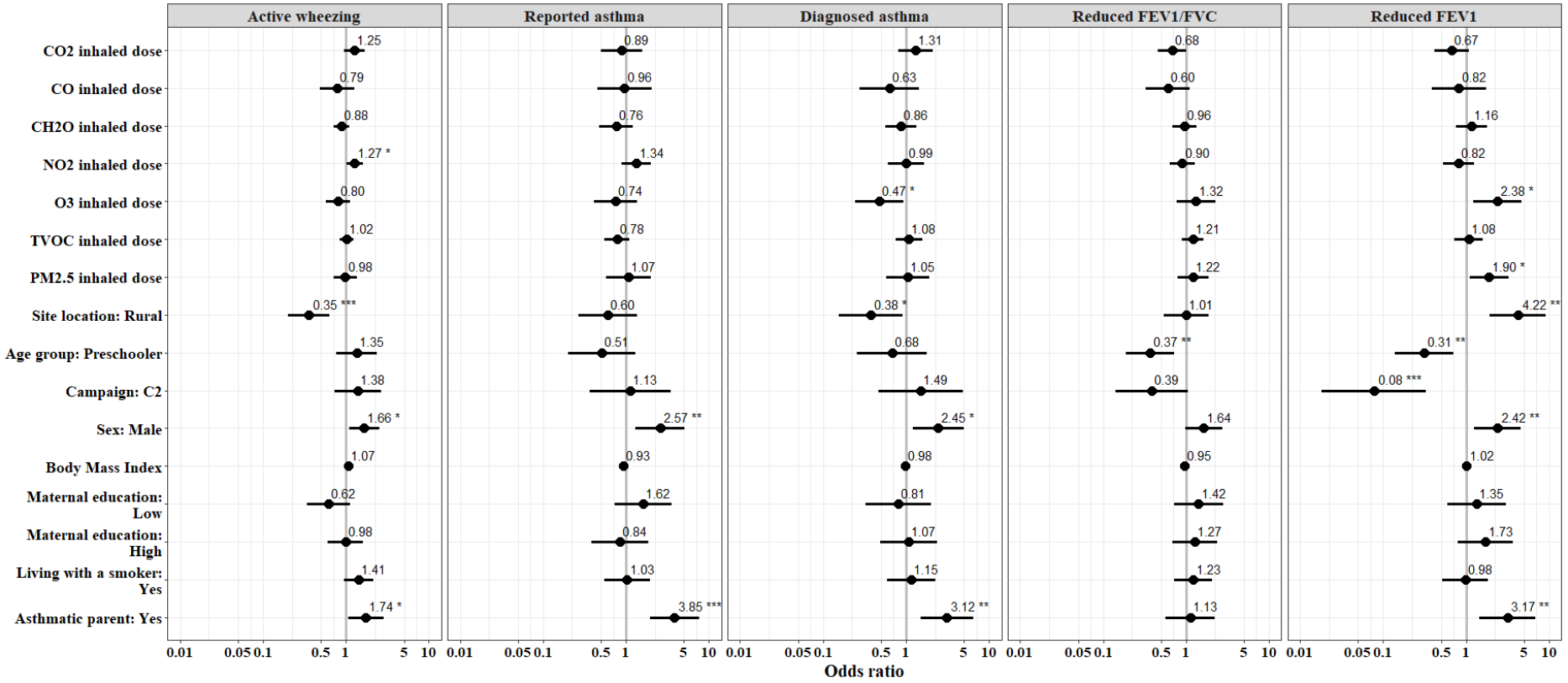


Figure 2 – Results from the multipollutant multivariate logistic regression models (adjusted odds ratio and 95% confidence intervals), when considering inhaled dose of indoor air pollutants scaled by the interquartile range and all the studied respiratory health outcomes (active wheezing, reported asthma, diagnosed asthma, reduced FEV_1/FVC and reduced FEV_1). * *p*-value < 0.05; ** *p*-value < 0.01; *** *p*-value < 0.001.

In these models, each IQR increase of exposure or inhaled dose was not associated with the odds increase of either reported/diagnosed asthma or reduced FEV_1/FVC. Nevertheless, in these multipollutant models, each IQR increase of NO_2 exposure (OR = 1.35 (1.00, 1.81)) and inhaled dose (OR = 1.27 (1.02, 1.59)) were both significantly associated with increased odds of active wheezing, while each IQR increase of both O_3 and $PM_{2.5}$ exposures (OR = 2.64 (1.24, 6.08), and OR = 1.98 (1.26, 3.10), respectively) and inhaled doses (OR = 2.38 (1.23, 4.63), and OR = 1.90 (1.11, 3.25), respectively) were significantly associated with reduced FEV_1. The latter was also found for unipollutant models. Similar results were also obtained from exposure and inhaled dose models of association.

To test for possible bias from non-randomised population selection, a sensitivity analysis was performed by testing the multipollutant multivariate logistic regression models (inhaled dose scaled by interquartile range) for all the studied health outcomes, for a stratum of the study population (female). Although with lower significance, results were quite similar to those obtained from the main analysis with the whole study population, confirming randomization in the selection of the study population (Figure S9, Supplementary Material).

In the same multipollutant approach, and although not always statistically significant, high (above the median) indoor air pollutants' exposures seemed to be associated with: i) active wheezing, namely due to NO_2 and TVOC; ii) diagnosed asthma, namely due to CO_2 and formaldehyde; iii) reduced FEV_1/FVC, namely due to formaldehyde and O_3 exposures (and TVOC inhaled dose, although not exposure); and iv) reduced FEV_1, namely due to CO_2, CO, formaldehyde, O_3 and $PM_{2.5}$ exposures (the same except CO_2 in the case of inhaled doses). Although not the same, results from exposure and inhaled dose models of association were similar for active wheezing, reduced FEV_1/FVC and reduced FEV_1 outcomes, while results were different for reported or diagnosed asthma outcomes.

448 Regarding covariates in these multipollutant models, site location had a statistically significant
449 contribution in most associations, with urban areas increasing the odds of all the studied health
450 outcomes except for reduced FEV_1. Being male and having at least one asthmatic parent also
451 increased the odds of all outcomes. Age group was also relevant, especially in reduced
452 FEV_1/FVC and reduced FEV_1 in which primary school children had statistically significant
453 increased odds of having those outcomes when compared with pre-schoolers.
454 Multinomial logistic regression models were used to estimate the effect of indoor air pollutants'
455 exposure/ inhaled dose on the probability that asthma diagnosed is in a particular category: no
456 asthma (as reference), asthma with aeroallergen sensitization and asthma without aeroallergen
457 sensitization. These results are summarised in Table 4 for $PM_{2.5}$ inhaled dose model and in
458 Tables S7 and S8 (Supplementary Material) for $PM_{2.5}$ exposure model and PM_{10} exposure and
459 inhaled dose models, respectively. Although not statistically significant, each IQR increase in
460 particulate matter exposure was associated with a higher increase in the odds of having asthma
461 diagnosed with aeroallergen sensitization (OR = 1.83 (0.90, 3.73) for $PM_{2.5}$; OR = 2.06 (0.83,
462 5.09) for PM_{10}) than of having asthma diagnosed without aeroallergen sensitization (OR = 1.08
463 (0.58, 2.00) for $PM_{2.5}$; OR = 1.18 (0.55, 2.55) for PM_{10}). Some covariates showed different
464 influence in the two studied categories of the outcome (diagnosed asthma with aeroallergen
465 sensitization, and diagnosed asthma without aeroallergen sensitization). In some cases, they had
466 a significantly higher influence on asthma without aeroallergen sensitization than in asthma
467 with aeroallergen sensitization, namely (as $PM_{2.5}$ inhaled dose model): i) having at least one
468 asthmatic parent (OR = 4.34 (1.35, 13.95), and OR = 2.10 (0.58, 7.61), respectively); and ii)
469 having a dog at home in child's first year of life (OR = 5.33 (1.46, 19.44), and OR = 0.38 (0.04,
470 3.63), respectively). In other cases, those covariates had significantly higher influence on
471 asthma with aeroallergen sensitization than on asthma without aeroallergen sensitization,
472 namely: i) being pre-schooler (OR = 0.04 (0.00, 0.43), and OR = 0.78 (0.22, 2.84),

473 respectively); and ii) being male (OR = 4.09 (1.09, 15.42), and OR = 1.51 (0.48, 4.71)). Identical
474 results were obtained for exposure and PM_{10} models.
475
476 **Table 4 –** Results from the multinomial logistic regression models for $PM_{2.5}$ inhaled dose:
477 adjusted odds ratio (aOR) for pollutant exposure, 95% confidence interval, and significance (*p*-
478 value)

Predictors	**%**	**Inhaled dose models**			
		Category 1 (asthma with aeroallergen sensitization)		**Category 2 (asthma without aeroallergen sensitization)**	
		aOR (95% CI)	***p*-value**	**aOR (95% CI)**	***p*-value**
$PM_{2.5}$ exposure / inhaled dose	-	1.81 (0.73-4.51)	0.202	1.11 (0.52-2.36)	0.786
Site location: Rural	40.2	0.33 (0.08-1.36)	0.125	0.86 (0.25-2.95)	0.805
Age group: Pre-schooler	42.3	0.04 (0.00-0.43)	**0.008**	0.78 (0.22-2.84)	0.711
Maternal education: Low	28.5	2.44 (0.55-10.79)	0.241	0.37 (0.09-1.55)	0.174
Maternal education: High	39.6	1.20 (0.27-5.35)	0.807	0.35 (0.10-1.29)	0.115
Living with a smoker: Yes	41.1	1.12 (0.35-3.62)	0.852	1.67 (0.55-5.11)	0.365
Sex: Male	49.0	4.09 (1.09-15.42)	**0.037**	1.51 (0.48-4.71)	0.482
Body Mass Index, *mean (sd)*	17.0 (3.0)	0.94 (0.77-1.16)	0.590	1.08 (0.91-1.29)	0.389
Asthmatic parent: Yes	15.1	2.10 (0.58-7.61)	0.258	4.34 (1.35-13.95)	**0.014**
Cat at home in child's 1st year	12.3	1.14 (0.20-6.38)	0.882	0.55 (0.10-3.14)	0.500
Cat at home in previous year	21.4	1.62 (0.41-6.46)	0.494	2.51 (0.71-8.84)	0.153
Dog at home in child's 1st year	21.1	0.38 (0.04-3.63)	0.401	5.33 (1.46-19.44)	**0.011**
Dog at home in previous year	28.2	0.48 (0.09-2.62)	0.396	0.98 (0.27-3.53)	0.978
Contact with farm animals in child's 1st year	20.9	1.64 (0.38-7.05)	0.507	0.33 (0.06-1.75)	0.194

479 aOR – adjusted odds ratio; CI – Confidence interval
480
481 **4. Discussion**
482 This study added new findings to the state-of-the-art. In the present study, exposures were
483 strongly correlated with inhaled doses in all the studied pollutants, and similar results were also
484 obtained from exposure and inhaled dose models of association, although inhalation exposure
485 models do not strictly take into account the inhaled dose of compounds, thus neglecting
486 inhalation rates and the bodyweight of the individuals.
487 Despite covering most of the relevant indoor air pollutants, this study did not found significant
488 associations between inhaled dose and childhood asthma prevalence. Still, it found significant
489 associations between inhaled dose to indoor air pollutants in nursery and primary schools and
490 other respiratory health issues in early childhood: reported wheezing (due to NO_2 exposure)
491 and reduced FEV_1 (due to $PM_{2.5}$ and O_3 exposure). In fact, and although NO_2 and O_3

concentrations indoor the studied nursery and primary schools were always below the 200 µg m^{-3} threshold (respectively from WHO and Portuguese legislation), children's exposure to them in schools seemed to be associated with increased odds of having those respiratory health issues during childhood. However, it is important to keep in mind that reduced FEV_1 might also reflect reduced lung growth, as in this study 64.0% of those with reduced FEV_1 also had reduced FEV_1/FVC.

