Impact of environmental exposures on exhaled breath and lung function: NELA Birth Cohort

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Shareable abstract (@ERSpublications)
There is a significant association between toluene and benzene in exhaled breath and air pollution (infants and mothers) and smoking status (mothers), and between toluene and $FEV_{0.5}$ in infants. Breath analysis could be used as a biomonitoring tool. https://bit.ly/3SjBevS

Cite this article as: Sola-Martínez RA, Jiménez-Guerrero P, Sánchez-Solís M, *et al*. Impact of environmental exposures on exhaled breath and lung function: NELA Birth Cohort. *ERJ Open Res* 2025; 11: 00597-2024 [DOI: 10.1183/23120541.00597-2024].

Received: 13 June 2024
Accepted: 17 July 2024

Abstract

Introduction Exposure to environmental factors (*i.e.* air pollution and second-hand tobacco smoke) have been associated with impaired lung function. However, the impact of environmental factors on lung health is usually evaluated separately and not with an exposomic framework. In this regard, breath analysis could be a noninvasive tool for biomonitoring of global human environmental exposure.

Methods Data come from 337 mother–child pairs from the Nutrition in Early Childhood Asthma (NELA) birth cohort. Levels of BTEX (benzene, toluene, ethylbenzene and xylenes) in exhaled breath from mothers and children at 3 months after birth were estimated using gas chromatography–mass spectrometry. Short-term residential exposures (breath sampling day and 15 days before breath sampling) to nitrogen dioxide, particulate matter ($PM_{2.5}$) and ozone were determined by chemical dispersion/transport modelling. Forced vital capacity, forced expiratory volume in 0.5 s ($FEV_{0.5}$) and forced expiratory flow at 75% of FVC and at 25%–75% of FVC were measured in infants according to the raised-volume rapid thoracoabdominal compression technique.

Results The results showed significant associations between short-term exposure to external agents and levels of benzene and toluene in exhaled breath. It was observed that exhaled levels of benzene and toluene were influenced by smoking status and outdoor air pollution in mothers, and by air pollution in infants (3 months of age). No significant relationship was observed between exposure to maternal tobacco smoking and/or short-term air pollution and lung function in healthy infants. However, there was a significant relationship between $FEV_{0.5}$ and exhaled toluene in children.

Discussion These findings indicated a significant relationship between environmental exposures and exhaled levels of benzene and toluene, suggesting that breath analysis could be a helpful exposure biomonitoring tool.

Introduction

Chronic respiratory diseases, including asthma, are highly prevalent worldwide and cause huge economic burden on health services. Exposure to external agents such as second-hand tobacco smoke and air pollutants in childhood could impair lung development and promote the onset of chronic respiratory pathologies [1–3]. Although the effect on lung function in children of maternal smoking [4, 5] and air pollution exposure [6, 7]

has been previously evaluated, additional studies on the interaction between the two factors are needed. Furthermore, a comprehensive approach such as the assessment of the exposome or all human environmental exposures is essential to unravel the early life origin of many respiratory diseases [8, 9].

In this regard, it has been suggested that breath analysis could be a useful tool for personal exposome monitoring [10]. Human exhaled breath can be collected by noninvasive sampling methods, and a large number of volatile organic compounds (VOCs) of both endogenous and exogenous origin can be detected [11, 12]. Most exogenous VOCs come from the inhalation of indoor and outdoor air pollutants, such as compounds from tobacco smoke, compounds derived from fossil fuel combustion or household products, among others. As a result, exogenous VOC analysis in exhaled breath could be used to determine the real internal exposure level of the organism (biomonitoring of human exposure), instead of the traditional ambient air monitoring that only reflects external exposure level [13]. Of particular note as potential indicators of air pollution are benzene, toluene, ethylbenzene, p/m-xylene and o-xylene, which are often grouped under the name BTEX. These exogenous VOCs have previously been linked to smoking status, indoor air exposure and crude oil/fuel exposure. Toluene, ethylbenzene and xylenes are used as solvents in different industries, and benzene is considered a human carcinogen [13–17].

The main objective of the present study was to examine whether exposure to external agents (air pollution and maternal smoking) can be biomonitored by analysis of BTEX in exhaled breath. Additionally, we studied the relationship between lung function of healthy infants (3 months of age) and both environmental exposures and exhaled BTEX levels. We used data from mothers and children of the Nutrition in Early Childhood Asthma (NELA) birth cohort [18].

Methods

Study design and participants

The present study involved mother–child pairs from a prospective birth cohort, NELA. All details concerning the NELA study (protocol of the study, sample size, recruitment process, inclusion and exclusion criteria, and methodology of data collection) have been described in Morales *et al.* [18]. Recruitment of subjects was conducted during the routine fetal anatomy scan in the 20th week of pregnancy at the Maternal-Fetal Unit of the "Virgen de la Arrixaca" University Clinical Hospital (Murcia, Spain) between March 2015 and April 2018. The NELA study has performed several follow-up points of the recruited subjects: follow-up visit 1 (at 20–24 weeks of pregnancy), follow-up visit 2 (at 32 weeks of pregnancy), follow-up visit 3 (at delivery), follow-up visit 4 (3 months after childbirth), follow-up visit 5 (18 months after childbirth), follow-up visit 6 (5 years after childbirth). A follow-up visit 7 (8 years after childbirth) is planned. Exhaled breath samples from mother–child pairs were collected at follow-up visit 4 between May 2017 and October 2018. Lung function tests were performed on infants when they were 3–8 months old [19]. The study protocol was assessed and approved by the Ethics Committee of the "Virgen de la Arrixaca" University Clinical Hospital in accordance with the guidelines of the Declaration of Helsinki (CEIC 09/14). Written informed consent was obtained from the mothers both at the time of recruitment and prior to infant lung function tests.

Exposure assessment to residential outdoor air pollutants

In the NELA study, the residential exposure of mother–child pairs to air pollutants was estimated by means of chemical dispersion/transport models. The information on residential address was gathered during follow-up visits. The Weather Research and Forecasting (WRF)+CHIMERE modelling system was implemented to determine outdoor air pollutant concentrations [18, 20]. The regional meteorological model Advanced Research Weather Research and Forecasting (WRF-ARW) Model v3.6.1 was used to provide the meteorology to the chemistry transport model [21]. WRF has been coupled off-line on an hourly basis to CHIMERE chemistry transport model [22]. WRF fields are interpolated to CHIMERE working grids. The chemistry transport model includes gas-phase chemistry and aerosols; and heterogeneous chemistry distinguishes among different chemical aerosol components. The physicochemical options for the regional modelling system can be found in Domínguez-Morueco *et al.* [23]. Geo-codification of the residential address by Geographic Information System (GIS) was used for atmospheric pollution extraction in the selected period. In the present study, short-term exposure to air pollution was determined in two periods: on the day of follow-up visit 4 (3 months after birth) and 15 days before follow-up visit 4 (figure 1b). Short-term mean concentrations of air standards (nitrogen dioxide (NO_2), particulate matter ($PM_{2.5}$) and ozone (O_3)) [24] at the family home of each mother–child pair were estimated during the two selected periods. For each air pollutant, exposure was classified as high or low/medium based on whether the estimated mean concentrations at the residential address are above or below the 90th percentile (supplementary figure S1) [25].

