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Abstract

Background: Short-term exposure to air pollution has adverse effects among patients with asthma, whether long-term exposure to air pollution is a cause of adult-onset asthma is unclear.

Objective: To investigate the association between air pollution and adult onset asthma.

Methods: Asthma incidence was prospectively assessed in six European cohorts. Exposures studied were annual average concentrations at home addresses for nitrogen oxides assessed for 23,704 participants (including 1,257 incident cases) and particulate matter assessed for 17,909 participants through ESCAPE land-use regression models, and traffic exposure indicators. Meta-analyses of cohort-specific logistic regression on asthma incidence were performed. Models were adjusted on age, sex, overweight, education and smoking and included city/area within each cohort as a random effect.

Results: In this longitudinal analysis, asthma incidence was positively, but not significantly, associated with all exposure metrics, except for PM_{coarse} . Positive associations of borderline significance were observed for NO_2 (adjusted OR = 1.10; 95% CI: 0.99, 1.21 per $10 \mu g/m^3$; $p=0.10$) and NO_x (1.04; 95% CI: 0.99, 1.08 per $20 \mu g/m^3$; $p=0.08$). Non-significant positive associations were estimated for PM_{10} (1.04; 95% CI: 0.88, 1.23 per $10 \mu g/m^3$), $PM_{2.5}$ (1.04; 95% CI: 0.88, 1.23 per $5 \mu g/m^3$), $PM_{2.5absorbance}$ (1.06; 95% CI: 0.95, 1.19 per $10^{-5}/m$), traffic load (1.10; 95% CI: 0.93, 1.30 per four million vehicles x m/day on major roads in a 100m buffer) and traffic intensity (1.10; 95% CI: 0.93, 1.30 per 5,000 vehicles/day on the nearest road). A non-significant negative association was estimated for PM_{coarse} (0.98; 95% CI: 0.87, 1.14 per $5 \mu g/m^3$).

Conclusions: Results are suggestive of a deleterious effect of ambient air pollution on asthma incidence in adults. Further research with improved personal-level exposure assessment (versus residential exposure assessment only) and phenotypic characterization is needed.

Introduction

Asthma has a high prevalence, of 5 to 10% (Eder et al. 2006) and in 2010 ranked as the 28th leading cause of disability-adjusted life years worldwide (Murray et al. 2012). Asthma is a heterogeneous disease that may appear at any age (most often in childhood), can persist, possibly remit, or show a variable disease activity over time (Strachan et al. 1996, Wenzel 2012). The complexity of this chronic disease is particularly challenging and more research is needed on the environmental determinants of the disease (and not only of acute triggers of attacks), as the increase in asthma incidence over the last decades (Eder et al. 2006) strongly suggests a role of environmental factors. The role of air pollutants in triggering asthma exacerbations in young and adult asthma patients is established (Peel et al. 2005; Sunyer et al. 1997). Several studies support the role of air pollution in the development of asthma in childhood (Anderson et al. 2013; McConnell et al. 2010), but not all (Mölter et al. 2014). The role of air pollution in adult-onset asthma (i.e. asthma incidence) has been investigated in only a few studies (Anderson et al. 2013; Jacquemin et al. 2012; Young et al. 2014) and should not be extrapolated from studies in children because childhood-onset and adult-onset asthma are two distinct asthma phenotypes that have, at least partly, different clinical, biological, and genetic characteristics (Wenzel 2012). Among studies in adults only four studies have used individually assigned air pollution estimates at home addresses. A small Swedish case-control study (203 cases and 203 controls) suggested an association of traffic-related NO₂ with asthma incidence, but the study lacked statistical power (Modig et al. 2006). The Respiratory Health in Northern Europe (RHINE) study including 3,824 participants (Modig et al. 2009) and the European Community Respiratory Health Survey (ECRHS) including 4,185 participants (Jacquemin et al. 2009a) reported also a positive

association between NO₂ and asthma incidence. The Swiss study on air pollution and health in adults (SAPALDIA) found similar results but only in never smokers and using source-specific models of local traffic-related particulate matter (PM) as a marker of exposure (Künzli et al. 2009). A recent US study suggested an association of PM_{2.5} with incident asthma in women (Young et al. 2014). Two recent reviews concluded that the existing evidence suggests a possible role of air pollution in adult-onset asthma but that the evidence is not conclusive as the studies lacked of power, suggesting the need for larger cohorts (Anderson et al. 2013; Jacquemin et al. 2012).

The European Study of Cohorts for Air Pollution Effects (ESCAPE) developed, for the first time at large scale, fully standardized air pollution measurement, modelling, and assignment methods to individually characterize home outdoor exposure (Beelen et al. 2013; Eeftens et al. 2012). We took advantage of a follow-up of more than 10 years in 23,704 adults in six prospective cohorts from eight countries to assess the association between long-term exposure to ambient air pollution and asthma incidence in adulthood.

