The combined effect of air pollution and house dust mite exposure on the airways

Efeito conjunto da exposição à poluição do ar e aos ácaros do pó sobre as vias aéreas

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ABSTRACT

Introduction: Asthma is a chronic respiratory disease which may be associated with environmental factors. In addition to allergens, other factors such as those related to air quality could be associated with worsening of symptoms. **Aim:** This study aimed to evaluate the effect of individual exposure to air pollutants on lung function in a group of children with a previous history of wheezing, also taking house dust mite exposure into account. **Methods:** A group of 51 children with a prior history of wheezing was selected through the ISAAC questionnaire. They were followed prospectively by means of standardised medical evaluations that included spirometry, dust-mite-exposure assessment and calculation of individual exposure to a wide range of air pollutants: PM_{10}, O_3, NO_2, benzene, toluene, xylene, ethylbenzene and formaldehyde. **Results:** There were a great number of mattresses with a medium or high degree of mite infestation. In general, except for PM_{10}, air pollutant exposure did not reach high levels. In the multivariate analysis, pollutants including PM_{10}, NO_2, benzene, toluene and ethylbenzene plus dust mite exposure were associated with lung function deterioration. **Conclusion:** This study reinforces the importance of air pollution exposure in wheezing children. This exposure, similarly to that to dust mites, seems to have an impact on the airways.

Keywords: Air pollution, asthma, house dust mites, spirometry, VOCs.

RESUMO

Introdução: A asma é uma doença respiratória crónica, cujo agravamento pode estar associado a factores ambientais, entre os quais os relacionados com a qualidade do ar. **Objectivo:** O presente trabalho pretendeu avaliar o efeito da exposição individual a poluentes atmosféricos em termos de função respiratória, num grupo de crianças com história de sibilância, entrando em consideração com o grau de infestação de ácaros do pó doméstico. **Métodos:** Um grupo de 51 crianças com história de sibilância, seleccionadas através do questionário do estudo ISAAC, foi acompanhado prospectivamente num estudo com medidas repetidas, que envolveu avaliações médicas padronizadas que incluíram a realização de espirometria, avaliação da exposição aos ácaros do pó e cálculo do valor de exposição individual a uma variedade de poluentes do ar: PM_{10}, O_3, NO_2, benzeno, tolueno, xileno, etilbenzeno e formaldeído. **Resultados:** Observou-se uma elevada percentagem de colchões com um grau de infestação de ácaros médio ou elevado. Com excepção dos valores de PM_{10}, os valores de exposição aos poluentes do ar não alcançaram valores elevados. Na análise multivariável, tanto os poluentes (designadamente PM_{10}, NO_2, benzeno, tolueno e etilbenzeno) como o grau de infestação de ácaros do pó associaram-se a deterioração da função pulmonar. **Conclusão:** O presente trabalho vem reforçar o interesse da exposição aos poluentes do ar em crianças com história de sibilância, que à semelhança do que acontece com os ácaros do pó influenciam as vias aéreas.

Palavras-chave: Ácaros do pó, asma, COVs, espirometria, poluição do ar.

INTRODUCTION

Asthma is a chronic inflammatory respiratory disease clinically hallmarked by recurrent episodes of cough, wheezing and dyspnoea. For several decades now the theory that environmental factors such as air quality may contribute to worsening of symptoms has been studied.

The vast majority of asthma cases, particularly in children, are described as allergic asthma[1]. In these cases there is a sensitisation to aeroallergens which could be associated with a worsening of symptoms[1]. House dust mites are the prime source of aeroallergen sensitisation in western countries and are mainly found in indoor environments[2].

The relationship between exposure to the allergen and worsening of asthma symptoms has been accepted over the last few decades by the medical community and the population at large and constitutes a proven justification for the worsening of respiratory symptoms[2,3]. In addition to aeroallergens, however, other environmental factors became associated with worsening of respiratory disease. A raft of studies[4] has shown that environmental pollution can lead to worsened asthma symptoms. The air we breathe contains a variety of environmental pollutants coming from both natural[5] and anthropogenic sources[6], arising from industry, home, or car exhaust emissions. These pollutants, traditionally considered an environmental problem, are becoming increasingly seen as a public health problem causing increased human mortality and morbidity[7].

In addition, indoor air quality in terms of pollutants has been associated with respiratory complaints in both studies into the workplace and those assessing home environments[8].