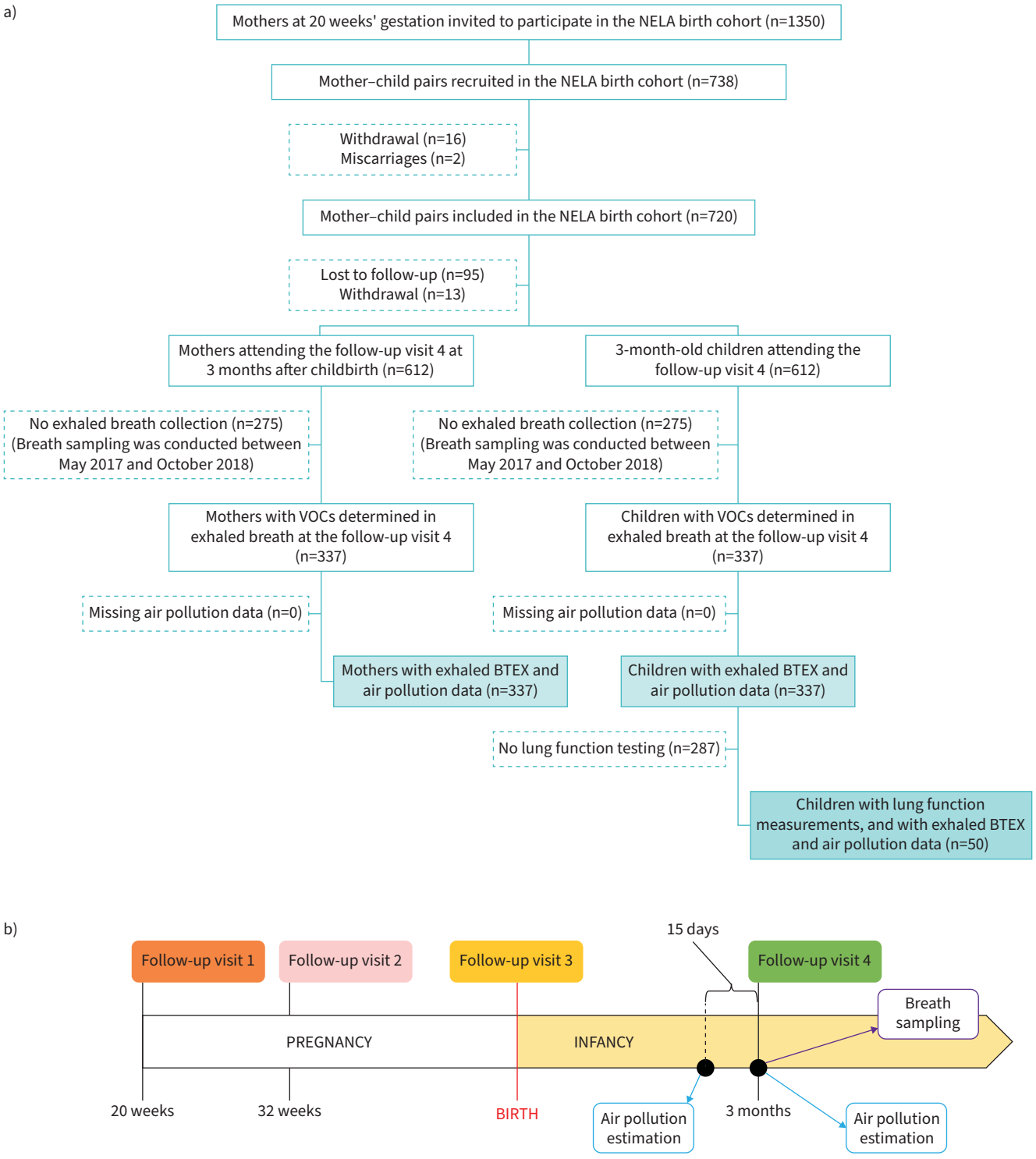

FIGURE 1 Study flow diagram. a) Study population, exclusions and missing data. Subjects of the present study are included in Nutrition in Early Childhood Asthma (NELA) birth cohort [18]. b) Flow chart of residential air pollution estimation and breath analysis. VOCs: volatile organic compounds; BTEX: benzene, toluene, ethylbenzene and xylenes (p-xylene, m-xylene and o-xylene). Follow-up visit 4 was conducted between September 2015 and October 2018, and breath sampling started in May 2017 [48].

Information on maternal smoking status and external agent exposure

Trained interviewers performed a structured questionnaire to mothers at each follow-up visit of the NELA study [18]. During follow-up visit 4, information about smoking status of mothers (smoker: yes/no) was

compiled. Thus, subjects were divided into two groups ("exposure to external agents" and "no exposure to external agents") based on maternal smoking habits and exposure to outdoor air pollutants at the residential address. The "exposure to external agents" group consisted of subjects exposed to high concentration of residential air pollution using a unique threshold (⩾90th percentile) to any of the air pollutants (NO_2, $PM_{2.5}$ and O_3), and/or is a smoker (maternal smoking in children). The group "no exposure to external agents" included subjects exposed to a low/medium concentration of three air pollutants and nonsmokers (no maternal smoking in children). The "exposure to external agents" group was subdivided into three categories ("exposure to air pollution", "exposure to tobacco smoking" and "exposure to tobacco and air pollution") according to smoking habits of the mothers.

Information on other variables

Information on other variables was obtained using structured questionnaires administered in person in different follow-up visits of the NELA study. In follow-up visit 1 this included: age of mother; pre-pregnancy body mass index (BMI) of mother; social class of mother (defined as occupation during pregnancy based on the highest social class by using a widely used Spanish adaptation of the international ISCO88 coding system: I–II, managers/technicians; III, skilled; IV–V, semiskilled/unskilled; and unemployed) [26]; educational level of mother (incomplete secondary or less, complete secondary, and university); asthma in the father (yes/no); asthma in the mother (yes/no); allergic rhinitis in the father (yes/no); allergic rhinitis in the mother (yes/no); maternal smoking during pregnancy (yes/no); information about domestic heating (oil heater (yes/no), thermal plates (yes/no), electric heater (yes/no), heat pump (yes/no), gas stove (yes/no), wood burning (yes/no) and diesel burning (yes/no)); and information about domestic cooking systems (gas cooker by city gas (yes/no), gas cooker by butane (yes/no) and electric cooker (yes/no)). In follow-up visit 3 this included: sex of child; birthweight of child; and birth height of child. In follow-up visit 4 this included: number of cohabitant smoker(s); number of cigarettes smoked in total by cohabitant daily; home smoking policy (total smoking ban, partial smoking ban and no smoking ban), number of cigarettes smoked by mothers daily; paternal smoking (yes/no); number of cigarettes smoked by fathers daily; and season at breath sampling (winter, December–February; spring, March–May; summer, June–August; and autumn, September–November). In addition, before lung function testing, the weight and height of the children were measured.