Methods

Study population and assessment of asthma incidence

Six prospective cohorts from 24 areas in eight countries contributed to the analysis of asthma incidence in adulthood over a 10-year period. Three of these cohorts (ECRHS (The European Community Respiratory Health Survey II 2002), the French Epidemiological study on the Genetics and Environment of Asthma (EGEA, (Siroux et al. 2009)) and SAPALDIA (Ackerman-Liebrich et al. 2005)) were respiratory epidemiological cohorts, with detailed information regarding respiratory symptoms, bronchial challenge tests, and sensitization. The three others were general health cohorts. The study on the influence of Air pollution on Lung function,

Inflammation and Aging (SALIA, Schikowski et al. 2010) and SAPALDIA were originally designed to investigate effects of air pollution. ECRHS, SAPALDIA and the Medical Research Council's National Survey of Health and Development (NSHD, Kuh et al. 2011) corresponded to a representative sample of subjects of predefined areas. The Etude Epidémiologique auprès de femmes de la Mutuelle Générale de l'Education Nationale (E3N, Clavel-Chapelon et al. 1997) and SALIA were conducted in elderly women. EGEA included by design a high proportion of relatives of asthma patients recruited in chest clinics. ECRHS, EGEA and SAPALDIA were initiated in the 90's and followed-up between 9 and 12 years later. NSHD is a birth cohort of participants born in 1946 and with over 20 regular follow-ups since then, for this analysis baseline was considered in 1989 and follow-up in 1999. E3N women were recruited in 1990, and followed-up every two years, the last follow up included for this analysis is the one from 2008. SALIA women were recruited in 1985, a questionnaire follow-up was conducted in 2006 and a second in 2007 to 2010. More detailed information on each study is provided in see Supplemental Material, Table S1; see Supplemental Material, Figure S1.

For each cohort, the absence of asthma at baseline and the incidence of asthma during follow-up were defined as shown in Supplemental Material Table S2, according to each cohort variables' availability. Two principles were followed regarding the assessment of asthma: harmonisation across cohorts and optimal use of available information. Depending on the cohort, asthma was defined by two standardized questionnaires: the British Medical Research Council questionnaire (Samet et al. 1978), which originated in the 1960s, and the ECRHS questionnaire (Burney et al. 1994), designed in the 1990s. For all studies, asthma incidence was defined only in subjects without asthma at baseline. To further improve the specificity of our asthma incidence definition (Sunyer et al. 2007; Pekkanen et al. 2005) we also excluded from the population at risk of new-

onset asthma any participant who reported at baseline three out of five asthma-like symptoms in the last 12 months (wheeze and breathlessness; chest tightness; attack of shortness of breath at rest; attack of shortness of breath after exercise; woken by attack of shortness of breath) - this was possible in three of the six cohorts (ECRHS, EGEA, SAPALDIA) as this information was available (Boudier et al. 2013). Flow charts and criteria used to classify asthma for each cohort are provided in Supplemental Material (Figures S1a-f and Table S2). In ECRHS, SAPALDIA, EGEA, objective asthma related traits were available. Methacholine bronchial provocation tests were performed and bronchial responsiveness defined when the provocative dose to decrease by 20% the forced expiratory flow volume in 1 second was below or equal to 1mg cumulative dose of methacholine. Allergic sensitization was assessed as at least one skin prick test or at least one specific Immunoglobulin E > 0.35 U/ml (see Table 1 for details). In all studies, hay fever was recorded by questionnaire at baseline and follow-up. Eczema was assessed in some studies. Moving status was defined based on the available data, considering addresses (geocodes) when baseline address was available and reported move assessed through questionnaire otherwise. Ethical approval was obtained for each cohort/centre from the appropriate institutional or regional ethics committee, and written consent was obtained from each participant.

The covariates were chosen based on evidence from previous studies (Jacquemin et al. 2009a; Modig et al. 2009; Künzli et al.2009) but also taking into account the assessment and quality of available data within the ESCAPE cohorts. Smoking (current, former, never), maximum educational level (low, medium, high) and overweight (BMI < 25, ≥ 25 kg/m², except in ECRHS where an additional missing category was created due to more than 20% of missing for this variable) were considered in the analysis.

City/area refers to the city in ECRHS, EGEA, E3N and SAPALDIA, and the country in NSHD (England, Wales and Scotland). All SALIA participants came from one area.

Exposure data

NO₂/NO_x measurements were conducted in three seasons in 2010 or 2011 using passive samplers in the 24 areas. Areas refers to cities (with or without its metropolitan area) in most of the cases, except in UK where it is the whole country and in the Ruhr region in Germany where it is an urban area including several cities. PM monitoring campaigns were conducted in 12 areas. Exposure estimates at the participants' addresses at follow-up (NO₂, NO_x, PM₁₀, PM_{2.5}, PM_{2.5}absorbance, PM_{coarse}) derived from land use regression (LUR) models were used as primary exposure covariates (Beelen et al. 2014; Eeftens et al. 2012) (<http://www.escapeproject.eu>).