As indoor air is a complex mixture of several gaseous compounds and suspended particulate matter, results of the association from multipollutant models have not always been similar to those from unipollutant models. This evidenced confounding effects on estimates between the air pollutants, indicating that multipollutant studies of association should be favoured to avoid biases.

Some findings from the present study were comparable to those from previous studies in the literature. Annesi-Maesano et al. (2012) also reported poor air quality in French primary schools, which varied significantly among schools and cities, related to an increased prevalence of clinical manifestations of asthma and rhinitis in schoolchildren. Moreover, previous findings from Rawi et al. (2015) indicated that the exposures to poor IAQ and increasing levels of indoor air pollutants' concentrations in pre-schools in Malaysia were associated with a reduction in lung function and with increasing reports of respiratory symptoms among pre-school children, namely wheezing ($PM_{2.5}$, PM_{10}, VOCs and CO). Another previous study, this time considering personal monitoring of 6-15 years old children living in the city of Rio de Janeiro, Brazil, also reported that even within acceptable levels most of the time, air pollution, especially PM_{10} and NO_2, was associated with a decrease in lung function (Castro et al., 2009). Findings from Mölter et al. (2013) also suggested that lifetime exposure to PM_{10} and NO_2 might be associated with reduced growth in FEV_1 in children when considering home, school and commuting between them. Ranzi et al. (2014) reported for outdoor air a clear link between exposure to NO_2

(estimated by land-use regression modelling) and respiratory symptoms in young children during their first 7 years of life, but only weak associations that seemed to increase with age. Mölter et al. (2015) reported no statistically significant association between exposure to selected ambient air pollution metrics (estimated by land-use regression modelling) and childhood asthma (although mainly positive associations were found) in a meta-analysis of five birth cohorts located in five large conurbations in Europe. In agreement, previous published studies reported that asthma exacerbation, severe respiratory symptoms and moderate airway obstruction on spirometry were observed in children due to various sources of indoor air pollution in households and schools (Liu et al., 2018).

Findings from this study also seemed to indicate that children sensitised to aeroallergens are more likely to develop childhood asthma due to indoor air pollutants' exposure in nursery and primary schools than those that are not sensitised. Dust mites, pollens and animal dander are among those common aeroallergens, which were often found on desktop surfaces in pre-schools and elementary schools (Kanchongkittiphon et al., 2014). Previous studies in literature also identified significant positive associations among $PM_{2.5}$ and NO_2 and sensitised asthmatics (Annesi-Maesano et al., 2012).

In this study, respiratory symptoms were common at younger ages (pre-schoolers), but they might indicate other pathologies rather than asthma (Yeh et al., 2011). Wheeze is the most common symptom associated with asthma in children aged 5 years old or younger. It might occur in several different patterns, but a wheeze that occurs recurrently, during sleep, or with triggers such as activity, laughing, or crying, might be consistent with a diagnosis of asthma. However, wheezing in this age group is a highly heterogeneous condition, and not all wheezing indicate asthma. Many young children may wheeze with viral infections, typically with upper respiratory tract infections (respiratory syncytial virus and rhinovirus).

Although results showed a strong correlation between reported and diagnosed asthma, a higher reported asthma prevalence evidenced misdiagnosed asthma in the study population. In this study, reported asthma represented those who answered "Yes" to the question "Does the child have or ever had asthma?", and those were probably diagnosed by outdated criteria or by criteria merely based on the history of characteristic symptoms without lung function testing or any other medical test to assist the diagnosis. Lung function testing is not easily accessible to Portuguese children, especially in rural areas. There were a limited number of studies in the literature comparing urban with rural areas, but, in general, children from urban sites presented higher asthma prevalence and asthma-like symptoms (Oluwole et al., 2018) as in the present study. Higher asthma prevalence in older children (primary school age) might be explained by the asthma prevalence continuous increase during childhood reported in previous studies (Bjerg-Backlund et al., 2006), although it might also be explained by a higher robustness in asthma diagnosis given child's increase capability of using diagnostic adjuncts. Children under 5 years old present a number of special challenges regarding pulmonary function testing and asthma diagnosis (Beydon et al., 2007), but previous recent studies including from the authors revealed its feasibility (Branco et al., 2020). In fact, including children from different ages allowed understanding variances at different childhood stages and influences of different exposure patterns.

Higher inhaled dose of CO_2 in younger ages (pre-schoolers) in comparison with older children (primary school age) was in agreement with previous studies reporting high levels of CO_2 in classrooms (Branco et al., 2015b; Mainka and Zajusz-Zubek, 2015) and could have been mainly caused by overcrowding and deficit air exchange (insufficient ventilation) (Branco et al., 2019). Pre-schoolers' classrooms were usually more crowded and less ventilated to keep the thermal comfort – to prevent heat loss in cold season and heat incoming in the warm season. As younger children are more susceptible to temperature changes, there are usually more concerns about

thermal comfort with them than with older ones. Moreover, younger children usually have activities with greater mobility, thus contributing also to higher particulate matter exposure and higher inhalation rates, concomitantly with a lower body weight, leading to higher inhaled doses. Those aspects together with specific activities and sources (painting, crafts, specific furniture, among others) in classrooms for pre-schoolers might have contributed to their higher exposure to other gaseous indoor air pollutants (VOCs and formaldehyde), namely VOCs and formaldehyde, in comparison with older children (primary school) (Branco et al., 2019). In previous studies from the authors, particulate matter ($PM_{2.5}$ and PM_{10}) was mainly originated in indoor sources, while NO_2 was expected to come mainly from indoor sources in canteens, and mainly from outdoor air in the other cases (classrooms and dormitories) (Branco et al., 2014a; Branco et al., 2019; Nunes et al., 2015; Sousa et al., 2012b). On the other hand, CO and O_3 seemed to have been greatly influenced by outdoor air penetration explaining the observed differences between urban and rural sites (Nunes et al., 2016).

Although not considered a pollutant per se in indoor environments, CO_2 is often considered a useful indicator for adequate ventilation (Salthammer et al., 2016). However, results indicated that CO_2 was not significantly associated with the increase in the odds of having any of the studied respiratory asthma outcomes. Thus, studies of the association between indoor air pollutants' exposures in school indoor environments and children's respiratory health should not be limited to CO_2 as a global indicator of IAQ.

The objectives of this study were achieved. Nevertheless, it is not free from limitations that should be taken into account when interpreting its findings. This study was designed as a cross-sectional study, mainly to allow comparing/adjusting many different variables at the same time with little or no additional cost, in comparison with longitudinal study design. Still, with this type of design authors may not provide definite information about cause-and-effect relationships, as it was not possible to know when asthma was developed. In future studies, a

longitudinal approach should be favoured. Although sample size allowed to have acceptable statistical power, a bigger sample size would allow performing stratifications of the study population, namely by site location (urban and rural) and by age group (pre- and primary schoolchildren) to deepen the analysis.

This study did not collect information on the history of other respiratory illnesses such as bronchitis or pneumonia which might also be linked to reduced FEV_1, neither on viral respiratory infections which might be linked to wheezing instead of asthma. Although used as an outcome, parent-reported wheezing was not confirmed by a clinician in this study, thus it might have included some error as parents might describe any noisy breathing as "wheezing" (Mellis, 2009). This study did not also consider complete information about individual's atopy, as information about eczema, itchy rash or even parents' history of atopic disease were not collected. Lung function was only assessed (by spirometry) in children reporting symptoms or reporting previously diagnosed asthma in the questionnaires, which limited the analysis of the impact of indoor air pollutants on both reduced FEV_1/FVC and reduced FEV_1 as there were no asymptomatic population as reference. Aeroallergen sensitization was only assessed (skin prick tests) in the first campaign, which limited the number of individuals in the study population in multinomial logistic regression modelling, thus reducing the statistical significance of their results.

This study has considered relevant confounders for the studied associations, namely site location, child's age, gender, BMI and family history of asthma, and the scope of this study was only indoor scholar microenvironments in nursery and primary schools. However, previous studies have linked exposure to outdoor air with adverse respiratory health outcomes. Outdoor air pollution contributes as a major source for IAP, particularly in schools, where fireplaces do not exist, cooking is confined to the kitchen (not used by children), and smoking is not allowed. Although time-activity-location patterns indicated that children spent less than 10% of the day

outdoors and less than 1 hour per day in transport (commuting), children's exposure in those environments might introduce some confounding effect in the associations studied. Due to the lack of that exposure data, models were not controlled for them, which is a limitation of this study. Not considering the confounding effect of exposure to outdoor air, might explain the negative statistically significant associations (OR < 1) found between asthma outcomes and O_3 in some specific multipollutant models (Sousa et al., 2013; Sousa et al., 2009). Likewise, home exposure was not possible to quantify, although it could have also introduced confounding in the studied associations. While models were adjusted for relevant indirect measures of home exposure, namely mother education as a measure of the family socioeconomic status, exposure to tobacco smoke at home, contact with pets and farm animals, other potential confounders missed including cooking, ventilation, heating and moulded spots or leaking ceiling.

Additionally, using a microenvironmental modelling approach is not free from bias, although it is considered the best cost-effective approach to estimate children's exposure to air pollution (Branco et al., 2014b). Thus, it might be important to validate these results with personal monitoring in a future study. Moreover, accompanying parent-based diaries with wearable sensors containing accelerometer and GPS might be an option in future studies to improve data of time-activity-location patterns.

5. Conclusions

This study represented the complex mixture of several air pollutants that occur in indoor air by considering multipollutant models of association. Nevertheless, and although this study covered most of the considered major indoor air pollutants of nursery and primary schools environments, overall it found no evidence of a significant association with the prevalence of childhood asthma. However, other asthma-related outcomes were associated with children's exposure to IAP in nursery and primary schools, namely reported active wheezing associated

641 with higher NO_2 and reduced FEV_1 associated with higher O_3 and $PM_{2.5}$. Although NO_2 and O_3
642 were always below thresholds, and their exceedances were not common indoors in schools, this
643 study suggests they seemed to have a negative impact on children's respiratory health.
644 Moreover, this study evidenced that children sensitised to common aeroallergens are more
645 likely to develop asthma during childhood for being exposed to particulate matter in nursery
646 and primary schools. These findings support the urgent need for mitigation measures to reduce
647 indoor air pollution in schools, especially particulate matter, to reduce its health burden to
648 children. Future research should consider a longitudinal design to study causality, and to allow
649 assessing the impact that IAP on asthma at pre-school age will have on the impact on primary
650 school age.
651

652 **Acknowledgements**

653 The authors are grateful to the children and their parents/caregivers, as well as to the governance
654 bodies and staff of all the nursery and primary schools' involved in this study. This work was
655 financially supported by: project UIDB/00511/2020 - Laboratory for Process Engineering,
656 Environment, Biotechnology and Energy – LEPABE funded by national funds through
657 FCT/MCTES (PIDDAC); project PTDC/SAU-SAP/121827/2010 funded by FCT, COMPETE,
658 QREN and EU; grant SFRH/BD/97104/2013 for PTBS Branco, funded by FCT, POPH/QREN
659 and European Social Fund (ESF).