Indoor environments have undergone huge changes over the last few decades. A vast range of new materials has been introduced in the forms of furniture, paints, varnishes and cleaning products, and a growing trend towards less ventilation of indoor spaces in attempts to conserve buildings' energy efficiency has been seen. These changes in ventilation were born of changes both in building construction and their inhabitants' habits. There is acknowledgment in the scientific community of the deleterious effects of environmental pollution[9], recognised by the World Health Organization[4]. Despite that, there is not as yet an adequate transposition of these worries, principally those to do with indoor air quality, to the population at large.

Our group concluded[10,11] that individual exposure to air pollution impacts on the airways of children with a history of wheezing. This study seeks to assess if the effect of individual exposure to air pollutants on respiratory function persists, also taking house dust mite exposure into account.

MATERIAL AND METHODS

The methodology used was developed as part of the Saud'Ar Project: Health is the air which we breathe. It is a prospective panel study which was performed in Viseu, Portugal. Viseu is a non-industrial city of approx. 50,000 inhabitants situated in the northern midlands. The city was chosen as the site of the study as this is an emerging city with a young population compared to other Portuguese cities.

Medical assessment protocol

The Project's methodology was drawn up[10,11]. Once approved by the Ethics Committee of the Hospital de São Teotónio, the study was launched, involving using the ISAAC – International Study of Asthma and Allergies in Childhood – questionnaire. This was given out in Decem-

ber 2005 to all children of four primary schools in Viseu, aiming to identify children with a history of wheezing in the last 12 months. Children's parents/guardians filled in the questionnaire. The schools chosen were selected because of their capacity and location. The two largest schools in the city centre were selected (urban schools) and the two largest beyond the Viseu ring road (suburban schools).

Parents gave their informed written consent and then it was suggested to parents of children with a history of wheezing for their children to take part in the study. The study was composed of four visits as follows: January 2006 (Visit 1), June 2006 (Visit 2), January 2007 (Visit 3) and June 2007 (Visit 4). All children taking part were assessed in the same week of the month at the Pulmonology Unit of the Hospital de São Teotónio. Exclusion criteria were any neuromuscular or psychiatric diseases, or any other lung disease which was not asthma.

At every visit, children underwent a clinical evaluation followed by same-day spirometry with bronchial challenge test.

Sensitivity to aeroallergens was evaluated on first and last visit using skin prick tests. Skin tests (Leti®, Madrid, Spain) were carried out withthe following extracts: positive control (histamine 10mg/ml), negative control (phenolated solution), *Dermatophagoides pteronyssinus, Dermatophagoides farinae*, dog dander, cat dander, German cockroach, alternaria, grass pollen mix, olive tree pollen, parietaria, cypress tree pollen, birch tree pollen and oak tree pollen. Extracts were introduced into the skin using disposable lancets (Stallerpoint®, Stallergenes, Paris, France). A wheal with redness with mean diameter over 3mm was considered positive.

Guided by the medical team, the suggestion to assess the degree of infestation of house dust mites in the children's mattresses was put to parents/guardians.

Spirometry with bronchial challenge test

This exam was performed in line with American Thoracic Society and European Respiratory Society guidelines[12]. The spirometrer was a Vitalograph® Compact (Buckingham, UK). Spirometry was performed prior to and 15 minutes after administration of bronchodilator (200µg of salbutamol). The results were expressed in percentage of the expected score in line with the Polgar reference equations[13]. For the final analysis we considered as variables FEV_1 (forced expiratory volume in one second), FEV_1/FVC (forced vital capacity) ratio, $FEF_{25-75\%}$ (forced expiratory flow occurring in the middle 50% of the patient's exhaled volume) and Δ FEV_1 (FEV_1 variation after administration of bronchodilator).

Assessment of exposure to house dust mites

Allergenic exposure was measured using dust samples collected in line with standard aspiration criteria in a $1m^2$ area for two minutes[14]. Samples from the mattresses of children taking part were selected as these were felt to be the most representative sources of allergens[15]. Then a semiquantitative dose of guanine was used, the Acarex Test® (Allergopharma, Reinbek, Germany). The colorimetric scale results were divided into 4 classes: no infestation, light infestation, medium infestation and heavy infestation. The Acarex Test® correlated with monoclonal antibody measurement[16]. Measurements of 2µg of Der p 1 per gram of dust (risk factor for sensitisation) corresponded to class 1 of the guanine test[17]. Measurements of 10µg of Der p 1 per gram of dust (risk factor for worsening of asthma symptoms) corresponded to class 3 of the guanine test[17]. The dust samples were collected by an environmental technician during Visits 1, 3 and 4. No evaluations were made during Visit 2.