Back-extrapolated exposure estimates for NO₂ and PM₁₀ were used for sensitivity analyses as ESCAPE air pollution measurement campaigns took place after the health surveys for most cohorts. The back extrapolated concentration was estimated by multiplying the modelled ESCAPE annual mean concentration with the ratio between average annual concentrations as derived from the routine monitoring site(s) for the period in the past and for the ESCAPE measurement period time (Beelen et al. 2014). Exposures were back extrapolated to the follow-up period using routinely available air pollution monitoring data but could not be extrapolated to baseline for all the areas because of a lack of earlier monitoring data for some cities, in particular for PM₁₀. Furthermore baseline addresses were not available in all the cohorts. Traffic exposure indicators, traffic intensity (on the nearest road) and traffic load (in a 100m buffer) were derived from geographic databases.

Data analysis

The following cohort-specific random-effects logistic regressions were performed for all air pollution metrics: unadjusted (model 1), adjusted for age and sex (model 2), and additionally adjusted for smoking, overweight, and education level at baseline (model 3, the main model).

Cox regression analysis was not used due to imprecision of the date of onset. The heterogeneity of the effect estimates between the cohorts was tested using χ^2 test. Meta-analytic estimates were estimated using fixed-effect models in the absence of heterogeneity between cohorts (p-value of heterogeneity >0.1), and using random-effect models when heterogeneity between cohorts was present. The I^2 statistic was calculated for quantifying heterogeneity. For meta-analyses of subgroups (age, sex and smoking status), meta-analytic stratum-specific estimates were derived and were compared between strata. Cohort-specific estimates in subgroup analyses were conducted using model 3, but without taking into account random effect per city/area as random effect models encountered convergence problems.

Because NO_2 is not measured near busy roads, models of associations with traffic variables were adjusted for background NO_2 . Random effects were used for the main relevant cluster for each cohort (city/area for E3N, ECRHS, NSHD, SAPALDIA, or family for EGEEA).

Sensitivity analyses were conducted: 1) to address the robustness of the association to a change in the window of exposure (by using back-extrapolated NO_2 and PM_{10}), 2) to address the possible impact of the exposure models' performance [by restricting analyses to areas where exposure models had the highest predictive value (cross-validation $R^2 > 0.6$)], 3) to better compare the NO_2 with the PM results (by restricting NO_2 analyses to participants that also had PM measurements), 4) to unmask a possible effect of one pollutant over the other using a two-pollutant model (NO_2 and PM_{10}), 5) excluding individuals with a self-reported age-at-onset 2

years or more prior baseline according to record at follow-up, in order to better capture adult onset asthma and not reappearance of childhood onset of asthma, (Jacquemin et al. 2009a) ; this analysis is referred as incidence with coherent age of onset in tables, 6) excluding individuals with exposures at both upper and lower 5% extremes of pollutant values, 7) adjusting for “study city/area” as a fixed effect instead of random effect, as used before (Jacquemin et al. 2009a) but debated (Neuhaus and Kalbfleish 1998). Stratified analyses were conducted by age (<50 or ≥ 50 years), sex, and smoking (ever or never smokers) and analyses restricted to non movers were conducted. We investigated the robustness of the meta-analyses estimates by excluding consecutively each cohort. We performed further analyses within the ECRHS cohort to allow direct comparison with a previous ECRHS publication (Jacquemin et al. 2009a) that estimated NO₂ using the APMospHERE (air pollution modelling for support to policy on Health and Environmental Risk in Europe) model, a 1x1-km surface model developed using GIS-based techniques (Vienneau et al. 2009).

All the results are shown for an increase of 10 µg/m³ of NO₂ and PM₁₀, 5 µg/m³ of PM_{2.5} and PM_{coarse}, 10⁻⁵/m¹ of PM_{2.5_}absorbance and 20 µg/m³ of NO_x. For traffic measures, the results are shown for an increase of 5000 vehicles/day for traffic intensity on the nearest road and four millions vehiclesxm/day for traffic load in major roads within a 100m buffer. Analyses used Stata, version 12 (StataCorp, College Station, Texas, USA).

Results

Population

The six cohorts contributed to 1,257 incident cases of asthma for the total population of 23,704 participants (Table 1). Cohorts differed by several characteristics, reflecting recruitment differences. Asthma incidence rates varied from 2.9/1,000/year in SAPALDIA to 8.3/1,000/year

in EGEA. In the three cohorts (ECRHS, EGEA, SAPALDIA) with available data, participants who developed asthma after baseline (i.e., incident asthma cases) were more likely than other participants to be classified as having bronchial hyperresponsiveness (BHR) at baseline (28% versus 9% with a positive methacholine test), and were even more likely to have BHR at follow-up (40% compared with 9%). Compared to subjects who did not develop asthma, those with incident asthma exhibited more allergic sensitization, before (baseline) and after (follow-up) the onset of asthma. Hay fever was twice as common among participants with incident asthma compared to those without asthma (except for NSHD and EGEA at baseline).