References

Amato, F., Rivas, I., Viana, M., Moreno, T., Bouso, L., Reche, C., et al., 2014. Sources of indoor and outdoor PM2.5 concentrations in primary schools. Science of The Total Environment. 490**,** 757-765.

Annesi-Maesano, I., Agabiti, N., Pistelli, R., Couilliot, M. F., Forastiere, F., 2003. Subpopulations at increased risk of adverse health outcomes from air pollution. European Respiratory Journal. 21**,** 57s-63s.

Annesi-Maesano, I., Baiz, N., Banerjee, S., Rudnai, P., Rive, S., the, S. G., 2013. Indoor Air Quality and Sources in Schools and Related Health Effects. Journal of Toxicology and Environmental Health, Part B. 16**,** 491-550.

Annesi-Maesano, I., Hulin, M., Lavaud, F., Raherison, C., Kopferschmitt, C., de Blay, F., et al., 2012. Poor air quality in classrooms related to asthma and rhinitis in primary schoolchildren of the French 6 Cities Study. Thorax. 67**,** 682-688.

Beydon, N., Davis, S. D., Lombardi, E., Allen, J. L., Arets, H. G., Aurora, P., et al., 2007. An official American Thoracic Society/European Respiratory Society statement: pulmonary function testing in preschool children. Am J Respir Crit Care Med. 175**,** 1304-45.

Bjerg-Backlund, A., Perzanowski, M. S., Platts-Mills, T., Sandstrom, T., Lundback, B., Ronmark, E., 2006. Asthma during the primary school ages--prevalence, remission and the impact of allergic sensitization. Allergy. 61**,** 549-55.

Branco, P., Alvim-Ferraz, M. C. M., Martins, F. G., Ferraz, C., Vaz, L. G., Sousa, S. I. V., 2020. Asthma in urban and rural pre- and primary schoolchildren according to the latest GINA definition. Allergy.

684 Branco, P. T., Nunes, R. A., Alvim-Ferraz, M. C., Martins, F. G., Sousa, S. I., 2015a. Children's
685 exposure to indoor air in urban nurseries - Part II: Gaseous pollutants' assessment. Environ Res.
686 142**,** 662-670.

687 Branco, P. T. B. S., Alvim-Ferraz, M. C. M., Martins, F. G., Sousa, S. I. V., 2014a. Indoor air
688 quality in urban nurseries at Porto city: Particulate matter assessment. Atmospheric
689 Environment. 84**,** 133-143.

690 Branco, P. T. B. S., Alvim-Ferraz, M. C. M., Martins, F. G., Sousa, S. I. V., 2014b. The
691 microenvironmental modelling approach to assess children's exposure to air pollution – A
692 review. Environmental Research. 135**,** 317-332.

693 Branco, P. T. B. S., Alvim-Ferraz, M. C. M., Martins, F. G., Sousa, S. I. V., 2015b. Children's
694 exposure to indoor air in urban nurseries-part I: CO2 and comfort assessment. Environmental
695 Research. 140**,** 1-9.

696 Branco, P. T. B. S., Alvim-Ferraz, M. C. M., Martins, F. G., Sousa, S. I. V., 2019. Quantifying
697 indoor air quality determinants in urban and rural nursery and primary schools. Environmental
698 Research. 108534.

699 Branco, P. T. B. S., Nunes, R. A. O., Alvim-Ferraz, M. C. M., Martins, F. G., Ferraz, C., Vaz,
700 L. G., et al., 2016. Asthma prevalence and risk factors in early childhood at Northern Portugal.
701 Revista Portuguesa de Pneumologia (English Edition). 22**,** 146-150.

702 Breysse, P. N., Diette, G. B., Matsui, E. C., Butz, A. M., Hansel, N. N., McCormack, M. C.,
703 2010. Indoor Air Pollution and Asthma in Children. Proceedings of the American Thoracic
704 Society. 7**,** 102-106.

Castro, H. A., Cunha, M. F., Mendonca, G. A., Junger, W. L., Cunha-Cruz, J., Leon, A. P., 2009. Effect of air pollution on lung function in schoolchildren in Rio de Janeiro, Brazil. Rev Saude Publica. 43**,** 26-34.

Cui, X., Li, Z., Teng, Y., Barkjohn, K. K., Norris, C. L., Fang, L., et al., 2020. Association Between Bedroom Particulate Matter Filtration and Changes in Airway Pathophysiology in Children With Asthma. JAMA Pediatrics. 174**,** 533-542.

Day, D. B., Xiang, J., Mo, J., Li, F., Chung, M., Gong, J., et al., 2017. Association of Ozone Exposure With Cardiorespiratory Pathophysiologic Mechanisms in Healthy Adults. JAMA Internal Medicine. 177**,** 1344-1353.

Decreto-Lei nº 79/2006. Ministério das Obras Públicas, Transportes e Comunicações. Diário da República - I Série, Nº 67, 2416-2468.

Ferrero, A., Esplugues, A., Estarlich, M., Llop, S., Cases, A., Mantilla, E., et al., 2017. Infants' indoor and outdoor residential exposure to benzene and respiratory health in a Spanish cohort. Environmental Pollution. 222**,** 486-494.

GINA, Global Initiativa for Asthma. Global Strategy for Asthma Management and Prevention, 2018. Available from: www.ginasthma.org. 2018.

Goldizen, F. C., Sly, P. D., Knibbs, L. D., 2016. Respiratory effects of air pollution on children. Pediatr Pulmonol. 51**,** 94-108.

Götschi, T., Heinrich, J., Sunyer, J., Künzli, N., 2008. Long-Term Effects of Ambient Air Pollution on Lung Function: A Review. Epidemiology. 19**,** 690-701.

Hauptman, M., Phipatanakul, W., 2015. The school environment and asthma in childhood. Asthma research and practice. 1**,** 12.

He, L., Li, Z., Teng, Y., Cui, X., Barkjohn, K. K., Norris, C., et al., 2020. Associations of personal exposure to air pollutants with airway mechanics in children with asthma. Environment International. 138**,** 105647.

Huang, S., Garshick, E., Weschler, L. B., Hong, C., Li, J., Li, L., et al., 2020. Home environmental and lifestyle factors associated with asthma, rhinitis and wheeze in children in Beijing, China. Environmental Pollution. 256**,** 113426.

Kanchongkittiphon, W., Sheehan, W. J., Friedlander, J., Chapman, M. D., King, E. M., Martirosyan, K., et al., 2014. Allergens on desktop surfaces in preschools and elementary schools of urban children with asthma. Allergy. 69**,** 960-3.

Khreis, H., Kelly, C., Tate, J., Parslow, R., Lucas, K., Nieuwenhuijsen, M., 2017. Exposure to traffic-related air pollution and risk of development of childhood asthma: A systematic review and meta-analysis. Environment International. 100**,** 1-31.

Liu, H.-Y., Dunea, D., Iordache, S., Pohoata, A., 2018. A Review of Airborne Particulate Matter Effects on Young Children's Respiratory Symptoms and Diseases. Atmosphere. 9**,** 150.

Mainka, A., Zajusz-Zubek, E., 2015. Indoor Air Quality in Urban and Rural Preschools in Upper Silesia, Poland: Particulate Matter and Carbon Dioxide. International Journal of Environmental Research and Public Health. 12**,** 7697.

Mellis, C., 2009. Respiratory noises: how useful are they clinically? Pediatr Clin North Am. 56**,** 1-17, ix.

Migueres, M., Dávila, I., Frati, F., Azpeitia, A., Jeanpetit, Y., Lhéritier-Barrand, M., et al., 2014. Types of sensitization to aeroallergens: definitions, prevalences and impact on the diagnosis and treatment of allergic respiratory disease. Clinical and Translational Allergy. 4**,** 1-8.

749 Mölter, A., Agius, R. M., de Vocht, F., Lindley, S., Gerrard, W., Lowe, L., et al., 2013. Long-
750 term exposure to PM10 and NO2 in association with lung volume and airway resistance in the
751 MAAS birth cohort. Environ Health Perspect. 121**,** 1232-8.

752 Mölter, A., Simpson, A., Berdel, D., Brunekreef, B., Custovic, A., Cyrys, J., et al., 2015. A
753 multicentre study of air pollution exposure and childhood asthma prevalence: the ESCAPE
754 project. European Respiratory Journal. 45**,** 610-624.

755 Norbäck, D., Lu, C., Wang, J., Zhang, Y., Li, B., Zhao, Z., et al., 2018. Asthma and rhinitis
756 among Chinese children — Indoor and outdoor air pollution and indicators of socioeconomic
757 status (SES). Environment International. 115**,** 1-8.

758 Norback, D., Lu, C., Zhang, Y., Li, B., Zhao, Z., Huang, C., et al., 2019. Onset and remission
759 of childhood wheeze and rhinitis across China - Associations with early life indoor and outdoor
760 air pollution. Environ Int. 123**,** 61-69.

761 Nunes, R. A. O., Branco, P., Alvim-Ferraz, M. C. M., Martins, F. G., Sousa, S. I. V., 2016.
762 Gaseous pollutants on rural and urban nursery schools in Northern Portugal. Environ Pollut.
763 208**,** 2-15.

764 Nunes, R. A. O., Branco, P. T. B. S., Alvim-Ferraz, M. C. M., Martins, F. G., Sousa, S. I. V.,
765 2015. Particulate matter in rural and urban nursery schools in Portugal. Environmental
766 Pollution. 202**,** 7-16.

767 Oluwole, O., Rennie, D. C., Senthilselvan, A., Dyck, R., Afanasieva, A., Adamko, D. J., et al.,
768 2018. Asthma diagnosis among children along an urban-rural gradient. J Asthma. 1-11.

769 Patelarou, E., Tzanakis, N., Kelly, F., 2015. Exposure to Indoor Pollutants and Wheeze and
770 Asthma Development during Early Childhood. International Journal of Environmental
771 Research and Public Health. 12**,** 3993.

772 Portaria nº 353-A/2013.Ministérios do Ambiente, Odenamento do Território e Energia, da
773 Saúde e da Solidariedade, Emprego e Segurança Social - Diário da República - 1ª Série, Nº 253,
774 6644(2)-6644(9).

775 Quanjer, P. H., Stanojevic, S., Cole, T. J., Baur, X., Hall, G. L., Culver, B. H., et al., 2012.
776 Multi-ethnic reference values for spirometry for the 3–95-yr age range: the global lung function
777 2012 equations. Eur Respir J. 2012.

778 Ranzi, A., Porta, D., Badaloni, C., Cesaroni, G., Lauriola, P., Davoli, M., et al., 2014. Exposure
779 to air pollution and respiratory symptoms during the first 7 years of life in an Italian birth cohort.
780 Occup Environ Med. 71**,** 430-6.

781 Rawi, N. A., Jalaludin, J., Chua, P. C., 2015. Indoor Air Quality and Respiratory Health among
782 Malay Preschool Children in Selangor. Biomed Res Int. 2015**,** 248178.

783 Sá, J., Branco, P., Alvim-Ferraz, M., Martins, F., Sousa, S., 2017. Evaluation of Low-Cost
784 Mitigation Measures Implemented to Improve Air Quality in Nursery and Primary Schools.
785 International Journal of Environmental Research and Public Health. 14**,** 585.