Exhaled breath analysis

The protocols used for breath sampling, breath analysis and data preprocessing were explained in detail in Sola-Martínez *et al*. [27]. The mixed expiratory breath portion was collected from both mothers and 3-month-old infants in gas sampling bags. Whereas exhaled breath was collected in infants using a face mask coupled to a 400-mL Quintron bag (QuinTron Instrument Company) and subsequently transferred to a 1-L Tedlar bag (Supelco), exhaled breath of mothers was directly collected in 1-L Tedlar bags. In infant breath sampling, this additional step was performed because they are passive subjects and the 400-mL Quintron® bags offer minimal resistance to filling. All breath samples were collected in the same location. Moreover, a room air sample was also collected for each human exhaled breath sample by an Easy-VOC syringe (Markes International) to assess the influence of the environmental air from the sampling room. Both human exhaled breath and air room samples were stored in thermal desorption tubes (Tenax TA/carbograph 5td, Markes International) prior to analysis. The samples were analysed by a thermal desorption system coupled with gas chromatography–single quadrupole mass spectrometry (TD-GC/q-MS). Raw mass spectrometry data were preprocessed using a workflow developed by our group [27], which allows a matrix to be generated with the intensities of the features or ionic fragments (mass/charge or m/z signals) detected in the samples, to determine to which compound each feature belongs and the identification of the compounds. The compounds were identified by matching with the National Institute of Standard and Technology (NIST) spectral library based on mass spectra and retention indexes. Chemical standards (C7–C30 saturated alkanes standard and VOC calibration standard, Sigma-Aldrich) were used for retention indexes calculation. For the present study, characteristic features of benzene (feature m/z=78), toluene (feature m/z=91), ethylbenzene (feature m/z=91) and xylene (feature m/z=91) were selected from the generated matrix. A log10 transformation was applied on the intensity values of the selected features. For the calculation of xylene intensity, the 91 m/z signal intensity values of all xylene isomers (m-xylene/p-xylene and o-xylene) were considered.

Lung function measurements

Lung function measurements of children were carried out in the Pediatric Respiratory Unit of the "Virgen de la Arrixaca" Children's University Clinical Hospital as previously described [19]. Master-Screen BabyBody Plethysmograph (Jaeger) was used for lung function tests. During measurements, the infants were sedated (chloral hydrate 80–100 $mg \cdot kg^{-1}$), and a Neopuff infant resuscitator (Fisher & Paykel Healthcare) connected to a face mask was used to inflate the lung to a standard pressure of 30 cmH_2O.

In addition, oxygen saturation was carefully monitored until recovery from sedation. Forced vital capacity (FVC), forced expiratory volume in 0.5 s ($FEV_{0.5}$) and forced expiratory flow at 75% of FVC (FEF_{75}) and at 25%–75% of FVC (FEF_{25-75}) were obtained from maximal expiratory volume curves by the raised-volume rapid thoracoabdominal compression technique according to the American Thoracic Society/European Respiratory Society guidelines [19, 28, 29]. Z-score of lung function parameters (FVC, $FEV_{0.5}$, FEF_{75} and FEF_{25-75}) was determined according to Lum *et al.* [30].

Calculation of child anthropometric indicators

Z-scores of four anthropometric indicators (length/height-for-age, weight-for-age, weight-for-length and BMI-for-age) were calculated based on child data collected during follow-up visit 4 using the World Health Organization (WHO) Anthro Survey Analyser (www.who.int/tools/child-growth-standards/software). It is a tool designed to check the growth of children under 5 years.

Statistical analysis

The statistical analysis was conducted by version R 4.0.5. Data distribution was evaluated by Shapiro–Wilk tests or Lilliefors tests (nortest package; https://cran.r-project.org/package=nortest) for the choice of the most appropriate statistical tests (parametric or non-parametric tests). Associations between variables were assessed using Chi-square tests or Fisher's exact-tests (categorical variable *versus* categorical variable), Spearman's rank correlation tests (continuous variable *versus* continuous variable), t-tests or Mann–Whitney U-tests (continuous variable *versus* categorical variable with two groups), or ANOVA or Kruskal–Wallis tests followed by Bonferroni correction for multiple testing (continuous variable *versus* categorical variable with more than two groups). In addition, multiple linear regression models were constructed with each of the lung function parameters expressed in volume (millilitres) (dependent variable) and each of the selected VOCs (independent variables). The models included the following covariates: sex, height, weight and age at the time of lung function measurements. The significance threshold for all statistical analysis was $p<0.05$.

Results

Description of study participants

Study population, exclusions and missing data are summarised in figure 1a. Of the 738 mother–child pairs recruited in the NELA birth cohort [18], 612 attended the follow-up visit 4 (3 months after childbirth). The collection of exhaled breath began in May 2017, so that exhaled breath samples from 337 mother–child pairs were collected and analysed. Thus, exhaled breath samples and residential air pollution determinations (data on the day of breath sampling and 15 days before breath sampling) were obtained from 674 subjects (337 mothers and 337 children). In addition, lung function data ($FEV_{0.5}$ values) were

TABLE 1 Study population characteristics

Mothers included (n=337)	
Age years	33.0±4.3
BMI kg·m^{-2} before pregnancy	23.9±4.4
Social class	
Managers–technicians	123 (36.5)
Skilled	69 (20.5)
Semiskilled–unskilled	69 (20.5)
Unemployed	76 (22.6)
Educational level	
Incomplete secondary or less	57 (16.9)
Complete secondary	88 (26.1)
University	192 (57.0)
Smoker (yes)	50 (14.8)
Asthma (yes)	40 (11.9)
Allergic rhinitis (yes)	118 (35.0)
Children included (n=337)	
Males/females	158/179
Birthweight kg	3.224±0.491
Birth height cm	50.5±2.3
Mother/father with asthma (yes)	66 (19.6)
Mother/father with allergic rhinitis (yes)	169 (50.1)

Data are presented as n, n (%) or mean±SD. BMI: body mass index.

obtained from 50 of the 337 infants. Characteristics of the study population are outlined in table 1 and supplementary tables S1–S4. There was no significant relationship between exposure to external agents (air pollution, tobacco or both) and the percentage of mothers with asthma or allergic rhinitis, or the percentage of children with a parental history of asthma or allergic rhinitis (supplementary tables S1–S4, supplementary figures S2 and S3). However, there was a significant difference in the distribution of season at breath sampling between exposed and non-exposed groups during the follow-up visit 4, with a different observed frequency of breath collection being lower in winter and higher in summer than expected in exposed subjects (supplementary table S1).

Association between exposure to external agents and BTEX levels in exhaled breath

The effect of external agent exposure on BTEX levels in exhaled breath of mothers and children is shown in figures 2 and 3. The levels of the four VOCs were higher in the exhaled breath of subjects exposed to external agents than in non-exposed subjects in both periods (breath sampling day and 15 days before breath sampling). However, the differences between both groups (exposed and non-exposed) were only significant in the levels of benzene and toluene. Significantly elevated benzene levels were observed in children and in mothers exposed to external agents during the day of follow-up visit 4, and in mothers exposed to external agents during the 15 days prior to follow-up visit 4. Furthermore, significantly elevated toluene levels were observed in both children and mothers exposed to external agents in two selected periods. It is important to highlight that, no significant differences due to external agents were observed in BTEX levels in the environmental air samples from the breath sampling room (supplementary figures S4 and S5). Then, the influence of external agents (environmental pollution and maternal tobacco smoking) on exhaled BTEX levels was analysed separately (figures 4 and 5, supplementary figures S6 and S7). Significantly elevated levels of benzene and toluene were observed in subjects exposed to air pollution in both mothers and children in the two selected periods (figures 4 and 5). Furthermore, the effect of tobacco exposure was different in mothers and children. Whereas benzene and toluene levels were significantly higher in smoking mothers in the two periods, no differences were observed between children passively exposed to maternal tobacco smoking and non-exposed children. There was also no significant relationship between exhaled BTEX levels in children and maternal smoking during pregnancy, paternal smoking, or household environmental tobacco smoke (ETS) exposure (smoking status of cohabitants, smoking burden of cohabitants and home smoking policy) (supplementary table S5 and supplementary figures S8–S11).