Air pollution and traffic metrics

Mean and median air pollution exposures were lower for the NSHD cohort compared with the other five cohorts, though distributions overlapped among the cohorts (Figure 1 and Supplemental Material, Table S3). The highest mean NO₂ concentration was found in E3N (31 ± 13 µg/m³) and the lowest in NSHD (22 ± 7 µg/m³). For PM₁₀, the highest mean concentration was found in SALIA (27 ± 2 µg/m³) and the lowest in NSHD (16 ± 2 µg/m³). Cohort-specific IQRs indicated substantial variability in the exposure contrasts within cohorts, ranging from 8–20 µg/m³ and 2–8 µg/m³ for NO₂ and PM₁₀, respectively (see also Supplemental Material, Table S3). The highest correlation coefficients were always seen between NO₂ and NO_x ($r > 0.90$) (see Supplemental Material, Table S4). Correlation coefficients between NO₂ and PM₁₀ varied from 0.53 in E3N to 0.83 in SAPALDIA. Correlation coefficients between the different air pollutant concentrations and the traffic indicators showed wide between-cohort heterogeneity (from 0.06 for NO₂ and traffic intensity in NSHD to 0.81 for PM_{2.5_}absorbance and traffic load within a 100 m buffer in EGEA) (Table S4). All the LUR models had a leave-one-out cross validation R² above

50%, and most of them above 80% (Eeftens et al. 2012, Beelen et al. 2013) (see Supplemental Material, Table S5).

Associations between air pollutants and traffic metrics and asthma incidence

The unadjusted, simple (adjusted by sex and age) and fully adjusted models provided similar results in individual cohorts (Table 2). The fully adjusted meta-analytic estimate for NO₂ was positive (OR = 1.10; 95%CI: 0.99, 1.21; p=0.10). The association did not change when using the back-extrapolated NO₂ ESCAPE estimates (OR=1.10; 95%CI: 1.00, 1.20) (Table 2 and Figure 2). When adjusting by city/area as a fixed effect (instead of random effect), the OR for NO₂ increased to 1.14 (95%CI: 1.01, 1.29) (Table 3), changes being mainly driven by an increased association estimate in ECRHS (OR = 1.41; 95%CI: 1.10, 1.80 instead of OR = 1.07; 95% CI: 0.92, 1.23).

NO₂ estimates were positive in all sensitivity and stratified analyses (Table 3). Using the stricter definition of asthma incidence with coherent age of onset did not modify the associations but confidence intervals were wider as power was decreased (Table 3). The analyses restricted to non-movers or after the exclusion of the 5% extreme value of the pollutants tended to decrease the associations (OR=1.04; 95% CI: 0.98, 1.10 and 1.03; 95% CI: 0.97, 1.10; respectively) (Table 3). While cohort-specific association estimates suggested the possibility of between-cohort differences, with stronger estimates in the French EGEA cohort as compared to the others (Figure 2), heterogeneity among the cohorts was not statistically significant (Table 2). After consecutive exclusion of each cohort in the meta-analyses, the point estimate of the OR always remained positive, varying from 1.03 to 1.15, reaching significance for NO₂ after the exclusion of E3N (OR=1.15; 95% CI: 1.03, 1.27 with a decreased heterogeneity between cohorts' estimates) (Table 3) or SAPALDIA (OR=1.11; 95% CI: 1.00, 1.24). A trend for stronger

association between NO₂ and asthma incidence was observed in ever smokers as compared to never smokers (OR=1.13; 95% CI: 0.99, 1.29 and OR=1.01; 95% CI: 0.88, 1.16; respectively) (p-interaction = 0.35) (Table 3). Neither age nor sex modified the associations between NO₂ and asthma incidence (p-interaction were 0.88 and 0.66 respectively) (Table 3). Restricting the analyses either to centres with both NO₂ and PM₁₀ measurements or to areas with a high goodness of fit of the LUR models did not modify the associations between NO₂ and asthma incidence.

For PM₁₀, meta-estimates were similar and not significant in models with or without back-extrapolation, (OR=1.04; 95% CI: 0.87, 1.24 and OR=1.04; 95% CI: 0.88, 1.23; respectively) (Table 2). Except for PM_{coarse}, estimates were all positive but not significant, though borderline significant for NO_x (OR=1.04; 95% CI: 0.99, 1.08) (Table 2).

PM₁₀ estimates were positive, and tended to increase in any sensitivity analysis, except when excluding EGEA, but never reached significance (Table 3). The analyses restricted to non-movers or after the exclusion of the 5% extreme value of the pollutants tended to increase the associations (OR=1.12; 95% CI: 0.91, 1.37 and OR=1.11; 95% CI: 0.89, 1.37; respectively). In the stratified analyses, slightly stronger association between PM₁₀ and asthma incidence were observed in ever smokers as compared to never smokers (OR=1.17; 95% CI: 0.79, 1.74 and OR=1.01; 95% CI: 0.88, 1.16; respectively) and in women as compared to men (OR=1.07; 95% CI: 0.91, 1.26 and OR=1.00; 95% CI: 0.63, 1.59; respectively), whereas associations were similar for age < 50 and ≥ 50 years.

In the bi-pollutant model, the NO₂ estimate increased from 1.10 (95% CI: 0.99, 1.21) to 1.17 (95% CI: 0.99, 1.38) while the PM₁₀ estimate decreased from 1.04 (95% CI: 0.88, 1.23) to 0.98 (95% CI: 0.79, 1.21) (Table 3).