Protocol for evaluating the environmental pollutants

Measurements were made by the Department of Land Planning, Universidade de Aveiro. In sum, this involved assessing air quality, modelling the air quality and calculating individual exposure using the methodology already described[10,11]. Each child's daily activity profile (time-activity profile) was taken into account when calculating individual exposure to air pollutants. This allowed various microenviroenments frequented by the children and the time spent in each over a Monday-Friday school week to be identified. Evaluation of air quality in each microenvironment identified was carried out using an approach involving field measurements and modelling the air quality to map the areas in which it was not possible to perform measurements. The microenvironments studied were the children's homes and schools.

The pollutants to which individual exposure was calculated were PM_{10}, O_3, NO_2, BTEX (benzene, toluene, xylene, ethylbenzene) and formaldehyde. BTEX and formaldehyde are volatile organic compounds (VOCs).

Statistical analysis

An exploratory analysis of variables of interest was initially undertaken. The variables considered of interest were the spirometry parameters FEV_1, FEV_1/FVC ratio, $FEF_{25-75\%}$ and ΔFEV_1.

We used regression models which consider the structure of dependence existing between the measurements gleaned over time (longitudinal data models) to evaluate repeated measurements. These models' parameters were estimated using the Generalized Estimating Equations, GEE, as an (exchangeable) uniform working correlation matrix.

We first performed a univariate analysis to identify which variables in themselves were associated with each one of the answer variables. In addition to the pollutants and the degree of house dust mite exposure, the different independent variables evaluated were age, sex, height, weight, parental smoking history, parental level of schooling, aeroallergen sensitisation, number of visit, mean temperature, mean humidity relative to day of medical visit, any older siblings, mould or humidity in the home, and any fireplace or pets (cats and dogs) in the home.

The multivariate analysis studied the effect of the pollutant and the degree of exposure to the mites as well as other independent variables which were associated in the univariate analysis with the respective dependent (assuming solely for this effect $p < 0.15$). Age, sex and height were not part of the multivariable analysis relative to FEV_1 and $FEF_{25-75\%}$, as these answer variables were already part of these parameters. Consequently, the final models included in addition to pollutants and mite exposure were:

– Model with FEV_1 as a variable answer adjusted to the Visit, parental schooling level and any fungus/humidity in the home;
– Model with FEV_1/FVC as a variable answer adjusted to the Visit, any fungus/humidity in the home, and any fireplace in the home;
– Model with $FEF_{25-75\%}$ as a variable answer adjusted to the Visit, any older siblings, any mould or humidity in the home, and any fireplace in the home;
– Model with ΔFEV_1% as a variable answer adjusted to the Visit, age, parental schooling level, any mould or humidity in the home, and any fireplace in the home.

It is highlighted that these models considered only the data relating to Visits 1, 3 and 4, as the degree of mite exposure was not measured during Visit 2.

The regression coefficients and their respective 95% confidence intervals were calculated for an increase in exposure of 10 $\mu g.m^{-3}$.week of air pollutant.

The level of significance was set at 0.05. However, p values over 0.05 and under 0.1 were seen. We used the Stata (StataCorp LP, Texas, USA) program for Windows for data treatment.

RESULTS

Six hundred and forty-five of the 806 ISAAC study questionnaires given out to children at the participating schools were returned (80%). Of these, 77 (11.7%) complained of wheezing in the 12 months prior to receiving the questionnaire. Via a first phone call, 54 parents /guardians of children with wheezing showed interest in their children taking part in the study. This study took repeated measurements, and the final analysis only dealt with 51 children who participated in all stages. Table I describes the sample at study start. The majority of children were male (55%), with mean age at study start 7.3 ± 1.1 years old. Twenty-seven (53%) were sensitised to aeroallergens. The most common sensitisation found in the skin prick tests were to house dust mites (21 children) and grass pollens (18 children). The majority of children had no change in their lung function exam throughout the study (Table II).

The descriptive analysis of the degree of infestation found on the children's mattresses at each visit is shown in Table III. There was a total number of 131 measurements. It is highlighted that a high number of mattresses at each visit had a medium to high degree of infestation of mites. Table IV shows the individual exposure to pollutants at Visits 1, 3 and 4. The majority of exposure was low, with the exception of PM_{10} values.