Possible biases due to other sources of indoor air pollution such as domestic heating and cooking systems were also assessed (supplementary tables S6 and S7). Significant differences between groups (external agents *versus* no external agents) were observed in the percentage of families with butane gas and electric cookers: butane gas cookers were more frequent in the exposed group, and electric cookers in the non-exposed group. However, no significant relationship was observed between exhaled BTEX levels in children and mothers and the use of butane gas and electric cookers at home (supplementary figures S12–S17).

Association between exposure to external agents and lung function

No significant differences were observed in lung function parameters (FVC (z-score), $FEV_{0.5}$ (z-score), FEF_{75} (z-score) and FEF_{25-75} (z-score)) between children exposed and not exposed to external agents (figures 2 and 3). Furthermore, no significant influence was observed on any lung function parameter due to the exposure of the different external agents separately (supplementary figures S18 and S19).

Association between exposure to external agents and anthropometric indicators

There was no significant influence of the exposure to external agents on weight-for-age (z-score), weight-for-length (z-score) and BMI-for-age (z-score) in infants (supplementary figures S20–S23). Although there was a significant decrease in length/height-for-age (z-score) in the exposed group considering the residential air pollution of the day of the follow-up visit 4 (supplementary figures S20 and S22), no such effect was observed when considering residential air pollution of the previous 15 days (supplementary figures S21 and S23).

Association between lung function and levels of benzene and toluene in exhaled breath

The relationship between exhaled levels of benzene and toluene (VOCs significantly related to external agent exposure (figures 2 and 3)) and lung function parameters in children was assessed using three approaches. First, the correlation between lung function parameters (FVC (z-score), $FEV_{0.5}$ (z-score), FEF_{75} (z-score) and FEF_{25-75} (z-score)) and the levels of the selected VOCs (benzene and toluene) was studied (figure 6 and supplementary figure S24). Exhaled toluene was significantly negatively correlated with $FEV_{0.5}$ (z-score) (figure 6). Then, children were classified into three groups based on $FEV_{0.5}$ (z-score) values (supplementary figures S25C), and differences among the groups in exhaled levels of benzene and toluene were checked (supplementary figure S25). Significant differences between groups were found in

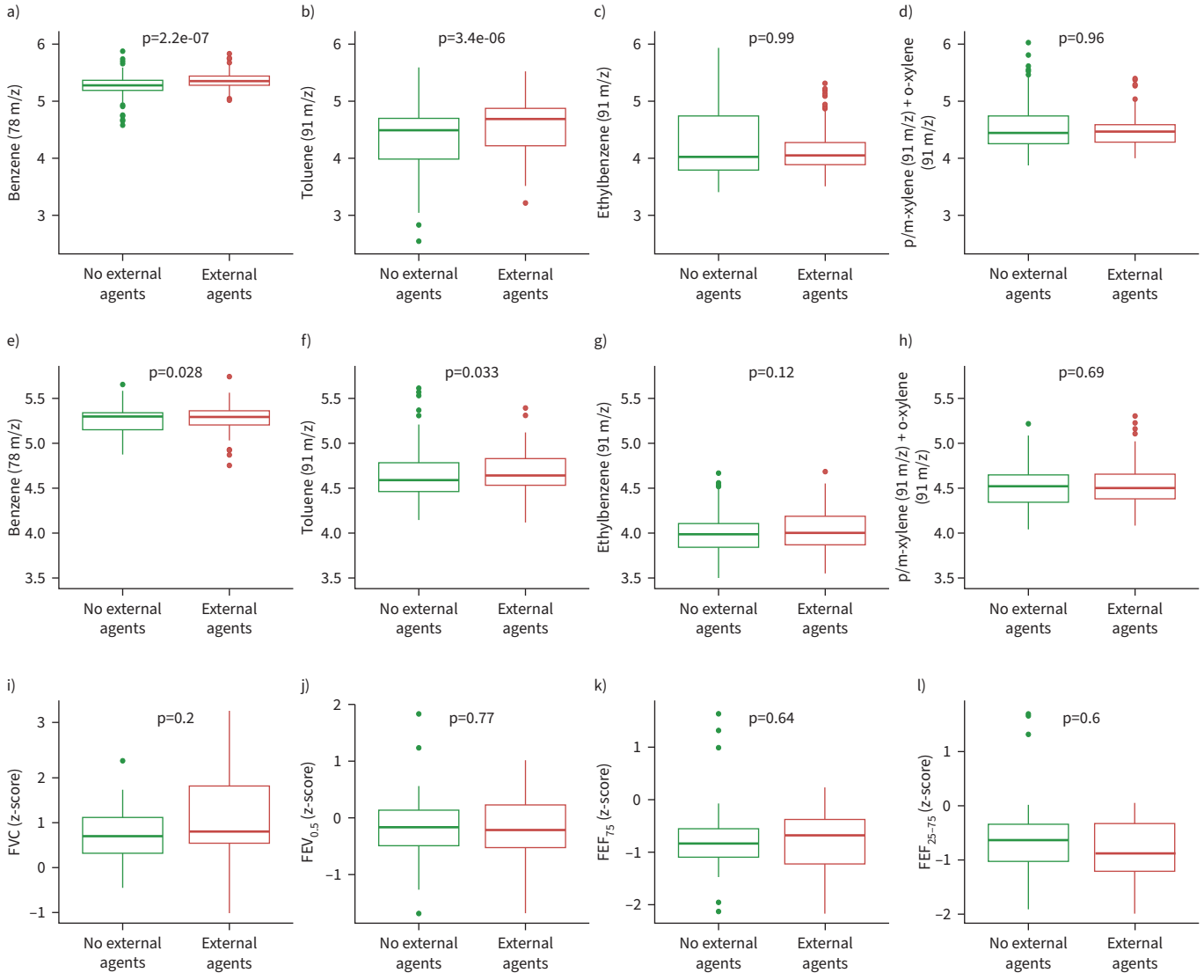

FIGURE 2 Effect of exposure to external agents during the breath sampling day on BTEX levels in exhaled breath and on lung function. Exposure to external agents involved exposure to high concentrations (90th percentile) of air pollutants (nitrogen dioxide, $PM_{2.5}$ or ozone) at the family home on the breath sampling day, and/or maternal smoking (active smoking in mothers and passive smoking in children). a) Influence of exposure to external agents on benzene levels in exhaled breath from mothers. b) Influence on toluene in exhaled breath from mothers. c) Influence on ethylbenzene in exhaled breath from mothers. d) Influence on xylenes (p/m-xylene and o-xylene) in exhaled breath from mothers. e) Influence on benzene in exhaled breath from children. f) Influence on toluene in exhaled breath from children. g) Influence on ethylbenzene in exhaled breath from children. h) Influence on xylenes (p/m-xylene and o-xylene) in exhaled breath from children. i) Influence of exposure to external agents on FVC (z-score) of children. j) Influence of exposure to external agents on $FEV_{0.5}$ (z-score) of children (n=50). k) Influence of exposure to external agents on FEF_{75} (z-score) of children (n=50). l) Influence of exposure to external agents on FEF_{25-75} (z-score) of children (n=50). BTEX: benzene, toluene, ethylbenzene and xylenes (p-xylene, m-xylene and o-xylene); FVC: forced vital capacity; $FEV_{0.5}$: forced expiratory volume in 0.5 s; FEF_{75}: forced expiratory flow at 75% of FVC; FEF_{25-75}: forced expiratory flow at 25%–75% of FVC.

exhaled toluene levels. Finally, the association between lung function and levels of benzene and toluene in exhaled breath was evaluated by multiple linear regression analysis (table 2). Thus, it was observed that lower values of $FEV_{0.5}$ were significantly associated with higher levels in exhaled breath of toluene.