The comparison of ECRHS results using ESCAPE NO₂ estimates or the previously published APMoSPHERE NO₂ estimates (Jacquemin et al. 2010) showed that the effect estimates were sensitive to both the analytic approach and the exposure models. Higher effect estimates were observed in the model with study/city used as fixed effect and/or when using the APMoSPHERE exposure model (see Supplemental Material, Table S6). For instance, the estimate based on the ESCAPE model and random effect on city was 1.04 (95% CI: 0.91, 1.20) and increased up to 1.94 (95% CI: 1.27, 2.96) in the model using the APMOSPHERE air pollution exposure and adjusted on city.

Discussion

In this longitudinal investigation, asthma incidence was positively associated with all exposure metrics, except with the coarse fraction of PM. The association was borderline statistically significant for a 10- $\mu\text{g}/\text{m}^3$ increase in NO₂ (OR=1.10 95%CI: 0.99, 1.21) and significant with back-extrapolated NO₂ (OR=1.10; 95%CI: 1.00, 1.20). Overall, these findings provide suggestive but no firm evidence for a role of ambient air pollution on asthma incidence in adults.

The main strengths of this study are a large population from a wide geographical area, including more than 23,000 participants from eight countries and more than 20 different cities across Europe using standardized air pollution estimates at the residential address for a variety of air pollutant metrics. This was achieved through a standardized procedure regarding air pollutants measurements, development of land use regression models and validation (Beelen et al. 2013,

Eeftens et al. 2012). The lack of highly significant associations in our findings is in line with three interpretations, namely that there is no such association, that pollutants affect only subgroups of adults, or that we were unable to reliably capture such association as a result of epidemiological bias or lack of power. Overall the validity of those LUR models, assessed with the R^2 (Table S5) were good, although this varied across study sites. We showed that restricting the NO_2 analyses to the centres with higher R^2 did not modify the results. A simulation study showed that LUR modelling with a small number of measurement sites may bias the health-effect estimates in the form of attenuation towards the null (Basagaña et al. 2013). The lack of association with PM may partly result from the small number of measurement sites for these pollutants. A further limitation was the long lag between the health assessments of most of our cohorts and the standardized ESCAPE measurement campaigns, reaching up to 20 years in some of the cohorts. The resulting exposure misclassification likely contributed to imprecise risk estimates and a bias toward the null (Basagaña et al. 2013). To investigate this, back-extrapolated exposure estimates to the follow-up periods for NO_2 were analysed. The odds ratio using back-extrapolated values then reached formal statistical significance, but the effect size, which mainly relied on within-city contrasts, was virtually identical to that in the initial analysis. The validity of back-extrapolation of LUR models is supported by a study showing a good correlation between the 1991 back-extrapolated NO_2 concentrations estimated from the 2009 LUR model and the NO_2 concentrations measured by monitoring sites in 1991 (Gulliver et al. 2013). However, back-extrapolated exposure estimates will not account for potential changes over time in spatial contrasts within cities, so their validity may vary by location and time. This is an inherent limitation of the ESCAPE project. Nevertheless, associations with other outcomes investigated in ESCAPE, including mortality (Beelen et al. 2014) and lung cancer incidence

(Raaschou-Nielsen et al. 2013), have been similar for exposures based on ESCAPE measurement period estimates and exposures based on back-extrapolated estimates. Caution is necessary when interpreting our findings. Although positive, associations with PM and traffic proximity were non-significant, which may indicate that these pollutants do not affect adult-onset asthma or that the analyses lacked statistical power to reliably estimate small effects among rather heterogeneous cohorts. The fact that the positive associations with NO₂ were the closest to statistical significance does not necessarily mean that NO₂ is the causal pollutant. It could reflect that our exposure model more accurately estimates the true exposure for this pollutant (which is supported by a trend for a higher R² cross validation of the LUR model for NO₂ as compared to PM₁₀ (Eeftens et al. 2012, Beelen et al. 2013)). Further, given the correlation between pollutant concentrations, we cannot estimate associations with individual pollutants that account for potential confounding by other pollutants. Moreover, no matter how good the exposure models are, there will always be limitations and potential bias in estimating association using exposure estimates only at home addresses that do not account for the individual space-time activity.

The design induces some limitation regarding the generalisability of our result to other European cities. For all cohorts, the first inclusion criteria was the availability of ESCAPE models, which varied from 20% for E3N (a national study) to 100% for SALIA and NSHD. At the whole cohort level, follow-up rates were less variable, varying between 60 and 80%, which represents a reasonable follow-up rate for such long term studies (Ackerman-Liebrich et al. 2005, Siroux et al. 2009, Antó et al. 2010, Schikowski et al. 2010, Kuh et al. 2011, Sanchez et al. 2013). Though our study is the largest ever conducted in Europe, with the greatest number of countries and areas, and our estimates did not indicate strong heterogeneity in associations across cohorts,

some caution is needed in extrapolating our results, in particular in relation to the heterogeneity between areas, and more importantly to the small sample size in each area.