As expected, multivariate analysis showed that the rising level of dust mite exposure negatively correlated with spirometry parameters, namely FEV_1/FVC (-1.35; CI 95%: -2.37 to -0.33; p = 0.009), $FEF_{25-75\%}$ (-2.14; CI 95%: -4.98 to -0.70; p = 0.023) and Δ FEV_1

Table I. Description of the sample

	Total
Total	51 (100%)
Sex	
Male	28 (54,9%)
Female	23 (45,1%)
Age in years	
Total (mean±SD)	7,3±1,1
Parental education level	
Primary or Secondary	23 (45,1%)
Complementary or University	28 (54,9%)
Sensitisation to aeroallergens	
No	24 (47%)
Yes	27 (53%)
Parental smoking	
No	41 (80,4%)
Yes	10 (19,6%)
Older siblings	
No	22 (43%)
Yes	29 (57%)
Mould or humidity at home	
No	45 (88%)
Yes	6 (12%)
Pets	
No	31 (61%)
Yes	20 (39%)
Fireplace at home	
No	23 (45%)
Yes	28 (55%)

Table II. Descriptive analysis of the answer variables (spirometry parameters). Results given as median [percentile 25 – percentile 75]

	Visit 1	Visit 2	Visit 3	Visit 4
Spirometry				
FEV_1%	102 [85,5-110]	99,5 [90,8-110]	95 [86-103]	96 [86,5-105]
FEV_1/FVC	0,78 [0,73-0,84]	0,80 [0,75-0,85]	0,79[0,74-0,83]	0,80 [0,76-0,84]
$FEF_{25-75\%}$	79,5 [61-105]	83 [63-108]	76 [63-95]	77 [64-101]
ΔFEV_1%	7 [3-14,5]	6 [3-13]	7,5 [5-13]	7 [3-11]

Table III. The degree of dust mite infestation of children's mattresses. Number of mattresses given for each visit and for each degree of infestation.

	Visit 1	Visit 3	Visit 4
Degree of infestation (n)			
Not measured	9	5	8
No infestation	4	10	12
Light infestation	13	10	11
Medium infestation	9	13	9
High infestation	16	13	11

(1.19; CI 95%: 0.03 to 2.36; p = 0.044). FEV_1 had a negative correlation coefficient, but this did not reach statistical significance (-1.29; CI 95%: -2.28 to -0.69; p = 0.202).

Models for each variable answer were created, bearing in mind these and other prior results[10,11] which found an association between deteriorated lung function and individual exposure to PM_{10}, NO_2, benzene, toluene, xylene

Table IV. Children's total exposure to the various air pollutants (µg.m-3. week) Results given as median [minimum – maximum] for each visit

	Visit 1	Visit 2	Visit 3	Visit 4
Exposure to:				
PM_{10}	66,3 [23,8 a 67,8]	40,0 [38 a 41,8]	66,0 [61,5 a 70,3]	37,8 [37,1 a 48,1]
O_3	18,4 [11,5 a 25,1]	26,0 [24,9 a 35,1]	35,9 [35,5 a 36,0]	44,5 [44,2 a 44,9]
NO_2	8,1 [5,0 a 8,6]	6,3 [5,8 a 7,5]	18,3 [16,9 a 20,4]	15,4 [14,0 a17,7]
Benzene	2,1 [1,2 a 25,7]	0,8 [0,5-3,5]	9,3 [3,6 a 39,2]	1,3[0,7 a 13,9]
Toluene	19,6 [3,3 a 91,7]	10,7 [3,8-90,9]	25,9 [10,3 a 108,1]	10,7 [3,8 a 33,5]
Xylene	7,4 [2,2 a 111,8]	5,2 [2,4-26,2]	4,9 [2,1 a 185,6]	10,8 [2,5 a 78,2]
Ethylbenzene	1,8 [0,6 a 33,5]	1,4 [0,7-5,2]	17,4 [7,6 a 60,6]	2,8 [0,8 a 16,0]
Formaldehyde	11,0 [5,2 a 28,9]	14,2 [6,1-29,1]	11,9 [2,9 a 29,5]	9,4 [2,3 a 30,1]

Table V. Multivariate analysis – association between exposure to air pollutants and spirometry variables adjusted for the degree of exposure to dust mites (regression coefficient, CI 95%, p value)