Discussion

In the current study, a significant association between toluene and benzene levels in exhaled breath and exposure to external agents (air pollution and maternal tobacco smoking) has been observed in both mothers and infants (figures 2 and 3). As can be seen in figures 4 and 5, not only were the levels of benzene and toluene in exhaled breath of the mothers influenced by environmental pollution, but also by

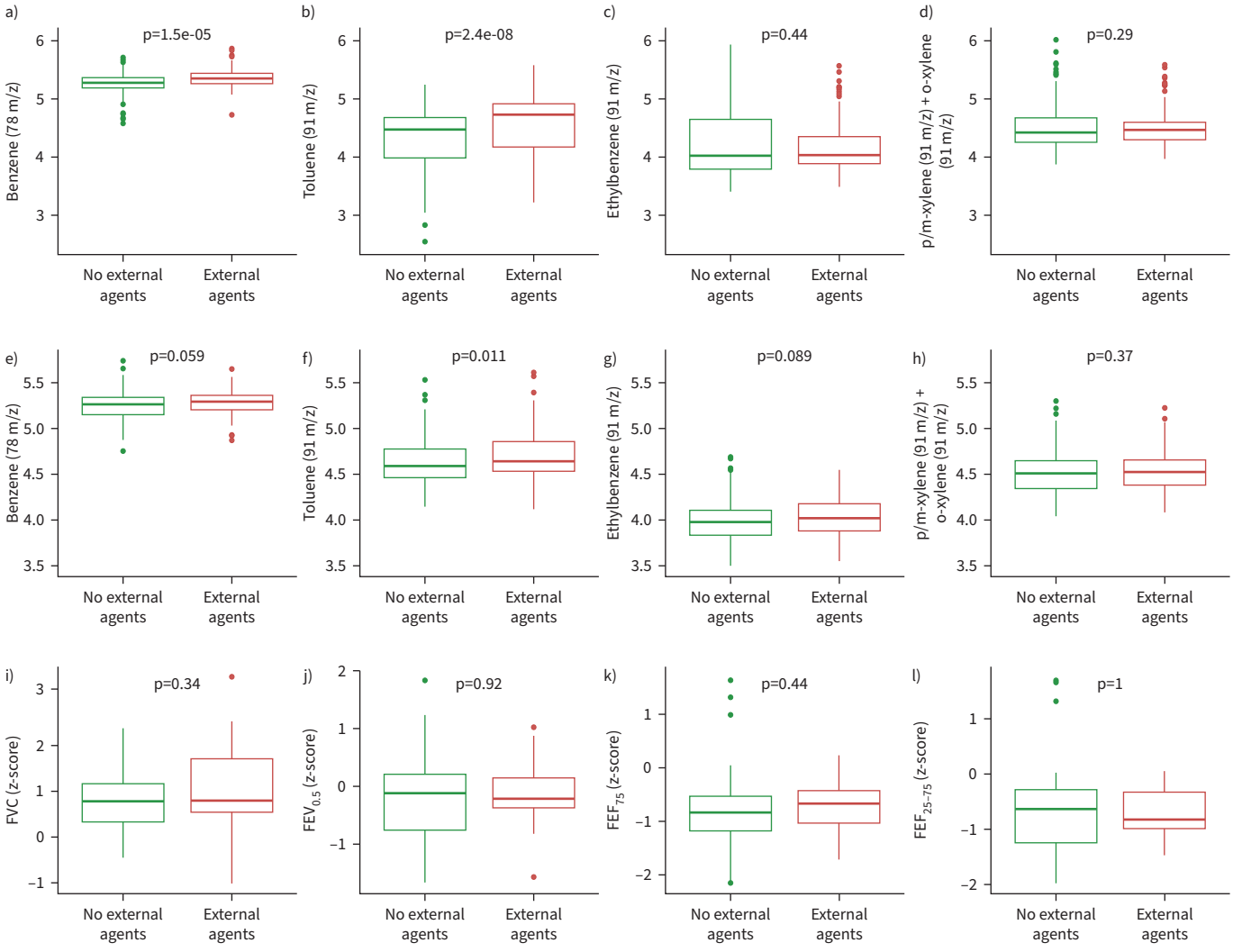

FIGURE 3 Effect of exposure to external agents during the 15 days before breath sampling on BTEX levels in exhaled breath and on lung function. Exposure to external agents involved exposure to high concentrations (90th percentile) of air pollutants (nitrogen dioxide, $PM_{2.5}$ or ozone) at the family home on the 15 days before breath sampling, and/or maternal smoking (active smoking in mothers and passive smoking in children). a) Influence of exposure to external agents on benzene levels in exhaled breath from mothers. b) Influence on toluene in exhaled breath from mothers. c) Influence on ethylbenzene in exhaled breath from mothers. d) Influence on xylenes (p/m-xylene and o-xylene) in exhaled breath from mothers. e) Influence on benzene in exhaled breath from children. f) Influence on toluene in exhaled breath from children. g) Influence on ethylbenzene in exhaled breath from children. h) Influence on xylenes (p/m-xylene and o-xylene) in exhaled breath from children. i) Influence of exposure to external agents on FVC (z-score) of children. j) Influence of exposure to external agents on $FEV_{0.5}$ (z-score) of children (n=50). k) Influence of exposure to external agents on FEF_{75} (z-score) of children (n=50). l) Influence of exposure to external agents on FEF_{25-75} (z-score) of children (n=50). BTEX: benzene, toluene, ethylbenzene and xylenes (p-xylene, m-xylene and o-xylene); FVC: forced vital capacity; $FEV_{0.5}$: forced expiratory volume in 0.5 s; FEF_{75}: forced expiratory flow at 75% of FVC; FEF_{25-75}: forced expiratory flow at 25%–75% of FVC.

smoking status. Benzene and toluene can be found in both indoor and outdoor air as they are by-products of tobacco smoke and traffic emissions [13, 16]. Thus, elevated levels of benzene and toluene in petrol station employees have been reported by Scheepers *et al.* [31]. In addition, significantly higher levels of benzene and toluene in the exhaled breath of smokers have also been observed [32]. In contrast, only significant differences in the levels of benzene and toluene in exhaled breath were observed between children exposed to environmental pollution and children not exposed to external agents. Therefore, postnatal maternal smoking did not have a significant effect on exhaled BTEX levels in infants (figures 4 and 5, supplementary figures S6 and S7). The smoking habits of the other cohabitants, the number of cigarettes smoked by cohabitants or home smoking policy also had no significant influence on the BTEX levels in exhaled breath of the children (supplementary figures S8–S11). In this regard, Scherer *et al.* [33] observed differences in exhaled benzene levels in a small population of children (4–15 years) between