Defining asthma incidence is more challenging than defining outcomes such as mortality (Beelen et al. 2014) or lung cancer (Raaschou-Nielsen et al. 2013). Furthermore, as adults may not remember early life wheezing, assessment of adult onset asthma is difficult (Strachan et al. 1996). A thorough comparison of questionnaires and protocols was undertaken to harmonise asthma definition across the various cohorts without losing valuable information. While only ECRHS and SAPALDIA were purposefully designed to assess asthma incidence, we were as rigorous as possible to identify only incident cases, by excluding participants who reported asthma or, when available, asthma-like symptoms at baseline from our study population. Bias in asthma diagnosis may have been introduced through both different cultural perceptions of asthma in the countries in which the cohorts were located, and the different questionnaires and diagnostic protocols used. In the largest cohort included, E3N, the validity of the simple asthma question used has been investigated in a subsample study, which showed good concordance with questions similar to those used in respiratory surveys and with dispensed asthma drug treatment (Sanchez et al. 2013). Due to a limited number of cohorts with bronchial challenge tests, we were unable to perform a sensitivity analysis defining asthma as new bronchial hyperresponsiveness plus symptoms, as used in a previous study of occupational risk factors for asthma (Kogevinas et al. 2007). However, for the three cohorts with information on bronchial hyperresponsiveness, the validity of our incident asthma classification was supported by the increase in bronchial hyperresponsiveness between baseline and follow-up among participants who developed asthma after the baseline examination.

Results should be interpreted in the context of current knowledge and research regarding asthma phenotypes. It is established that childhood onset asthma, compared to adulthood onset asthma, occurs more in males, is more often associated with allergic sensitization, childhood asthma also depends on specific genetic determinants (Bouzigon et al. 2008, Wenzel 2012). With the increase of childhood asthma, the potential reoccurrence of asthma in adulthood after remission becomes an increasing concern. Recent research on asthma temporal patterns and data-driven phenotyping conducted in four of the six cohorts included in the present analysis show the complexity of asthma variability over periods of around 10 years in adulthood (Sanchez et al, 2013, Boudier et al. 2013). Childhood only, adulthood only, old age only, mild (often forgotten) childhood asthma reappearing in adulthood, and persistent asthma throughout the life span, are various phenotypes which may depend on both genetic and environmental determinants of various critical windows of expression/exposure. The variability of asthma can be characterized according to different windows of time (Frey and Suki, 2008). They may be short (hours or days), often in relation to triggers of attacks, as well as long (months or years). Lessons from other environmental factors (smoking, occupation) have already shown effects on asthma through acute or sub chronic exposures. For example, there is increasing evidence of the role of occupational exposure in the various forms of work-related asthma, which encompasses both occupational asthma starting in adulthood and work-exacerbated asthma (Henneberger et al. 2011). The role of occupational exposure has clearly been evidenced in adult onset asthma assessed in a birth cohort followed-up till adulthood (Ghosh et al. 2013). The follow-up of the numerous birth cohorts initiated in the nineties, and still followed will likely help to understand the various evolutions of the disease. Our study considered multiple cohorts across Europe, which increased statistical power. However, this also gave potential for larger population heterogeneity, increasing the potential for

confounding and therefore bias in the effect estimates. Particular characteristics of each cohort may have influenced the results, such as the health consciousness and high education of the women in E3N or the greater baseline risk of asthma for members of asthmatic families in EGEA. Indeed, as shown in Figure 2, associations were usually largest in EGEA reaching statistical significance for NO₂ – although this finding was not robust to the exclusion of the 5% most extreme exposure values (data not shown). To investigate cohort-specific influences on results, we formally tested heterogeneity among cohorts and also looked at the robustness of the findings by removing each cohort in turn, which showed some modest variation.

Overall, nearly all the sensitivity and stratified analyses led to OR greater than 1. Results from stratified analyses should be interpreted with caution because of the limited number of incident cases in sub-groups in some cohorts, and none of the p-values for interaction were significant ($p > 0.35$). Surprisingly, the estimates tended to decrease when restricting the analysis to non-movers for NO₂ but not for PM₁₀. This could be due to the lower percentage of movers in E3N and the lack of standardization of moving assessment.

Our results were sensitive to the statistical approach chosen to account for the clustered data, namely using fixed versus random effects for study city/area. Which of the two modelling approaches provides more valid results is difficult to determine, but one factor may be the nature of the air pollutant variation in regards to the within vs. between-city/area. Fixed city/area effect models estimate purely within-city/area air pollution effects whereas random effect models estimate a weighted average of between- and within-city/area effects (Neuhaus and Kalbfleish 1998). The difference between both approaches within our analyses was driven by the ECRHS estimates, possibly explained by the higher between-city/area variation in air pollutant

concentration in this European cohort. Further analyses, including simulation studies, are warranted to better address this statistical issue in the context of the air pollution effect estimates.