	FEV_1%	FEV_1/FVC	$FEF_{25-75\%}$	ΔFEV_1%
PM_{10}	-1,83 (-3,38 a -0,28) p=0,021	-0,83 (-1,72 a 0,06) p=0,066*	-0,94 (-2,53 a 0,66) p=0,236*	1,07 (0,20 a 1,94) p=0,016
O_3	-5,81 (-12,95 a 8,52) p=0,320	-1,64 (-8,83 a 5,56) p=0,873*	-0,92 (-3,00 a 1,18) p=0,235*	-0,23 (-1,51 a 1,03) 0,475
NO_2	-7,39 (-12,96 a -1,82) p=0,009	-3,64 (-6,82 a -0,46) p=0,025*	-6,01 (-10,94 a -1,09) p=0,017*	2,44 (-0,39 a 5,28) p=0,092
Benzene	-5,23 (-8,01 a -2,45) p<0,001	-2,04 (-3,62 a -0,47) p=0,011*	-7,15 (-11,20 a -3,09) p=0,001*	3,19 (1,25 a 5,13) p=0,001
Toluene	-0,90 (-1,87 a 0,07) p=0,068	-0,24 (-0,74 a 0,25) p=0,357*	-0,79 (-2,15 a 0,66) p=0,157*	0,74 (0,16 a 1,32) p=0,012
Xylene	-0,16 (-1,09 a 0,78) p=0,500	0,09 (-0,47 a 0,49) p=0,644*	-0,22 (-1,57 a 1,14) p=0,725	-0,36 (-0,98 a 0,27) p=0,295
Ethylbenzene	-1,90 (-3,43 a -0,38) p=0,014	-0,58 (-1,43 a 0,27) p=0,277*	-2,69 (-4,86 a -0,52) p=0,015*	1,31 (0,21 a 2,42) p=0,020
Formaldehyde	-3,37 (-7,78 a 1,04) p=0,175	-0,02 (-2,12 a 2,08) p=0,467*	-1,64 (-7,80 a 4,51) p=0,297*	1,50 (-0,76 a 3,77) p=0,198

CI 95%: confidence interval 95%; *: variable relative to the degree of exposure to dust mites still has statistical significance when included in the model. Regression coefficient (CI 95%) represents the mean change in spirometry variables for an increase of 10 µg.m-3.week of pollutant

and ethylbenzene. The aim was to evaluate the joint effect of exposure to pollutants and degree of exposure to mites.

Table V shows the correlation coefficients resulting from the multivariate analysis, which include the effect of the pollutants and the degree of exposure to dust mites. It shows that the mean individual increase in exposure to PM_{10} in the weeks studied tended to be linked to the deterioration in lung function: a drop in FEV_1 (-1.83; CI 95%: -3.38 to -0.28), FEV_1/FVC (-0.83; CI 95%: -1.72 to 0.06) and a rise in ΔFEV_1 (1.07; CI 95%: 0.20 to 1.94). Associations between increased exposure to NO_2 and a drop in FEV_1 (-7.39; CI 95%: -12.96 to -1.82), FEV_1/FVC (-3.64; CI 95%: -6.82 to -0.46), $FEF_{25-75\,\%}$ (-6.01; CI 95%: -10.94 to -1.09), were also seen, as was an increase in ΔFEV_1 (2.44; CI 95%: -0.39 to 5.28). After adjustment, no association with ozone was seen.

Benzene, toluene and ethylbenzene were the VOCs with significant associations (Table V) between the rise in exposure to pollutants and changes in the airways, being the three of them related to deterioration in lung function. For benzene we found a significant decrease in FEV_1 (-5.23; CI 95%: -8.01 to -2.45), FEV_1/FVC (-2.04; CI 95%: -3.62 to -0.47), FEF25-75% (-7.15; CI 95%: -11.20 to -3.09), and a rise in ΔFEV_1 (3.19; CI 95%: 1.25 to 5.13). Toluene was associated with a drop in FEV_1 (-0.90; CI 95%: -1.87 to 0.07) and an increase in ΔFEV_1 (0.74; CI 95%: 0.16 to 1.32). For ethylbenzene we saw a drop in FEV_1 (-1.90; CI 95%: -3.43 to -0.38), $FEF_{25-75\%}$ (-2.69; CI 95%: -4.86 to -0.52) and a rise in ΔFEV_1 (1.31; CI 95%: 0.21 to 3.77).