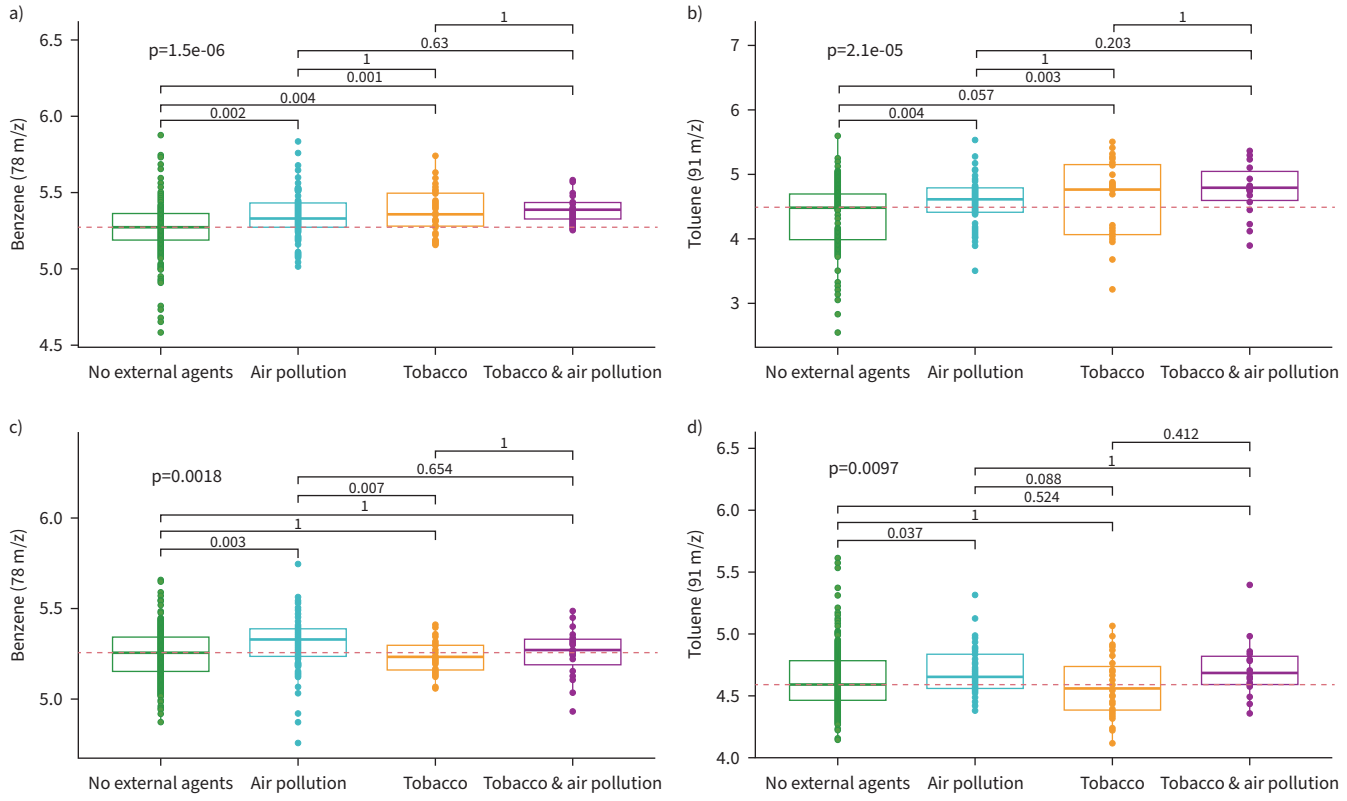

FIGURE 4 Impact of exposure to tobacco and to residential air pollution during the breath sampling day on levels of benzene and toluene in exhaled breath. Exposure to air pollution involved exposure to high concentrations (90th percentile) of air pollutants (nitrogen dioxide, $PM_{2.5}$ or ozone) at the family home on the breath sampling day. Tobacco exposure was considered active smoking in mothers and maternal smoking in children. a) Influence of tobacco and air pollution exposure on benzene levels in exhaled breath from mothers. b) Influence on toluene in exhaled breath from mothers. c) Influence on benzene in exhaled breath from children. d) Influence on toluene in exhaled breath from children. The red dotted line indicates the median of levels of benzene or toluene of the subjects who were not exposed to external agents.

residential locations (urban/suburban homes) but found no significant differences between nonsmoking and smoking homes. In contrast, Scheepers *et al.* [34] observed a significant impact on the exhaled breath of children (10–11 years) when they had cohabitants who smoked indoors (higher levels of toluene) and when they were exposed to ETS inside vehicles (higher levels of benzene). Previous studies in the paediatric population (5–11 years) have also shown significant associations between urinary unmodified benzene levels and both number of smokers in the houses and smoking burden [17, 35], as well as between second-hand smoke exposure and VOC metabolites benzene and toluene in urine [36]. So, it is likely that no significant relationship between exhaled BTEX levels and ETS exposure was detected in the present study because the children were 3 months old and could not have undergone a long period of exposure. In the next follow-up visits of the NELA study, we plan to continue to examine the possible impact on children's exhaled breath due to exposure to second-hand smoke.

Furthermore, in this study, the distribution of potential confounders between groups (external agents *versus* non-external agents) has been checked in detail in order to not bias the results (supplementary tables S1–S4, S6 and S7, and supplementary figures S2 and S3). A lower percentage in winter and a higher percentage in summer of subjects exposed to external agents on breath sampling day than expected was observed (supplementary table S1), which could be due to seasonal significant differences in mean concentration of air pollutants (NO_2, O_3 and $PM_{2.5}$) at the residential address (supplementary figures S26 and S27). The mean concentration of NO_2 and $PM_{2.5}$ was lower in winter and the mean concentration of O_3 was higher in summer. It has been previously reported that high temperature and strong solar radiation are linked to high O_3 concentrations [37]. Regarding household air pollution bias, no significant differences were observed in the distribution of domestic heating and cooking systems between the two groups, except for the use of butane gas cooker and electric cooker (supplementary tables S6 and S7). However, no significant association was found between the use of butane gas or electric cookers and