786 Salonen, H., Salthammer, T., Morawska, L., 2019. Human exposure to NO2 in school and office
787 indoor environments. Environment International. 130**,** 104887.

788 Salthammer, T., Uhde, E., Schripp, T., Schieweck, A., Morawska, L., Mazaheri, M., et al.,
789 2016. Children's well-being at schools: Impact of climatic conditions and air pollution.
790 Environment International. 94**,** 196-210.

791 Simoni, M., Annesi-Maesano, I., Sigsgaard, T., Norback, D., Wieslander, G., Nystad, W., et
792 al., 2010. School air quality related to dry cough, rhinitis and nasal patency in children.
793 European Respiratory Journal. 35**,** 742-749.

794 Sousa, S. I., Alvim-Ferraz, M. C., Martins, F. G., 2013. Health effects of ozone focusing on
795 childhood asthma: what is now known--a review from an epidemiological point of view.
796 Chemosphere. 90**,** 2051-8.

797 Sousa, S. I., Alvim-Ferraz, M. C., Martins, F. G., Pereira, M. C., 2009. Ozone exposure and its
798 influence on the worsening of childhood asthma. Allergy. 64**,** 1046-55.

799 Sousa, S. I., Ferraz, C., Alvim-Ferraz, M. C., Vaz, L. G., Marques, A. J., Martins, F. G., 2012a.
800 Indoor air pollution on nurseries and primary schools: impact on childhood asthma--study
801 protocol. BMC Public Health. 12**,** 435.

802 Sousa, S. I. V., Alvim-Ferraz, M. C., Martins, F. G., 2012b. Indoor PM10 and PM2.5 at
803 Nurseries and Primary Schools. Advanced Materials Research. 433-440**,** 385-390.

804 Thurston, G. D., Kipen, H., Annesi-Maesano, I., Balmes, J., Brook, R. D., Cromar, K., et al.,
805 2017. A joint ERS/ATS policy statement: what constitutes an adverse health effect of air
806 pollution? An analytical framework. Eur Respir J. 49.

807 Tsui, H.-C., Chen, C.-H., Wu, Y.-H., Chiang, H.-C., Chen, B.-Y., Guo, Y. L., 2018. Lifetime
808 exposure to particulate air pollutants is negatively associated with lung function in non-
809 asthmatic children. Environmental Pollution. 236**,** 953-961.

810 U.S. Environmental Protection Agency (EPA), 2011. Exposure Factors Handbook: 2011
811 Edition. National Center for Environmental Assessment, Washington, DC; EPA/600/R-
812 09/052F. Available from the National Technical Information Service, Springfield, VA, and
813 online at http://www.epa.gov/ncea/efh.

814 WHO, WHO guidelines for indoor air quality: selected pollutants. World Health Organisation,
815 Regional office in Europe, European Series, Copenhagen, Denmark, 2010.

816 Yeh, K. W., Ou, L. S., Yao, T. C., Chen, L. C., Lee, W. I., Huang, J. L., 2011. Prevalence and
817 risk factors for early presentation of asthma among preschool children in Taiwan. Asian Pac J
818 Allergy Immunol. 29**,** 120-6.

819

SUPPLEMENTARY MATERIAL

Impact of indoor air pollution in nursery and primary schools on childhood asthma

Pedro T.B.S. Branco[1], Maria C.M. Alvim-Ferraz[1], Fernando G. Martins[1],

Catarina Ferraz[2], Luísa G. Vaz[2], Sofia I.V. Sousa[1]*

[1] LEPABE – Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal

[2] Departamento de Pediatria (UAG-MC), Centro Hospitalar Universitário de São João (CHUSJ), Alameda Prof. Hernâni Monteiro, 4200-319, Porto, Portugal

*Corresponding author:

Telephone: +351 22 508 2262

Fax: +351 22 508 1449

E-mail address: sofia.sousa@fe.up.pt

Postal address: Rua Dr. Roberto Frias, 4200-465, E221, Porto, Portugal

Table S1– Aeroallergen sensitisation in the subpopulation which reported asthma and/or asthmatic symptoms (n = 341)

Allergen	Subpopulation (n=341)		Pre-schoolers (n=117)		Primary school children (n=224)		*p*-value	Urban (n=196)		Rural (n=145)		*p*-value
	n	%	n	%	n	%		n	%	n	%	
Dust mites	85	25	19	16	66	29	**0.01***	62	32	23	16	**< 0.01***
Pollens	37	11	20	17	17	8	0.37	42	21	27	19	0.62
Animal dander	50	15	17	15	33	15	1.00	26	13	24	17	0.49
Sensitisation												
Monosensitised	58	17	12	10	46	21	**0.02***	42	21	16	11	**0.02***
Polysensitised	62	18	18	15	44	20	0.41	37	19	25	17	0.81

* statistically significant (*p*-value < 0.05)

Table S2– Calculated hourly mean inhalation rates used to estimate daily inhaled doses

Hour	Pre-schoolers		Primary school children	
	Urban	Rural	Urban	Rural
0	0.273	0.272	0.288	0.288
1	0.273	0.272	0.288	0.288
2	0.273	0.272	0.288	0.288
3	0.273	0.272	0.288	0.288
4	0.273	0.272	0.288	0.288
5	0.273	0.272	0.288	0.288
6	0.279	0.274	0.289	0.296
7	0.363	0.375	0.382	0.455
8	0.628	0.638	0.633	0.652
9	0.657	0.670	0.662	0.661
10	0.660	0.673	0.659	0.790
11	0.671	0.665	0.660	0.661
12	0.660	0.657	0.668	0.675
13	0.638	0.690	0.671	0.814
14	0.707	0.654	0.668	0.663
15	0.700	0.665	0.678	0.673
16	0.867	0.677	0.698	0.811
17	0.981	0.767	0.801	0.670
18	1.082	1.095	1.022	0.859
19	0.884	1.049	1.095	0.922
20	0.798	0.844	0.825	0.782
21	0.661	0.628	0.670	0.677
22	0.290	0.292	0.307	0.299
23	0.286	0.286	0.297	0.294

Table S3 – Summary results of each unipollutant multivariate exposure and inhaled dose models, by considering continuous exposure/ inhaled dose scaled by the interquartile range (IQR): adjusted odds ratio (aOR) for pollutant exposure, its 95% confidence interval (CI), and significance (*p*-value)

Exposure model	**Active wheezing**		**Reported asthma**		**Diagnosed asthma**		**Reduced FEV_1/FVC**		**Reduced FEV_1**	
	aOR (95% CI)	***p*-value**	**aOR (95% CI)**	***p*-value**	**aOR (95% CI)**	***p*-value**	**aOR (95% CI)**	***p*-value**	**aOR (95% CI)**	***p*-value**
CO_2	0.90 (0.69,1.16)	0.406	0.69 (0.45,1.06)	0.082	0.94 (0.61,1.46)	0.797	0.85 (0.63,1.15)	0.293	0.99 (0.69,1.41)	0.951
CO	0.69 (0.46,1.03)	0.069	1.03 (0.57,1.85)	0.927	0.75 (0.40,1.39)	0.359	0.59 (0.38,0.91)	**0.015**	0.49 (0.30,0.81)	**0.005**
Formaldehyde	0.69 (0.50,0.96)	**0.019**	0.41 (0.20,0.82)	**0.003**	0.66 (0.37,1.21)	0.148	0.82 (0.53,1.26)	0.351	1.05 (0.63,1.73)	0.863
NO_2	1.17 (0.96,1.42)	0.120	1.03 (0.70,1.52)	0.882	0.89 (0.58,1.34)	0.560	1.33 (1.01,1.75)	**0.047**	1.30 (0.89,1.91)	0.185
O_3	1.06 (0.80,1.41)	0.668	1.16 (0.73,1.83)	0.537	0.82 (0.51,1.33)	0.426	1.46 (0.98,2.19)	0.060	2.71 (1.54,4.75)	< **0.001**
TVOC	1.12 (0.90,1.40)	0.330	0.69 (0.44,1.11)	0.098	0.83 (0.53,1.28)	0.379	1.15 (0.84,1.58)	0.373	0.90 (0.59,1.37)	0.615
$PM_{2.5}$	0.92 (0.72,1.17)	0.486	0.93 (0.63,1.36)	0.702	1.04 (0.70,1.54)	0.850	1.21 (0.93,1.59)	0.162	1.82 (1.34,2.48)	< **0.001**
PM_{10}	0.87 (0.64,1.17)	0.339	0.94 (0.58,1.52)	0.800	1.08 (0.66,1.75)	0.768	1.11 (0.78,1.58)	0.566	2.13 (1.42,3.18)	< **0.001**
Inhaled dose model	**Active wheezing**		**Reported asthma**		**Diagnosed asthma**		**Reduced FEV_1/FVC**		**Reduced FEV_1**	
	aOR (95% CI)	***p*-value**	**aOR (95% CI)**	***p*-value**	**aOR (95% CI)**	***p*-value**	**aOR (95% CI)**	***p*-value**	**aOR (95% CI)**	***p*-value**
CO_2	1.13 (0.90,1.43)	0.299	0.67 (0.43,1.06)	0.072	1.02 (0.68,1.52)	0.936	0.88 (0.67,1.14)	0.326	1.07 (0.79,1.44)	0.663
CO	0.85 (0.59,1.23)	0.396	1.06 (0.61,1.86)	0.837	0.78 (0.42,1.44)	0.419	0.62 (0.40,0.94)	**0.023**	0.43 (0.25,0.73)	**0.001**
Formaldehyde	0.88 (0.73,1.05)	0.133	0.54 (0.31,0.93)	**0.005**	0.80 (0.54,1.18)	0.206	0.92 (0.72,1.17)	0.463	1.06 (0.79,1.42)	0.723
NO_2	1.15 (0.99,1.33)	0.071	1.04 (0.78,1.39)	0.779	0.89 (0.64,1.24)	0.486	1.16 (0.94,1.43)	0.158	1.13 (0.84,1.52)	0.416
O_3	1.14 (0.90,1.45)	0.287	1.05 (0.69,1.60)	0.834	0.85 (0.54,1.33)	0.469	1.38 (0.96,1.99)	0.080	2.85 (1.70,4.77)	< **0.001**
TVOC	1.10 (0.95,1.28)	0.192	0.80 (0.58,1.11)	0.156	0.95 (0.71,1.25)	0.698	1.08 (0.90,1.31)	0.411	0.93 (0.72,1.20)	0.547
$PM_{2.5}$	0.98 (0.75,1.29)	0.904	0.84 (0.53,1.34)	0.461	0.99 (0.61,1.60)	0.975	1.08 (0.79,1.48)	0.612	1.94 (1.36,2.76)	< **0.001**
PM_{10}	0.95 (0.74,1.22)	0.658	0.87 (0.56,1.34)	0.523	1.01 (0.65,1.57)	0.962	0.96 (0.70,1.33)	0.819	1.86 (1.31,2.65)	< **0.001**

aOR – odds ratio; CI – Confidence interval

Table S4 – Summary results of each unipollutant multivariate exposure and inhaled dose models, considering exposure factorised by median as cutoff: adjusted odds ratio (aOR), its 95% confidence interval, and significance (*p*-value)