We further highlight that the effect of the degree of exposure to dust mites was significant in all the models for FEV_1/FVC and $FEF_{25-75\%,}$ as Table V shows. Figure 1 is a graph showing the effect of the degree of exposure to dust mites on the FEV_1/FVC ratio. The regression coefficients resulting from the univariate analysis and the multivariate analyses are presented, including the effect of the pollutants.

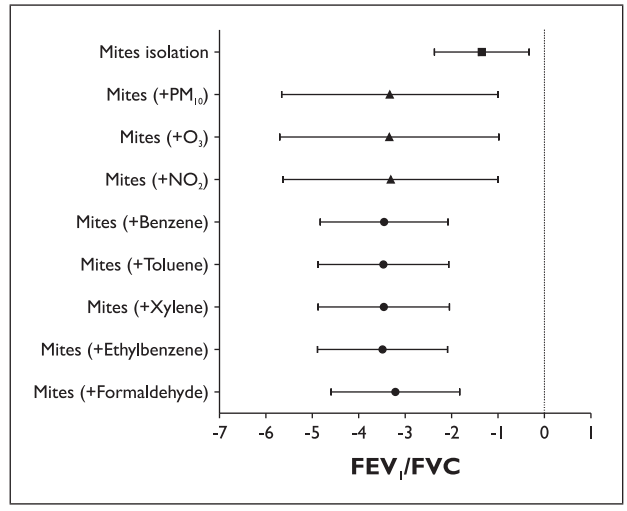

Figure 1. Regression coefficient (RC) and respective 95% confidence intervals relative to the effect of degree of exposure to dust mites on FEV1/FVC ratio. Presented are RC adjusted for the different pollutants (+ Pollutant) and RC for the univariate analysis of the mite exposure variable (isolation). Coefficients are interpreted as percentual changes in FEV1/FVC, by increase of class of exposure. The dotted line seen equals a lack of association. An RC to the left of this line shows the existence of association with decreased FEV1/FVC ratio while a result to the right of the line shows existence of association with an increase in FEV1/FVC ratio.

DISCUSSION

This study seeks to evaluate the effects of air pollution on the airways of children with wheezing also taking house dust mite exposure via the children's mattresses into account. To do so we selected a population of children with a history of wheezing, using an ISAAC study questionnaire, presuming this group would be more susceptible[18] to the effects of air pollution and house dust mites.

We found that a high number of mattresses of the participants had a medium to high degree of infestation of dust mites at each visit. We further found that increasing exposure to dust mites was, as expected[19], associated in a univarate analysis to a deteriorated lung function, namely the FEV_1/FVC ratio and $FEF_{25-75\%}$ and an increased bronchodilator response. This association reinforces the role of dust mites in the worsening of bronchial obstruction and its role in airway bronchomotor tone.

As dust mites are an important environmental factor in the worsening of respiratory disease, it is important to bear this factor in mind when assessing the role of environmental pollutants. It was this we sought to do, finding that exposure to both pollutants and dust mites was significant in the final model which included these variables. The results suggest that both types of exposure are important in a child with a history of wheezing.

The combined evaluation of the effects of air pollutants and allergens is well-known[20-22], but has not been pondered. Nielsen *et al.*'s[23] review concluded that the possible adjuvant effect of chemical and non-chemical exposure (with allergens the latter) on indoor air had little scientific support.

The majority of published studies on the impact of pollution on the airways have centred on a reduced number of pollutants, usually measured in outdoor air. Many of these studies started from the presupposition that the concentration of a pollutant in a determined site corresponded to the value of the exposure of that pollutant. Equally so, it is assumed that all the population close to the measurement point will have identical levels of exposure. We further add that given the great amount of time our children spend indoors, concentrations of pollutants measured in outdoor air do not reflect the real exposure to pollutants. This is particularly true of pollutants whose source of emission is found indoors.

Outdoor air quality has received special attention for many years, with legislation created to regulate the maximum limits of concentrations of classic pollutants, such as PM_{10}, ozone, NO_2, SO_2 and CO. When directing attention to indoor air quality, it is noticed that a high percentage of our time is spent indoors. VOCs are the more characteristic indoor air pollutants. Indoor air VOC concentrations can be 5-20 times higher than those found in outdoor air[8].

There are only a few Portuguese studies into indoor air pollution. The Habit'Ar[24] study into various pollutants in 557 homes on the Portuguese mainland found the indoor air quality was deficient in 60% of homes. Significant associations with the health parameters studied when the pollutants are assessed individually have not yet been reached. A further Portuguese study performed in Oporto schools[25] found increased CO_2 and VOCs in indoor air were associated with respiratory disease.