Compared to other published results for NO₂ our confidence intervals largely overlapped those from other studies (OR for 10 µg/m³ of NO₂ was 1.10 (95% CI: 0.99, 1.20) compared to 1.54 (95% CI: 1.00, 2.36) in RHINE (Modig et al. 2009) and 1.43 (95% CI: 1.02, 2.01) in ECRHS (Jacquemin et al. 2009a) and OR for 5.8 ppb of NO₂ (i.e. 11 µg/m³) 1.12 (95% CI: 0.96, 1.30) in the cohort of US women (Young et al. 2014)). Interestingly, the association with NO₂ tended to increase when controlling for PM₁₀ concentration. Two of the six cohorts included in our analyses had previously assessed associations between air pollution and asthma incidence in adults. In ECRHS a positive and significant association was found between individually assigned air pollution exposure derived from a 1x1 km air pollution map (APMoSPHERE) and asthma incidence defined in a similar way to ESCAPE (Jacquemin et al. 2009a) and also in an alternative way based on asthma symptoms (Jacquemin et al. 2009b). One possible reason for seeing consistently stronger associations with APMoSPHERE based analyses is that APMoSPHERE used air pollution data closer in time to the collection of health data. Alternatively, a spatially less resolved model may better account for the time activity patterns in adult populations, thus, “background” air pollutant exposure estimates could be a better proxy of the mean individual exposure as compared to the very local exposure estimates at the home address, produced by the ESCAPE modelling strategy. SAPALDIA (Künzli et al. 2009) reported significant associations between asthma incidence in never smokers and individually assigned changes in a specifically modelled marker termed “traffic related PM₁₀”. ESCAPE had no such marker therefore direct comparisons cannot be made. Moreover, only three SAPALDIA areas

were included in ESCAPE – and only one with PM – whereas all previous SAPALDIA results were based on the eight areas the cohort had been designed for in 1990.

~Various mechanisms have been proposed to explain the associations of air pollution with asthma. Active on-going research is being conducted to disentangle the various asthma phenotypes and assess which mechanisms may be specifically involved. As childhood onset asthma is more often associated with allergic sensitization, it could be hypothesized that allergic-related mechanisms influence childhood asthma relapsing in adulthood. However, recent results from ESCAPE did not show evidence in children up to 10 years of associations of air pollution exposure with allergic sensitization (Gruzieva et al. 2014). This suggests that non-allergic mechanisms, for which interest is increasing for asthma at any age, are particularly important to consider. Increased frailty of the epithelial barrier, inflammation, oxidative stress, interaction with genetic and epigenetic determinants have been proposed. Research in adults, including subjects from the cohorts included in our analysis, have suggested a role of air pollution in local inflammation measured in exhaled breath condensate and induced sputum (using ESCAPE exposure estimates) (Vossoughi et al. 2014), interaction with oxidative stress genes (Castro-Giner et al. 2009) or novel DNA methylation markers (Sofer et al. 2013). Ambitious programs with comprehensive environmental exposure assessment and biological markers are starting in childhood populations (Vrijheid et al. 2014). Altogether, adult onset asthma is only one of the various asthma phenotypes and comprehensive life course approaches should be developed at the environmental and phenotypic levels.

Conclusion

With more than 23,000 adults across Europe followed for 10 years, including 1257 incident cases of asthma, this is the largest study to estimate the association between traffic-related air

pollution, assessed using a standardized and validated method at the individual level, and asthma incidence in adults. Our findings provide suggestive but no firm evidence for a role of air pollution exposure on asthma incidence in adults. Further research with improved individual-level exposure assessment (taking into account for example time-activity patterns) and phenotypic characterisation in a life course perspective is needed to better understand the effect of air pollutants on asthma.

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Table 1. Characteristics of participants with NO₂ exposure estimates in the ESCAPE analyses, by study and outcome.