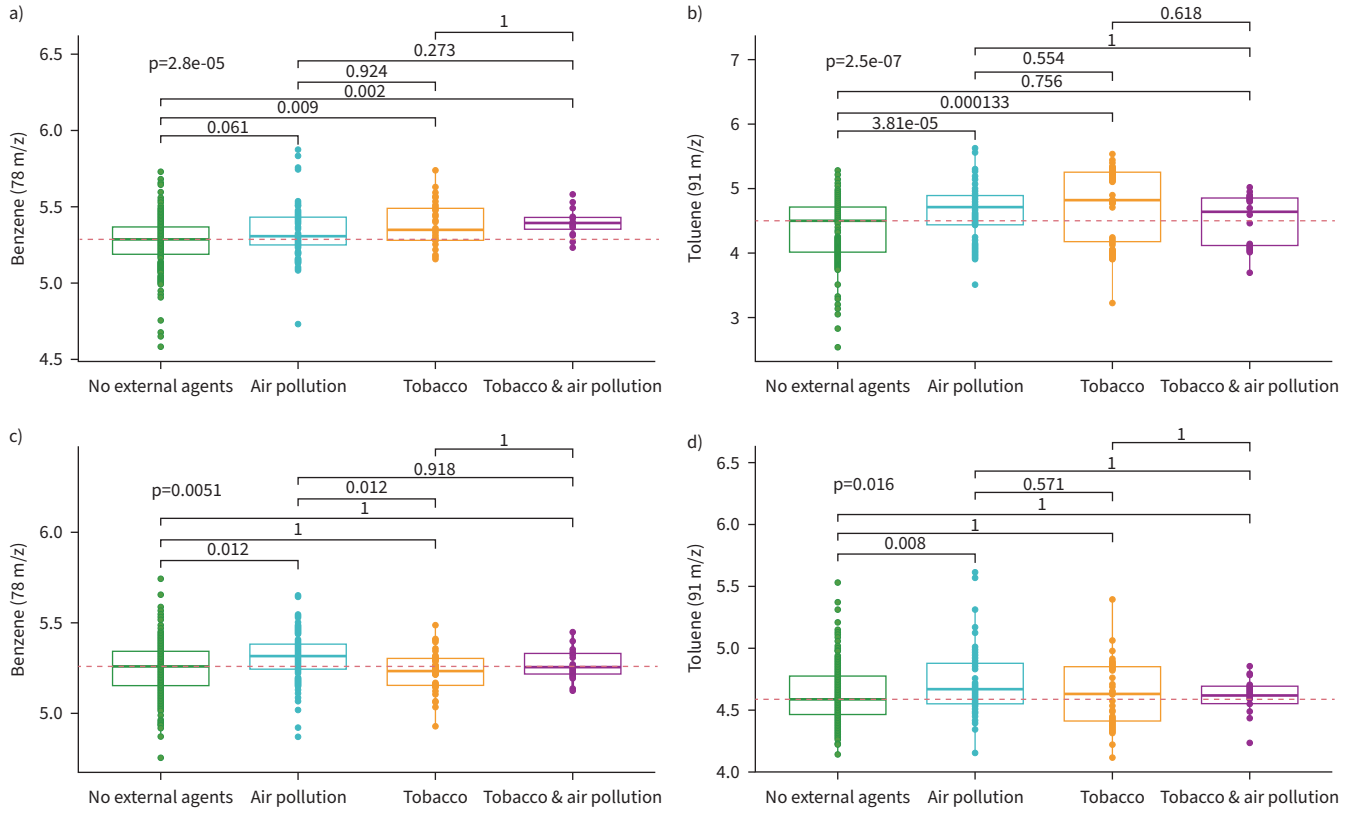

FIGURE 5 Impact of exposure to tobacco and to residential air pollution during the 15 days before breath sampling on levels of benzene and toluene in exhaled breath. Exposure to air pollution involved exposure to high concentrations (90th percentile) of air pollutants (nitrogen dioxide, $PM_{2.5}$ or ozone) at the family home on the 15 days before breath sampling. Tobacco exposure was considered active smoking in mothers and maternal smoking in children. a) Influence of tobacco and air pollution exposure on benzene levels in exhaled breath from mothers. b) Influence of tobacco and air pollution exposure on toluene levels in exhaled breath from mothers. c) Influence of tobacco and air pollution exposure on benzene levels in exhaled breath from children. d) Influence of tobacco and air pollution exposure on toluene levels in exhaled breath from children. The red dotted line indicates the median of levels of benzene or toluene of the subjects who were not exposed to external agents.

exhaled BTEX levels (supplementary figures S12–S17). In addition, in a previous study, we did not observe a significant impact of indoor dampness exposure on the exhaled levels of toluene, p/m-xylene and o-xylene in children and mothers from the NELA study [38]. However, other possible sources of indoor air pollution (*e.g.* floor or household cleaning products) might influence exhaled BTEX levels, as previously reported for urinary BTEX levels [14].

On the other hand, there was no significant relationship between exposure to external agents (maternal smoking and residential air pollution exposure) and lung function in the infants (figures 2 and 3, supplementary figures S18 and S19). Even though previous studies have observed a relationship between maternal smoking and infant lung function, most studies used urinary cotinine levels to classify mothers [39]. Turning to the effect of environmental pollution exposure, although the significant association between prenatal and early life air pollution exposure and respiratory outcomes has been widely reported in children, lung function measurements were usually performed in teenagers or in preschool-age and school-age children [7, 40–42]. Therefore, to our knowledge, it is the first study that assesses the impact of residential air pollution on the lung function of healthy infants <10 months old. Furthermore, a few studies showed no significant relationship between postnatal air pollution and lung function in children of different ages, such as preschool-age children [42] or term and preterm infants (44 weeks postconceptional age) [43].

Nevertheless, a significant association was found between lung function of children and levels of toluene in exhaled air (figure 6, table 2 and supplementary figure S25), which in turn are significantly related to exposure to air pollution (figures 4 and 5). One of the drawbacks of modelling residential air pollution exposure is that it does not consider that subjects move from their homes to other locations in their everyday lives (*e.g.* workplaces, nursery schools) [40]. Therefore, breath analysis is a noninvasive

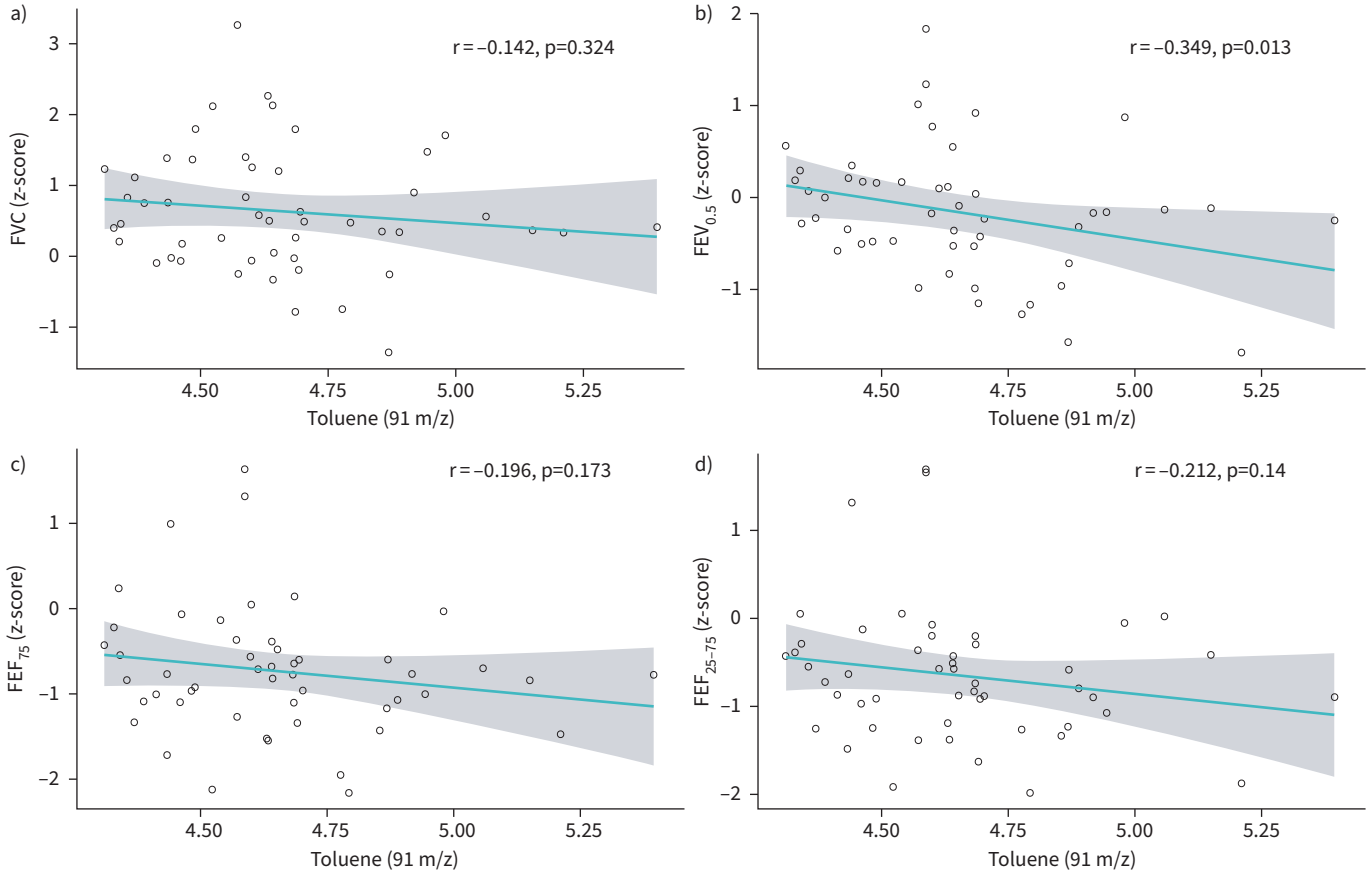

FIGURE 6 Relationship between lung function and levels of toluene in exhaled breath in infants (n=50). a) Correlation between FVC (z-score) and toluene levels in exhaled breath from children. b) Correlation between $FEV_{0.5}$ (z-score) and toluene levels in exhaled breath from children. c) Correlation between FEF_{75} (z-score) and toluene levels in exhaled breath from children. d) Correlation between FEF_{25-75} (z-score) and toluene levels in exhaled breath from children. FVC: forced vital capacity; $FEV_{0.5}$: forced expiratory volume in 0.5 s; FEF_{75}: forced expiratory flow at 75% of FVC; $FEF_{25–75}$: forced expiratory flow at 25–75% of FVC.