We further mention an ecological study carried out in Lisbon[26] which found associations between levels of particles measured in indoor air and visits to the A&E at Hospital de Dona Estefânia due to respiratory complaints. Overall, the published studies, and all published studies for VOCs, allow us to affirm that all studies treat exposure to air pollution in sectors: outdoor and indoor air. An asset of our study is that the associations reached are the result of a strategy of calculating exposure which involves the most important indoor environments frequented by the children but also measurements of outdoor air associated to techniques of modelling air quality.

We decided to perform a prospective study with repeated measurements using standardised medical evaluations, evaluations of exposure to different pollutants and also the degree of exposure to house dust mites. We found

that exposure to atmospheric pollutants has an important effect on the airways, even after adjusting for the degree of exposure to dust mites. This effect was marked for various pollutants studied, but the effect of VOCs stood out due to their original character: even at low concentrations these were associated with a worsening of the airways in a group of susceptible children. With the exception of tobacco smoke, indoor air pollution has not been approached in a systematic way.

Indoor environments have the greater concentrations of dust mites[15,21,27] of the main respiratory allergens. House dust mites, namely *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae* and *Lepidoglyphus destructor*, are believed capable of triggering asthma complaints in allergic patients[2,28]. Measurements of 10µg of Der p 1 per gram of dust come under class 3 of the Acarex Test®, corresponding to a concentration of allergen capable of inducing complaints[29,30]. While the results of the guanine quantification tests are not as precise as those obtained using quantification methods via monoclonal antibodies, it is a form of measurement which has been found valid[16,31].

CONCLUSION

Our study reinforces interest in air pollutants, mainly those associated with indoor environments, frequently forgotten and which could explain worsening in a child with wheezing. Children are frequently exposed to VOCs in several school activities or even at home, while working with glues, paints, plasticine or varnishes. Exposure to these pollutants, similarly to that seen in exposure to dust mites, seems to have an impact on the airways of children with wheezing. The effect of pollutants on the airways persists, even when taken into consideration the degree of exposure to house dust mites.

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REFERÊNCIAS

1. Bacharier LB, Boner A, Carlsen KH, Eigenmann PA, Frischer T, Gotz M, *et al.* Diagnosis and treatment of asthma in childhood: a PRACTALL consensus report. Allergy 2008;63:5-34.
2. Custovic A, Taggart SC, Francis HC, Chapman MD, Woodcock A. Exposure to house dust mite allergens and the clinical activity of asthma. J Allergy Clin Immunol 1996;98:64-72.
3. Crane J, Kemp T, Siebers R, Rains N, Fishwick D, Fitzharris P. Increased house dust mite allergen in synthetic pillows may explain increased wheezing. Bmj 1997;314:1763-4.
4. Carr D, von Ehrenstein O, Weiland S, Wagner C, Wellie O, Nicolai T, *et al.* Modeling annual benzene, toluene, NO2, and soot concentrations on the basis of road traffic characteristics. Environ Res 2002;90:111-8.
5. Almeida S, Farinha M, Ventura M, Pio C, Freitas M, Reis M, *et al.* Measuring air particulate matter in large urban areas for health effect assessment. Water Air Soil Pollut 2007;179:43-55.
6. Grahame TJ, Schlesinger RB. Health effects of airborne particulate matter: do we know enough to consider regulating specific particle types or sources? Inhal Toxicol 2007;19:457-81.
7. Eilstein D. Prolonged exposure to atmospheric air pollution and mortality from respiratory causes. Rev Mal Respir 2009; 26:1146-58.