Exposure model	**Active wheezing**		**Reported asthma**		**Diagnosed asthma**		**Reduced FEV_1/FVC**		**Reduced FEV_1**	
	aOR (95% CI)	***p*-value**	**aOR (95% CI)**	***p*-value**	**aOR (95% CI)**	***p*-value**	**aOR (95% CI)**	***p*-value**	**aOR (95% CI)**	***p*-value**
CO_2	0.80 (0.54,1.17)	0.248	0.56 (0.31,1.02)	0.056	0.90 (0.49,1.65)	0.725	0.81 (0.51,1.29)	0.373	1.06 (0.61,1.84)	0.827
CO	0.69 (0.46,1.04)	0.073	0.84 (0.44,1.58)	0.582	0.51 (0.26,1.01)	0.051	0.48 (0.28,0.79)	**0.004**	0.56 (0.31,0.99)	**0.047**
Formaldehyde	0.80 (0.51,1.24)	0.307	0.47 (0.23,0.95)	**0.030**	1.19 (0.60,2.39)	0.621	1.87 (1.07,3.26)	**0.028**	1.43 (0.75,2.73)	0.283
NO_2	1.62 (1.09,2.43)	**0.017**	0.90 (0.49,1.67)	0.748	0.89 (0.47,1.69)	0.729	1.48 (0.88,2.48)	0.135	1.36 (0.69,2.70)	0.371
O_3	1.24 (0.83,1.84)	0.297	1.24 (0.67,2.31)	0.494	1.14 (0.59,2.19)	0.694	1.36 (0.84,2.21)	0.210	2.70 (1.45,5.01)	**0.001**
TVOC	0.94 (0.64,1.37)	0.739	0.46 (0.25,0.85)	**0.011**	0.65 (0.34,1.21)	0.169	1.04 (0.65,1.67)	0.868	0.87 (0.50,1.53)	0.638
$PM_{2.5}$	0.77 (0.51,1.17)	0.225	1.07 (0.55,2.09)	0.837	0.94 (0.46,1.89)	0.857	1.14 (0.67,1.92)	0.627	2.43 (1.29,4.61)	**0.005**
PM_{10}	0.74 (0.49,1.12)	0.148	1.25 (0.64,2.45)	0.515	0.80 (0.40,1.61)	0.531	1.11 (0.65,1.89)	0.708	3.54 (1.82,6.88)	**< 0.001**
Inhaled dose model	**Active wheezing**		**Reported asthma**		**Diagnosed asthma**		**Reduced FEV_1/FVC**		**Reduced FEV_1**	
	aOR (95% CI)	***p*-value**	**aOR (95% CI)**	***p*-value**	**aOR (95% CI)**	***p*-value**	**aOR (95% CI)**	***p*-value**	**aOR (95% CI)**	***p*-value**
CO_2	0.81 (0.55,1.20)	0.285	0.58 (0.32,1.07)	0.079	1.01 (0.54,1.89)	0.974	0.74 (0.46,1.18)	0.201	0.64 (0.36,1.13)	0.123
CO	0.71 (0.47,1.06)	0.094	0.98 (0.52,1.84)	0.947	0.60 (0.31,1.17)	0.132	0.58 (0.35,0.97)	**0.035**	0.78 (0.44,1.39)	0.403
Formaldehyde	0.84 (0.54,1.29)	0.414	0.55 (0.28,1.10)	0.085	1.32 (0.67,2.60)	0.423	1.62 (0.95,2.75)	0.075	1.50 (0.82,2.75)	0.194
NO_2	1.57 (1.05,2.35)	**0.028**	0.99 (0.53,1.84)	0.976	1.08 (0.57,2.06)	0.805	1.70 (1.01,2.87)	**0.043**	1.03 (0.53,2.01)	0.932
O_3	1.19 (0.80,1.76)	0.399	0.99 (0.54,1.83)	0.984	0.78 (0.41,1.47)	0.437	1.55 (0.95,2.51)	0.075	2.61 (1.42,4.82)	**0.002**
TVOC	0.95 (0.65,1.39)	0.777	0.54 (0.30,0.97)	**0.039**	0.66 (0.36,1.24)	0.196	0.65 (0.40,1.04)	0.074	0.50 (0.28,0.89)	**0.017**
$PM_{2.5}$	0.99 (0.62,1.59)	0.982	1.04 (0.51,2.13)	0.909	1.14 (0.54,2.39)	0.736	1.15 (0.69,1.93)	0.595	1.72 (0.95,3.11)	0.069
PM_{10}	0.95 (0.60,1.51)	0.842	1.48 (0.72,3.07)	0.286	1.49 (0.71,3.15)	0.289	1.14 (0.68,1.91)	0.626	2.39 (1.30,4.41)	**0.005**

aOR – odds ratio; CI – Confidence interval

Table S5 – Summary results of each unipollutant multivariate exposure model, considering exposure factorised by threshold as cutoff: adjusted odds ratio (aOR), its 95% confidence interval, and significance (*p*-value)

	Active wheezing		**Reported asthma**		**Diagnosed asthma**		**Reduced FEV_1/FVC**		**Reduced FEV_1**	
	aOR (95% CI)	***p*-value**	**aOR (95% CI)**	***p*-value**	**aOR (95% CI)**	***p*-value**	**aOR (95% CI)**	***p*-value**	**aOR (95% CI)**	***p*-value**
CO_2	0.80 (0.54,1.17)	0.253	0.64 (0.35,1.16)	0.139	1.09 (0.59,1.99)	0.785	0.90 (0.57,1.42)	0.649	1.08 (0.62,1.88)	0.786
CO	-	-	-	-	-	-	-	-	-	-
Formaldehyde	0.43 (0.18,1.01)	**0.037**	0.22 (0.03,1.79)	0.086	0.29 (0.04,2.26)	0.159	0.72 (0.22,2.38)	0.581	1.34 (0.31,5.84)	0.704
NO_2	-	-	-	-	-	-	-	-	-	-
O_3	-	-	-	-	-	-	-	-	-	-
TVOC	2.82 (0.77,10.36)	0.139	a	a	2.18 (0.24,20.09)	0.524	2.93 (0.55,15.45)	0.218	2.06 (0.19,22.78)	0.575
$PM_{2.5}$	3.00 (0.70,12.94)	0.088	0.95 (0.19,4.64)	0.951	0.99 (0.20,4.81)	0.991	1.22 (0.39,3.76)	0.729	1.63 (0.42,6.35)	0.466
PM_{10}	1.24 (0.74,2.08)	0.409	1.31 (0.56,3.10)	0.522	1.01 (0.44,2.30)	0.990	1.29 (0.70,2.36)	0.411	2.73 (1.21,6.12)	**0.010**

aOR – odds ratio; CI – Confidence interval; a – no cases of reported asthma when exposure exceeded TVOC threshold

Table S6 –Summary results of each unipollutant multivariate exposure model, considering exposure factorised into those exposed to levels above (exposed at risk) or below the threshold (not exposed at risk): adjusted odds ratio (aOR), its 95% confidence interval, and significance (*p*-value)

	Active wheezing		**Reported asthma**		**Diagnosed asthma**		**Reduced FEV_1/FVC**		**Reduced FEV_1**	
	aOR (95% CI)	***p*-value**	**aOR (95% CI)**	***p*-value**	**aOR (95% CI)**	***p*-value**	**aOR (95% CI)**	***p*-value**	**aOR (95% CI)**	***p*-value**
CO_2	0.72 (0.49,1.07)	0.102	0.58 (0.32,1.05)	0.068	0.96 (0.52,1.75)	0.883	0.68 (0.42,1.09)	0.106	0.87 (0.50,1.51)	0.614
CO	-	-	-	-	-	-	-	-	-	-
Formaldehyde	0.58 (0.34,0.98)	**0.034**	0.42 (0.17,1.03)	**0.040**	0.59 (0.25,1.39)	0.205	0.42 (0.20,0.88)	**0.016**	1.09 (0.48,2.50)	0.832
NO_2	-	-	-	-	-	-	-	-	-	-
O_3	-	-	-	-	-	-	-	-	-	-
TVOC	0.79 (0.43,1.47)	0.454	0.42 (0.14,1.24)	0.086	0.45 (0.13,1.51)	0.151	0.63 (0.23,1.73)	0.357	0.09 (0.01,0.78)	**0.004**
$PM_{2.5}$	0.92 (0.60,1.41)	0.702	1.92 (0.86,4.30)	0.096	1.27 (0.58,2.75)	0.545	1.13 (0.65,1.97)	0.662	2.08 (1.04,4.14)	**0.034**
PM_{10}	0.74 (0.47,1.16)	0.187	0.78 (0.38,1.63)	0.510	0.82 (0.38,1.75)	0.595	1.08 (0.63,1.86)	0.778	3.19 (1.74,5.87)	< **0.001**

aOR – odds ratio; CI – Confidence interval

Table S7 – Results from the multinomial logistic regression models for $PM_{2.5}$ exposure: adjusted odds ratio (aOR) for pollutant exposure, 95% confidence interval, and significance (*p*-value)

Predictors	**Exposure models**			
	Category 1 (asthma with aeroallergen sensitisation)		**Category 2 (asthma without aeroallergen sensitisation)**	
	aOR (95% CI)	***p*-value**	**aOR (95% CI)**	***p*-value**
$PM_{2.5}$ exposure / inhaled dose	1.83 (0.90-3.73)	0.097	1.08 (0.58-2.00)	0.804
Site location: Rural	0.27 (0.06-1.24)	0.093	0.85 (0.24-2.96)	0.799
Age group: Pre-schooler	0.05 (0.01-0.46)	**0.008**	0.83 (0.26-2.68)	0.753
Maternal education: Low	2.43 (0.55-10.79)	0.243	0.38 (0.09-1.55)	0.175
Maternal education: High	1.19 (0.27-5.30)	0.816	0.35 (0.10-1.30)	0.117
Living with a smoker: Yes	1.18 (0.36-3.87)	0.785	1.68 (0.55-5.12)	0.363
Sex: Male	3.99 (1.06-14.95)	**0.040**	1.50 (0.48-4.70)	0.483
Body Mass Index	0.92 (0.76-1.12)	0.424	1.07 (0.90-1.28)	0.414
Asthmatic parent: Yes	2.36 (0.63-8.83)	0.202	4.36 (1.36-14.0)	**0.013**
Cat at home in child's 1st year	1.23 (0.22-6.96)	0.814	0.55 (0.10-3.14)	0.499
Cat at home in previous year	1.54 (0.38-6.19)	0.546	2.51 (0.71-8.84)	0.152
Dog at home in child's 1st year	0.38 (0.04-3.64)	0.401	5.35 (1.46-19.52)	**0.011**
Dog at home in previous year	0.49 (0.09-2.69)	0.409	0.97 (0.27-3.46)	0.962
Contact with farm animals in child's 1st year	1.47 (0.34-6.39)	0.605	0.33 (0.06-1.75)	0.195

aOR – adjusted odds ratio; CI – Confidence interval

Table S8 – Results from the multinomial logistic regression models for PM_{10} exposure and inhaled dose: adjusted odds ratio (aOR) for pollutant exposure, 95% confidence interval, and significance (*p*-value)