TABLE 2 Multiple linear regression analysis of the association between lung function and levels of benzene and toluene in exhaled breath of children (n=50)

	β	95% CI	p-value
$FEV_{0.5}$ mL			
Benzene (m/z=78)	−7.100	−63.848–49.649	0.802
Toluene (m/z=91)	**−29.359**	**−57.226– −1.491**	**0.039**
FVC mL			
Benzene (m/z=78)	−36.407	−112.634–39.821	0.341
Toluene (m/z=91)	−31.118	−69.662–7.426	0.111
FEF_{75} mL			
Benzene (m/z=78)	25.351	−108.338–159.039	0.704
Toluene (m/z=91)	−34.232	−102.439–33.976	0.317
FEF_{25-75} mL			
Benzene (m/z=78)	32.303	−154.190–218.800	0.729
Toluene (m/z=91)	−60.627	−155.069–33.814	0.202

Models adjusted for sex, height, weight and age of the children at the time of lung function assessment. p-values in bold denote statistical significance. β: regression coefficient; FVC: forced vital capacity; $FEV_{0.5}$: forced expiratory volume in 0.5 s; FEF_{75}: forced expiratory flow at 75% of FVC; $FEF_{25–75}$: forced expiratory flow at 25–75% of FVC.

biomonitoring method that allows a direct, personal and accurate determination of the real internal dose of pollutants in a human matrix. Thus, breath analysis allows for a holistic assessment of personal exposures to indoor and outdoor air pollutants (exposome), since it can monitor together the exposures to several factors that could contribute to the emission of the same VOCs and the exposures at different geographical locations visited by the subjects [10, 13]. In this sense, breath analysis has previously been used to assess indoor pollutant exposure [35, 38]. In addition, previous studies highlighted that breath analysis could also be implemented to check airway inflammation and oxidative stress [7, 19].

Regarding the relationship between exhaled toluene and lung function parameters (figure 6), toluene in exhaled breath correlates inversely and significantly with $FEV_{0.5}$ ($r= -0.349$, $p=0.013$) but not with FVC ($r= -0.142$, $p=0.324$), suggesting that this exogenous VOC relates more to an obstructive lung pattern than to a restrictive one [44]. Even though correlation between exhaled toluene levels and $FEV_{0.5}$ was weak ($r= -0.349$), it was confirmed by two other approaches (table 2 and supplementary figure S25). Other lung parameters, such as FEF_{25-75} and FEF_{75}, did not show significant correlation with toluene in exhaled breath, although there seems to be a parallel trend of both to follow the $FEV_{0.5}$–toluene interaction ($r= -0.212$, $p=0.140$ and $r= -0.196$, $p=0.173$ for FEF_{25-75} and FEF_{75}, respectively) (figure 6). To what extent $FEV_{0.5}$ and both FEF_{25-75} and FEF_{75} measure bronchoconstriction in the same way or if the two last parameters provide additional information to $FEV_{0.5}$ is debatable, at least in adults (when referring to forced expiratory volume in 1 s (FEV_1) instead of $FEV_{0.5}$) [45]. In children and adolescents, FEF_{25-75} seems to more sensitive than FEV_1 to the action of Tiotropium [46], perhaps reflecting that responses to either inhaled drugs or pollutants in peripheral and central airways may not be exactly the same. In fact, FEF_{25-75} and FEF_{50} (forced expiratory flow at 50% of FVC) obtained by means of the raised-volume rapid compression technique in infants have been shown to be better than $FEV_{0.5}$ or $FEV_{0.5}$/FVC in diagnosing bronchial obstruction [47], further indicating that central and peripheral airways may behave differently even in infants. Thus, bronchial reaction to toluene could be of different magnitude in every airway. Still, the sample size is limited, and larger numbers might dampen the somewhat diverse results found for $FEV_{0.5}$ and FEFs in the present study.

Strengths and limitations

This study has assessed, for the first time to our knowledge, the relationship among short-term exposure to external agents, BTEX levels in exhaled breath and lung function in infants. Moreover, the influence of exposure to different environmental factors (tobacco smoke and residential air pollution) on the levels of exogenous VOCs in exhaled breath from 674 subjects (337 mothers and 337 children) has been studied to examine the usefulness of breath analysis as an exposure biomonitoring tool. One of the strengths of this study was the measurement of lung function in healthy children under 10 months old. In addition, the current study is embedded in the NELA study, which will include several follow-up visits of the children during their school years and will provide extensive and valuable data.

However, the study has some limitations. Urinary cotinine levels were not measured in either children or mothers, which could have been helpful in ETS exposure assessment. Additionally, other possible sources of household air pollution not included in the questionnaires might be confounding factors with influence on exhaled breath samples. A major limitation of our study is that the number of children with lung function measurements was relatively small (n=50). The consent form for lung function measurement was not signed by many parents owing to the need for children to be sedated with chloral hydrate. The small number of subjects could reduce possible significant associations. In addition, the findings should be replicated in other cohorts.

Conclusions

The results of this study indicate that the levels of toluene and benzene in exhaled breath are significantly associated with short-term exposure to external agents, air pollution and smoking habits in mothers and air pollution in infants. In addition, it has been observed that there is a weak but statistically significant relationship between lung function and toluene in exhaled breath in healthy children. The present study highlights the usefulness of breath analysis as exposure biomonitoring tool and provides a basis for future research on the effects of postnatal environmental exposures on respiratory health in early childhood.

Provenance: Submitted article, peer reviewed.

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Ethics statement: The study protocol was assessed and approved by the Ethics Committee of the "Virgen de la Arrixaca" University Clinical Hospital in accordance with the guidelines of the Declaration of Helsinki (CEIC 09/14). Written informed consent was obtained from the mothers both at the time of recruitment and prior to infant lung function tests.

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Conflict of interest: All authors declare no conflict of interest related to the manuscript.

Support statement: This work was financed by the project PID2021-122202OB-I00 funded by MCIN/AEI/10.13039/501100011033 and by "ERDF A way of making Europe", by the European Union, and the project PI21/00243 funded by Institute of Health Carlos III. R.A. Sola-Martínez is the recipient of a grant "Margarita Salas" from the University of Murcia (133/MSJD/22), financed by the Ministry of Universities, by the European Union – NextGenerationEU, and the Recovery, Transformation and Resilience Plan, and A. Martínez-Vivancos is the recipient of an FPU-PhD fellowship from the University of Murcia. Funding information for this article has been deposited with the Crossref Funder Registry.

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