8. Rumchev K, Spickett J, Bulsara M, Phillips M, Stick S. Association of domestic exposure to volatile organic compounds with asthma in young children. Thorax 2004;59:746-51.
9. Cohen AJ, Ross Anderson H, Ostro B, Pandey KD, Krzyzanowski M, Kunzli N, *et al*. The global burden of disease due to outdoor air pollution. J Toxicol Environ Health A 2005;68:1301-7.
10. Martins P, Valente J, Papoila A, Caires I, Araújo-Martins J, Mata P, *et al*. Airways changes related to air pollution in wheezing children. Eur Respir J 2012;39:246-53.
11. Borrego C, Neuparth N, Carvalho A, Miranda A, Costa A, Carvalho A, *et al*. A saúde e o ar que respiramos. Um caso de estudo em Portugal. Lisboa, Edição Fundação Calouste Gulbenkian; 2008.
12. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, *et al*. Standardisation of spirometry. Eur Respir J 2005;26:319-38.
13. Quanjer PH, Borsboom GJ, Brunekreef B, Zach M, Forche G, Cotes JE, *et al*. Spirometric reference values for white European children and adolescents: Polgar revisited. Pediatr Pulmonol 1995;19:135--42.
14. Arbes SJ, Jr., Cohn RD, Yin M, Muilenberg ML, Burge HA, Friedman W, *et al*. House dust mite allergen in US beds: results from the First National Survey of Lead and Allergens in Housing. J Allergy Clin Immunol 2003;111:408-14.
15. Marks GB. House dust mite exposure as a risk factor for asthma: benefits of avoidance. Allergy 1998;53(48 Suppl):108-14.
16. Ransom JH, Leonard J, Wasserstein RL. Acarex test correlates with monoclonal antibody test for dust mites. J Allergy Clin Immunol 1991;87:886-8.
17. Quoix E, Le Mao J, Hoyet C, Pauli G. Prediction of mite allergen levels by guanine measurements in house-dust samples. Allergy 1993;48:306-9.
18. Schwartz J. Air pollution and children's health. Pediatrics 2004; 113(4 Suppl):1037-43.
19. Penagos M, Compalati E, Tarantini F, Baena-Cagnani CE, Passalacqua G, Canonica GW. Efficacy of mometasone furoate nasal spray in the treatment of allergic rhinitis. Meta-analysis of randomized, double--blind, placebo-controlled, clinical trials. Allergy 2008;63:1280-91.
20. Molfino NA, Wright SC, Katz I, Tarlo S, Silverman F, McClean PA, *et al*. Effect of low concentrations of ozone on inhaled allergen responses in asthmatic subjects. Lancet 1991;338:199--203.
21. Tunnicliffe WS, Burge PS, Ayres JG. Effect of domestic concentrations of nitrogen dioxide on airway responses to inhaled allergen in asthmatic patients. Lancet 1994;344:1733-6.
22. El-Sharif N, Abdeen Z, Barghuthy F, Nemery B. Familial and environmental determinants for wheezing and asthma in a case-control study of school children in Palestine. Clin Exp Allergy 2003;33:176--86.
23. Nielsen GD, Larsen ST, Olsen O, Lovik M, Poulsen LK, Glue C, *et al*. Do indoor chemicals promote development of airway allergy? Indoor Air 2007;17:236-55.
24. Morais de Almeida M, Lopes I, Nunes C. Caracterização da qualidade do ar interior em Portugal – Estudo HabitAR. Rev Port Imunoalergologia 2010;18:21-38.
25. Fraga S, Ramos E, Martins A, Samudio MJ, Silva G, Guedes J, *et al*. Indoor air quality and respiratory symptoms in Porto schools. Rev Port Pneumol 2008;14:487-507.
26. Moreira S, Silva Santos C, Tente H, Nogueira L, Ferreira F, Neto A. Morbilidade respiratória e exposição a partículas inaláveis na cidade de Lisboa. Acta Pediatr Port 2008;39:223-32.
27. Olsson S, van Hage-Hamsten M. Allergens from house dust and storage mites: similarities and differences, with emphasis on the storage mite Lepidoglyphus destructor. Clin Exp Allergy 2000;30:912--9.
28. Ronborg SM, Mosbech H, Poulsen LK. Exposure chamber for allergen challenge. A placebo-controlled, double-blind trial in house--dust-mite asthma. Allergy 1997;52:821-8.
29. Salo PM, Arbes SJ Jr, Crockett PW, Thorne PS, Cohn RD, Zeldin DC. Exposure to multiple indoor allergens in US homes and its relationship to asthma. J Allergy Clin Immunol 2008;121:678-684 e2.
30. Macher JM, Tsai FC, Burton LE, Liu KS. Concentrations of cat and dust-mite allergens in dust samples from 92 large US office buildings from the BASE Study. Indoor Air 2005;15(Suppl 9):82-8.
31. van der Brempt X, Haddi E, Michel-Nguyen A, Fayon JP, Soler M, Charpin D, *et al*. Comparison of the ACAREX test with monoclonal antibodies for the quantification of mite allergens. J Allergy Clin Immunol 1991;87(1 Pt 1):130-2.