Predictors	Exposure models				Inhaled dose models			
	Category 1 (asthma with aeroallergen sensitisation)		Category 2 (asthma without aeroallergen sensitisation)		Category 1 (asthma with aeroallergen sensitisation)		Category 2 (asthma without aeroallergen sensitisation)	
	aOR (95% CI)	*p*-value	aOR (95% CI)	*p*-value	aOR (95% CI)	*p*-value	aOR (95% CI)	*p*-value
PM_{10} exposure / inhaled dose	2.06 (0.83-5.09)	0.118	1.18 (0.55-2.55)	0.667	1.59 (0.68-3.69)	0.281	1.15 (0.57-2.32)	0.688
Site location: Rural	0.30 (0.07-1.27)	0.103	0.84 (0.24-2.92)	0.783	0.36 (0.09-1.42)	0.145	0.85 (0.25-2.94)	0.802
Age group: Pre-schooler	0.05 (0.01-0.47)	**0.008**	0.83 (0.26-2.68)	0.756	0.04 (0.00-0.47)	**0.009**	0.77 (0.22-2.71)	0.685
Maternal education: Low	2.42 (0.55-10.74)	0.244	0.37 (0.09-1.52)	0.168	2.42 (0.55-10.7)	0.244	0.36 (0.09-1.53)	0.167
Maternal education: High	1.15 (0.26-5.13)	0.855	0.35 (0.10-1.30)	0.117	1.17 (0.26-5.22)	0.838	0.35 (0.10-1.28)	0.114
Living with a smoker: Yes	1.12 (0.34-3.64)	0.854	1.68 (0.55-5.13)	0.362	1.08 (0.34-3.50)	0.894	1.68 (0.55-5.11)	0.363
Sex: Male	3.91 (1.04-14.67)	**0.043**	1.50 (0.48-4.69)	0.487	4.03 (1.07-15.13)	**0.039**	1.50 (0.48-4.70)	0.485
Body Mass Index	0.92 (0.76-1.13)	0.433	1.08 (0.91-1.28)	0.399	0.94 (0.76-1.15)	0.543	1.09 (0.91-1.30)	0.368
Asthmatic parent: Yes	2.18 (0.59-8.01)	0.242	4.35 (1.35-14.0)	**0.014**	1.99 (0.55-7.14)	0.291	4.33 (1.35-13.93)	**0.014**
Cat at home in child's 1st year	1.26 (0.22-7.12)	0.794	0.55 (0.10-3.15)	0.500	1.16 (0.21-6.45)	0.869	0.55 (0.10-3.15)	0.502
Cat at home in previous year	1.61 (0.40-6.47)	0.500	2.54 (0.72-8.98)	0.148	1.66 (0.42-6.62)	0.470	2.53 (0.72-8.94)	0.150
Dog at home in child's 1st year	0.39 (0.04-3.71)	0.412	5.35 (1.47-19.53)	**0.011**	0.39 (0.04-3.69)	0.411	5.33 (1.46-19.40)	**0.011**
Dog at home in previous year	0.48 (0.09-2.62)	0.394	0.98 (0.27-3.49)	0.972	0.47 (0.09-2.58)	0.387	0.99 (0.28-3.57)	0.992
Contact with farm animals in child's 1st year	1.51 (0.35-6.55)	0.581	0.33 (0.06-1.73)	0.190	1.65 (0.38-7.13)	0.501	0.33 (0.06-1.74)	0.192

aOR – adjusted odds ratio; CI – Confidence interval

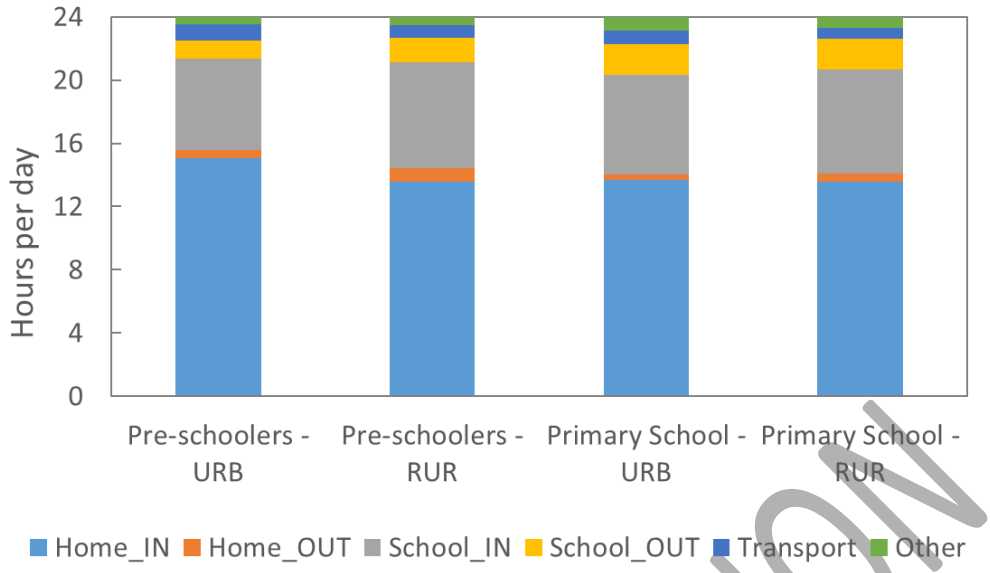


Figure S1 – Time spent in each major microenvironment, on a typical weekday, by pre-schoolers and primary school children, from both urban and rural sites

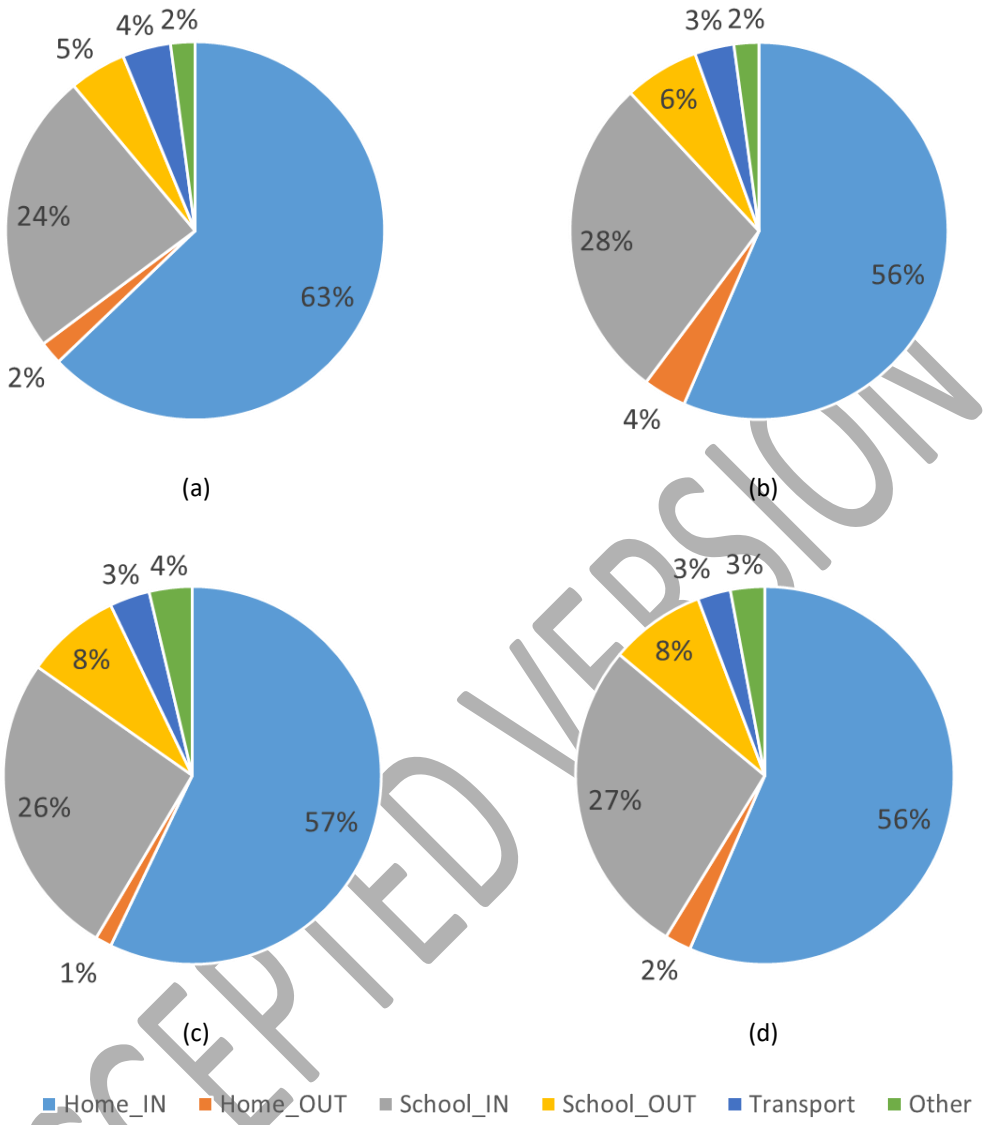


Figure S2 – Proportion (%) of time of a typical weekday (24-hour) spent in each major microenvironment by: (a) Pre-schoolers from urban sites; (b) Pre-schoolers from rural sites; (c) Primary school children from urban sites; and (d) Primary school children from rural sites.

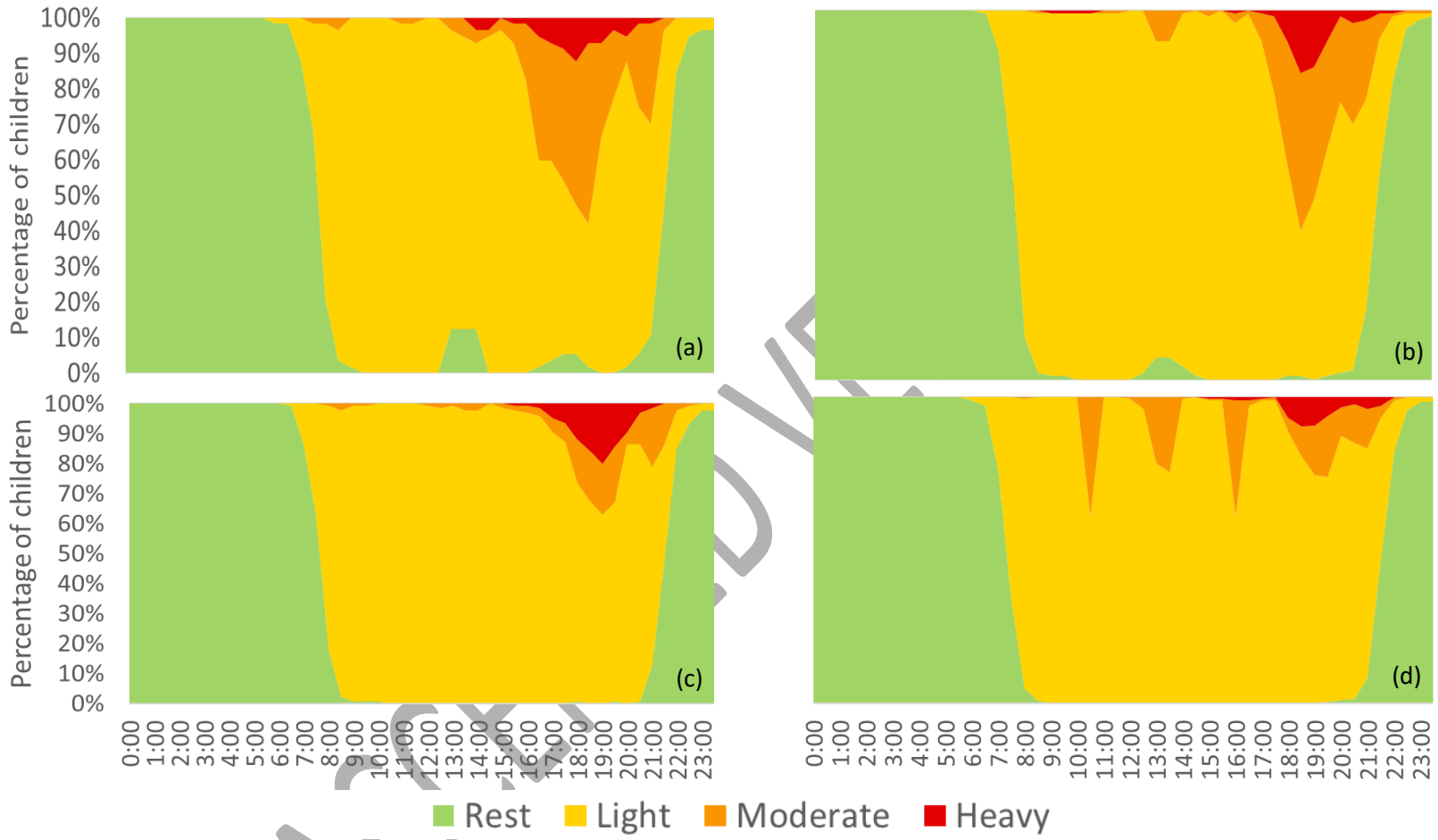


Figure S3 – Daily time-activity patterns of a typical weekday (24-hour) of: (a) Pre-schoolers from urban sites; (b) Pre-schoolers from rural sites; (c) Primary school children from urban sites; and (d) Primary school children from rural sites. Activities were classified into rest (sleep/ nap or sedentary/ passive), light intensity, moderate intensity and heavy (high intensity) according to the literature (U.S. Environmental Protection Agency (EPA) 2011).

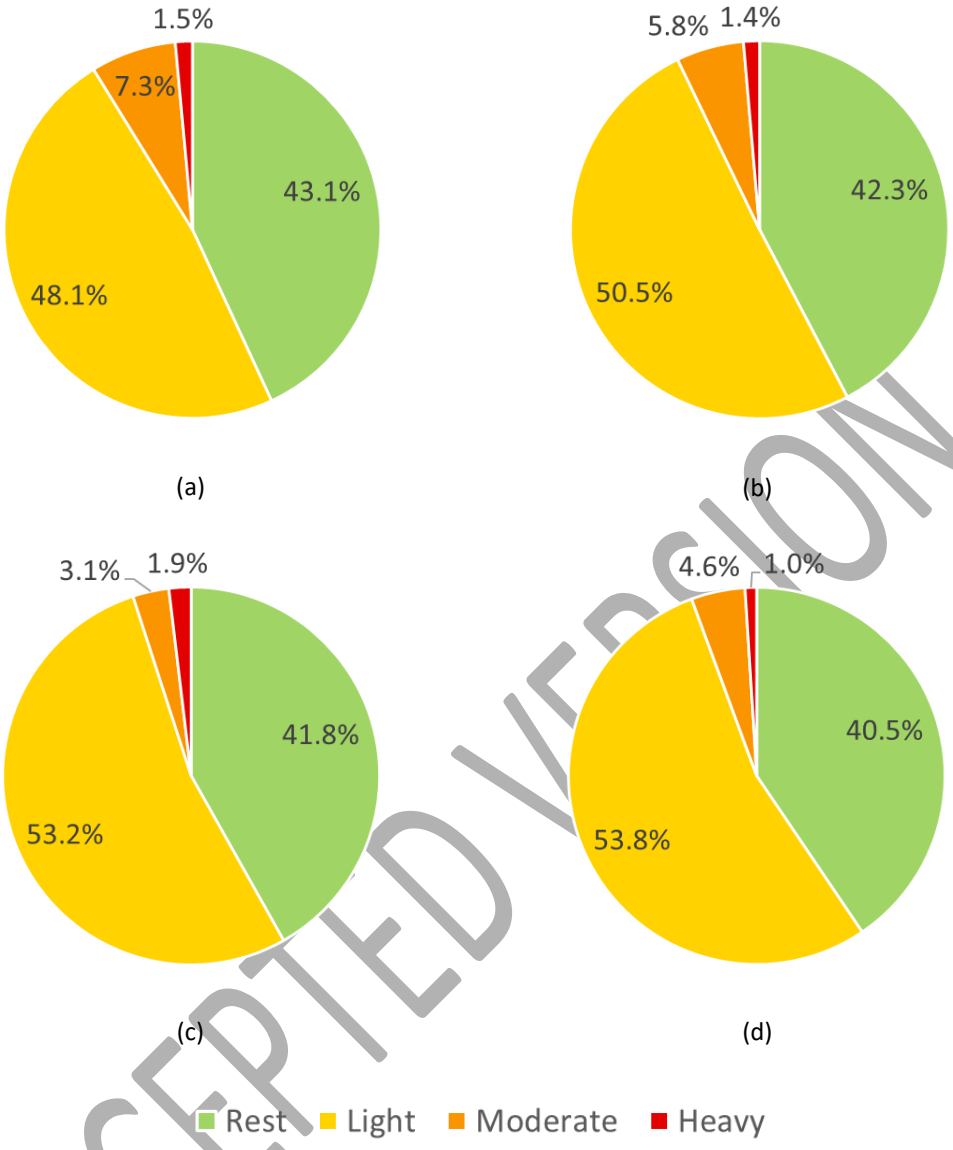


Figure S4 – Proportion (%) of time of a typical weekday (24-hour) spent in each type of activity by: (a) Pre-schoolers from urban sites; (b) Pre-schoolers from rural sites; (c) Primary school children from urban sites; and (d) Primary school children from rural sites.

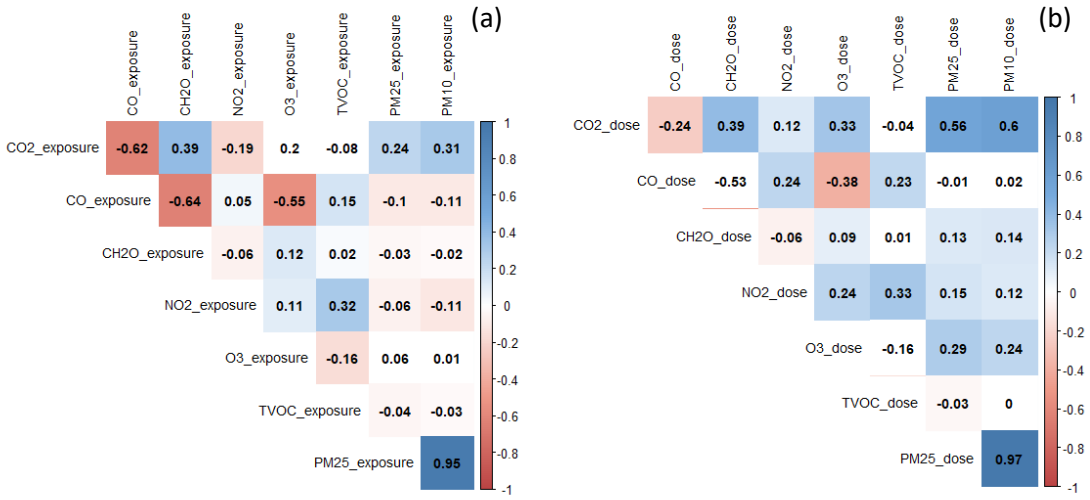


Figure S5 – Correlograms with Spearman's rank correlation coefficients between indoor air pollutants: (a) exposure; and (b) inhaled dose.

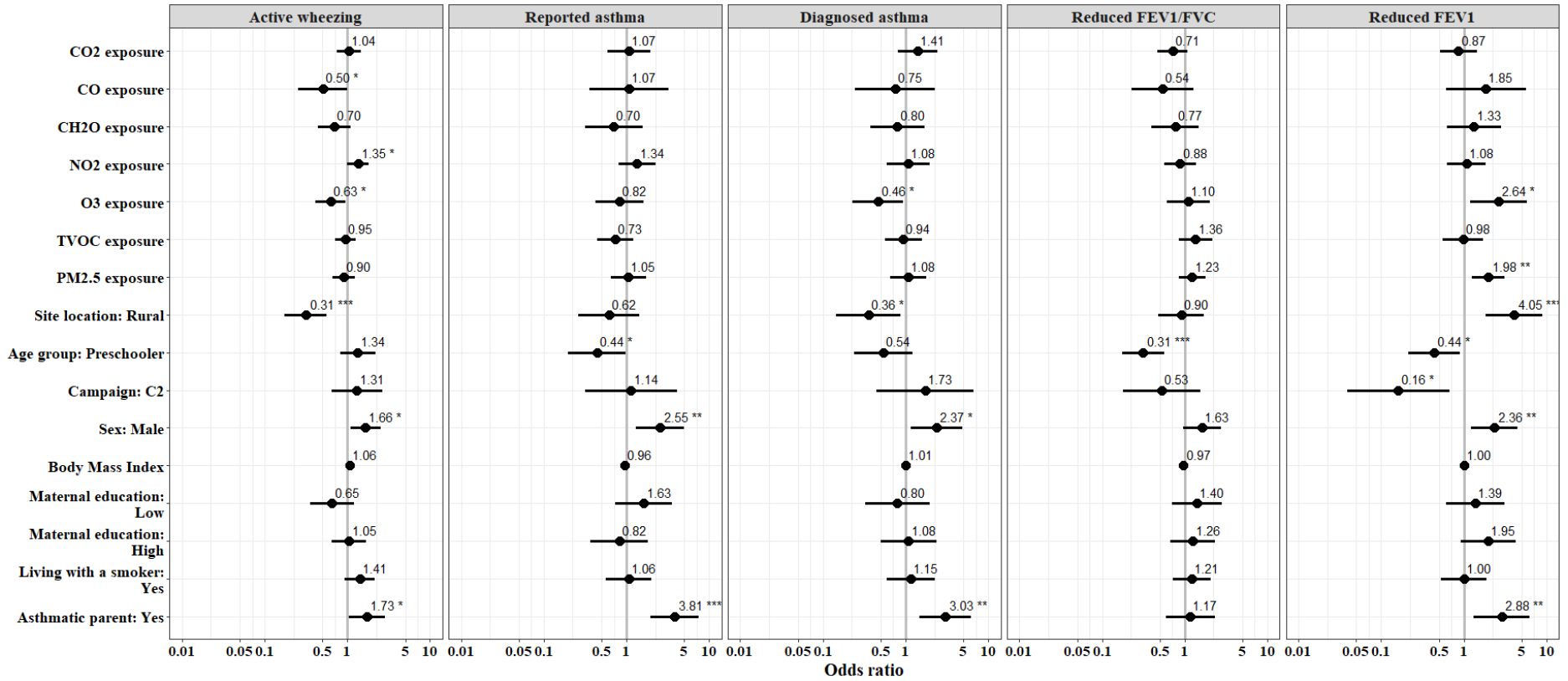


Figure S6 – Results from the multipollutant multivariate logistic regression models (adjusted odds ratio and 95% confidence intervals), when considering exposure to indoor air pollutants scaled by interquartile range and all the studied respiratory health outcomes (active wheezing, reported asthma, diagnosed asthma, reduced FEV_1/FVC and reduced FEV_1). * *p*-value < 0.05; ** *p*-value < 0.01; *** *p*-value < 0.001.

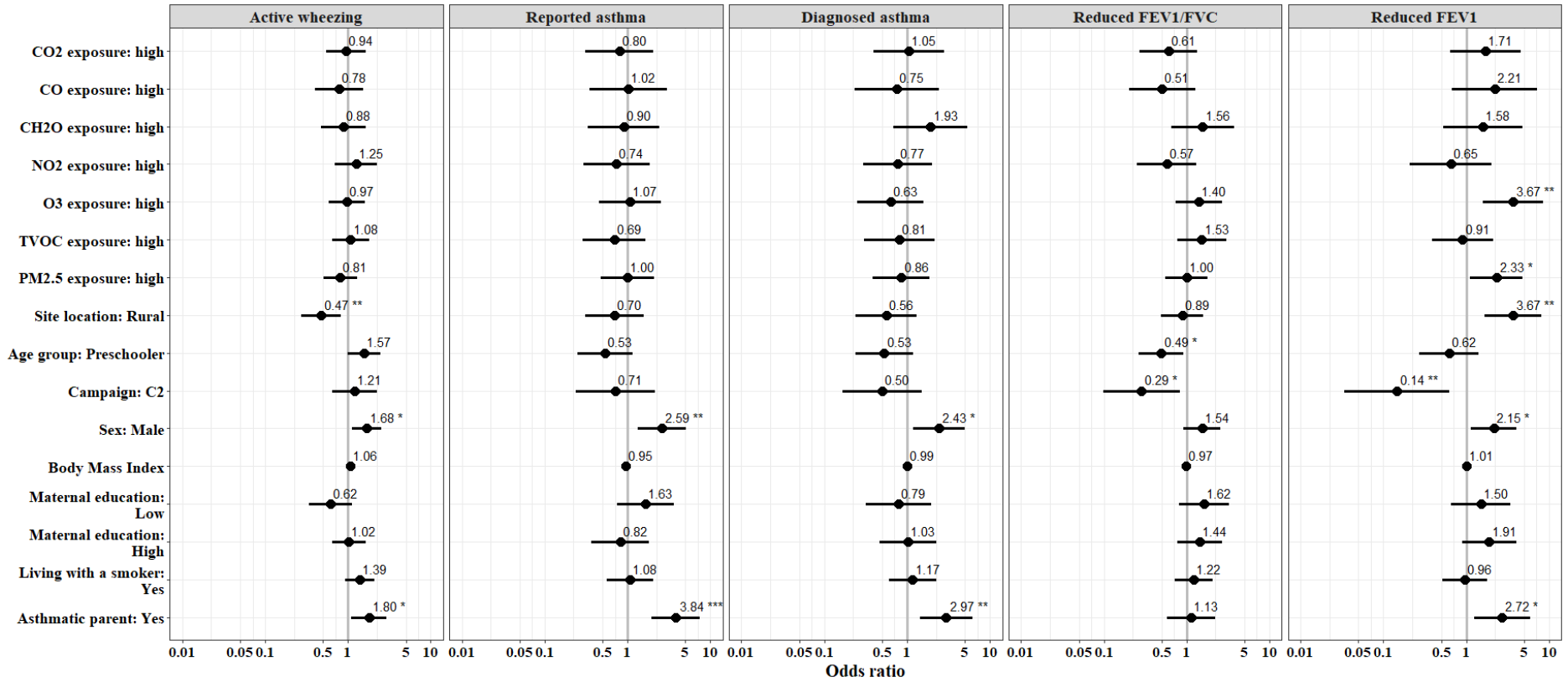


Figure S7 – Results from the multipollutant multivariate logistic regression models (adjusted odds ratio and 95% confidence intervals), when considering exposure to indoor air pollutants factorised by median as cutoff and all the studied respiratory health outcomes (active wheezing, reported asthma, diagnosed asthma, reduced FEV_1/FVC and reduced FEV_1). * *p*-value < 0.05; ** *p*-value < 0.01; *** *p*-value < 0.001.

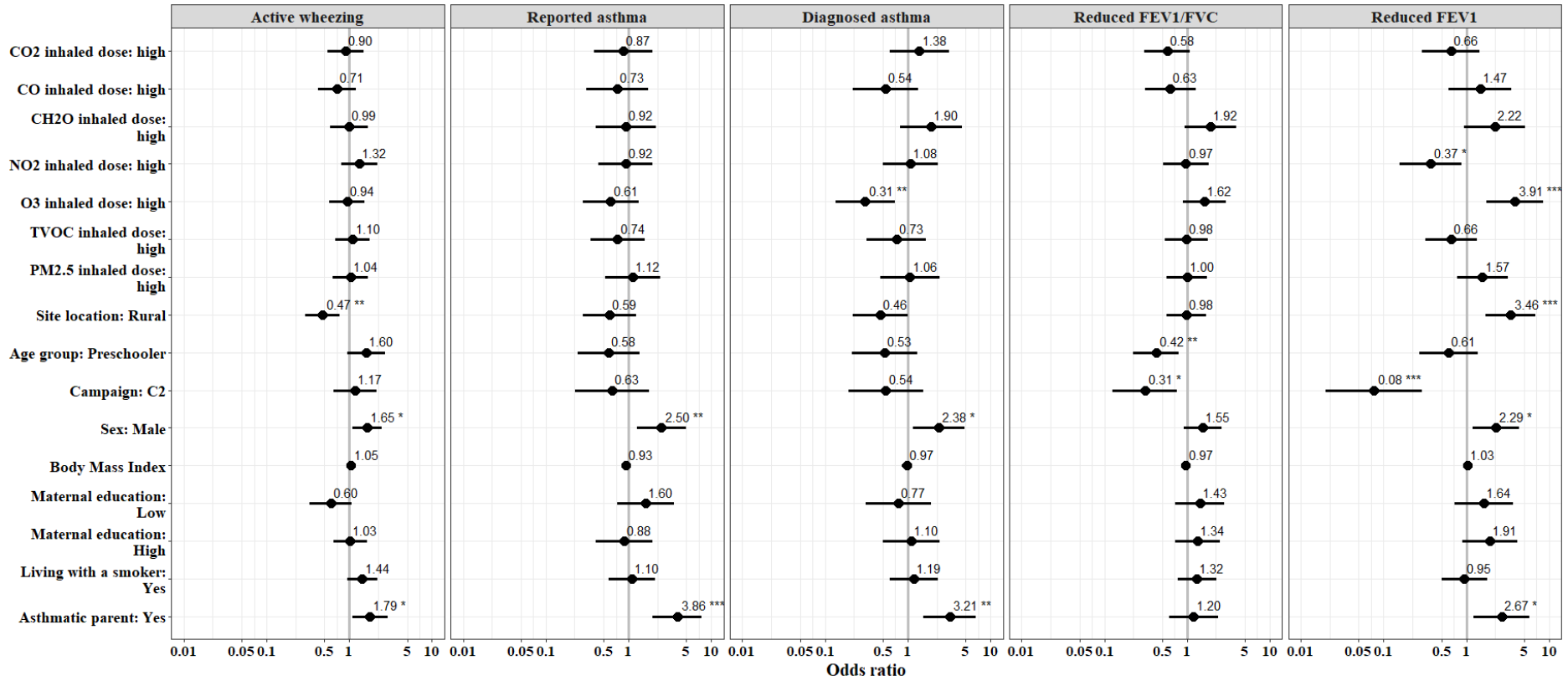


Figure S8 – Results from the multipollutant multivariate logistic regression models (adjusted odds ratio and 95% confidence intervals), when considering inhaled dose of indoor air pollutants factorised by median as cutoff and all the studied respiratory health outcomes (active wheezing, reported asthma, diagnosed asthma, reduced FEV_1/FVC and reduced FEV_1). * *p*-value < 0.05; ** *p*-value < 0.01; *** *p*-value < 0.001.

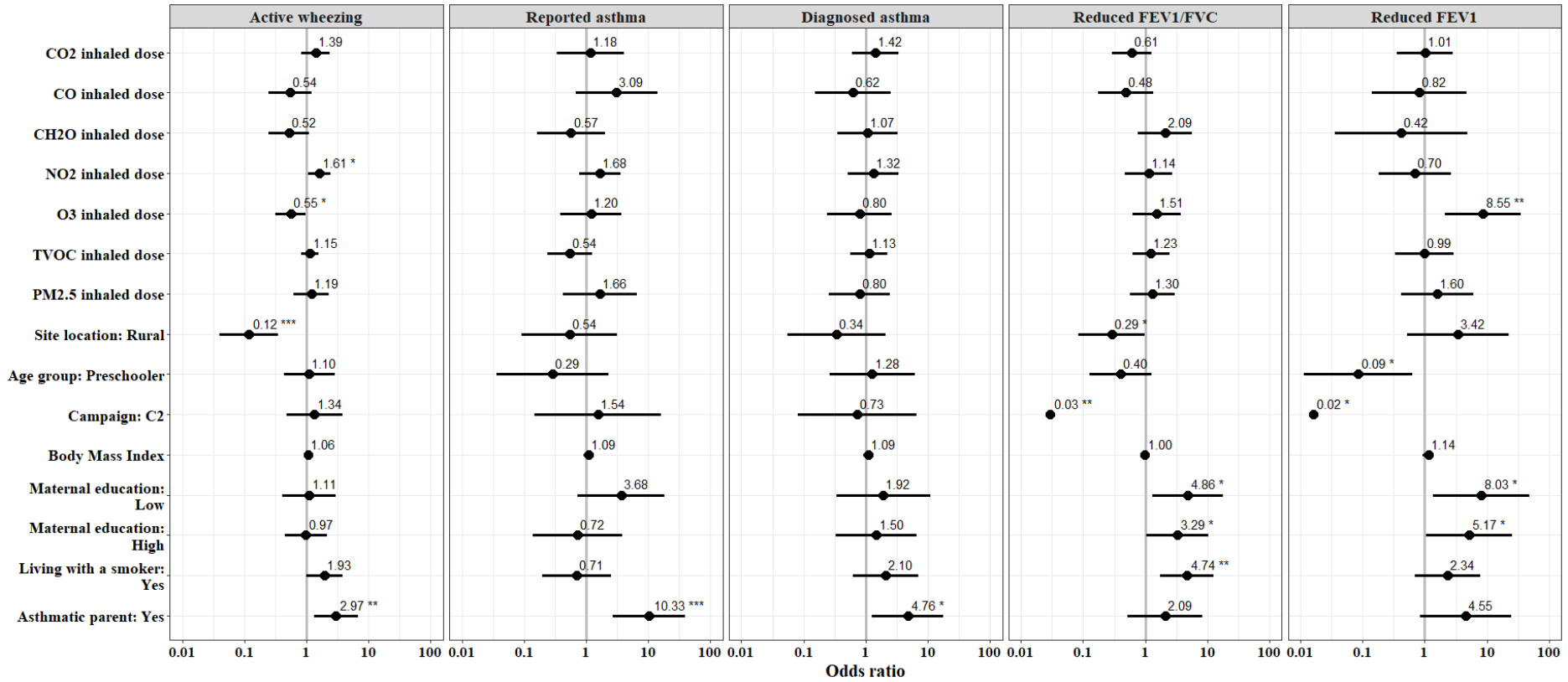


Figure S9 – Sensitivity analysis. Results applied to a stratum of the study population (female), from the multipollutant multivariate logistic regression models (adjusted odds ratio and 95% confidence intervals), when considering inhaled dose of indoor air pollutants scaled by interquartile range and all the studied respiratory health outcomes (active wheezing, reported asthma, diagnosed asthma, reduced FEV_1/FVC and reduced FEV_1). * *p*-value < 0.05; ** *p*-value < 0.01; *** *p*-value < 0.001.