Characteristic	All (23,704)		ECRHS (3,802)		EGEA (517) ^a		E3N (12,763)		NSHD (2,339)		SALIA (2,073)		SAPALDIA (2,210)	
	No asthma	Incident asthma	No asthma	Incident asthma	No asthma	Incident asthma	No asthma	Incident asthma	No asthma	Incident asthma	No asthma	Incident asthma	No asthma	Incident asthma
n	22447	1257	3,657	145	468	49	12,012	751	2,245	94	1,925	148	2,140	70
Female, %	82	89	52	67†	54	57	100	100	52	60	100	100	53	61
Age at baseline (years), mean ± sd	42	46	34±7	34±7	41±12	36±13*	49±7	49 ±6*	43±0	43±0	54±1	55±1	42±12	38±11†
Age≥50 at baseline, %	35	36	0	0	23	14*	43	38*	0	0	100	100	31	16*
Age at follow-up (years), mean ±sd	60	60	43±7	42±7	52±12	47±13*	65±7	64±6	53±0	53±0	71±3	72±3*	53±12	49±11
BMI at baseline (kg/m ²), mean ±sd	23	24	24±4	24±5	23±3	23±5	22±3	23±3*	25±4	27±5*	27±4	27±4	24±4	24±4
BMI>=25 at baseline, %	26	30	33	35	26	31	13	18*	45	67*	67	66	31	29
Smoking status at baseline														
Current smoker, %	22	22	36	30	25	47*	17	19	26	29	11	16	36	24
Former smoker, %	29	29	21	26	24	10*	34	34	42	39	9	5	21	27
Never smoker, %	49	49	43	45	51	43*	50	46	31	32	80	79	43	49
Maximum education at baseline or follow-up														
Low level, %	12	13	23	28	26	17	2	3*	41	51	22	25	7	9
Medium level, %	24	21	34	28	22	15	6	8*	48	42	49	51	62	60
High level, %	64	66	43	45	52	67	91	88*	11	8	29	24	30	31
Movers (between baseline and follow-up), %	33	33	45	42	45	55	27	31	39	37	18	15	48	50
Asthma related variables														
Methacholine test ^a , baseline, n	4837	197	2,871	112	385	38	NA	NA	NA	NA	NA	NA	1,581	47
PD20≤ 1mg, %	9	28	8	38*	12	29*	NA	NA	NA	NA	NA	NA	9	6
Methacholine test ^b , follow up, n	3499	147	2,197	94	264	25	NA	NA	NA	NA	NA	NA	1,038	28
PD20≤ 1mg, %	9	40	10	44*	12	48*	NA	NA	NA	NA	NA	NA	5	18*
SPT/splgE ^c , baseline, n	5207	228	2,937	119	457	49	NA	NA	NA	NA	NA	NA	1,813	60
Allergic sensitization, %	27	50	25	52*	35	45	NA	NA	NA	NA	NA	NA	28	50*
SPT/splgE ^c , follow-up, n	4684	194	2,859	112	371	40	NA	NA	NA	NA	NA	NA	1,454	42
Allergic sensitization, %	27	55	24	55*	33	55*	NA	NA	NA	NA	NA	NA	30	55*
Hay fever at baseline, %	13	26	19	46*	25	35	11	25*	16	19	5	10*	17	40*
Hay fever at follow-up, %	11	27	21	54*	29	64*	5	17*	23	40*	5	19*	18	51*
Eczema at baseline, %	34	43	33	43*	23	29	NA	NA	NA	NA	NA	NA	38	51*
Eczema at follow-up, %	27	36	35	48*	25	37	NA	NA	NA	NA	4	13*	35	57*

BMI: body mass index; NA: Not available; PD20: The dose of methacholine required to produce a 20% fall in the forced expiratory volume in 1 second; sd: standard deviation

Percentages are column percentages.

^aIn EGEA, the 517 participants belong to 372 families, 24% of the participants had at least one parent with asthma. ^bBronchial hyperresponsiveness was defined dichotomously as PD20 ≤1 mg (the common dose used in all three studies (ECRHS, EGEA and SAPALDIA)).

^cAllergic sensitization at baseline and follow-up for ECRHS, EGEA and SAPALDIA was defined as at least one skin prick test (SPT) positive or at least one specific Immunoglobulin E (IgE) > 0.35 U/mL . In ECRHS allergic sensitization was defined at baseline as any positive SPT (7 allergens tested) and allergic sensitization at follow-up was defined as any specific IgE concentration > 0.35 U/mL (4 IgEs tested). In EGEA and SAPALDIA, allergic sensitization at baseline or follow-up was defined as any positive SPT (EGEA: 11 and 12 allergens tested at baseline and follow-up, respectively; SAPALDIA: 8 allergens tested).

*p < 0.05 comparing cohort participants with and without incident asthma

Table 2. Meta-analyses of associations between air pollutants and traffic indicators and the risk for asthma incidence.

Exposure	Increase	OR (95% CI)			Heterogeneity between cohorts (model 3)	
		Model 1	Model 2	Model 3	I-squared	p
NO _x , number of participants		23,693	23,693	22,814		
NO ₂	10 µg/m ³	1.11 (1.00,1.23)	1.04 (0.99,1.09)	1.10 (0.99,1.21)	46.2%	0.10
NO ₂ back extrapolated to follow up	10 µg/m ³	1.10 (1.00,1.21)	1.04 (0.99,1.09)	1.10 (1.00,1.20)	49.6%	0.08
No _x	20 µg/m ³	1.09 (1.00,1.18)	1.04 (0.99,1.08)	1.04 (0.99,1.08)	39.8%	0.14
PM, number of participants		17,798 ^b	17,798 ^b	17,098 ^b		
PM ₁₀	10 µg/m ³	1.05 (0.89,1.24)	1.05 (0.89,1.24)	1.04 (0.88,1.23)	0.0%	0.44
PM ₁₀ back extrapolated to follow up	10 µg/m ³	1.04 (0.88,1.24)	1.04 (0.88,1.24)	1.04 (0.87,1.24)	0.0%	0.78
PM _{coarse}	5 µg/m ³	0.98 (0.86,1.12)	0.98 (0.86,1.12)	0.99 (0.87,1.14)	0.0%	0.61
PM _{2.5}	5 µg/m ³	1.11 (0.80,1.54)	1.04 (0.88,1.23)	1.04 (0.88,1.23)	24.2%	0.25
PM _{2.5} substance	10 ⁻⁵ /m	1.05 (0.94,1.16)	1.05 (0.94,1.17)	1.06 (0.95,1.19)	44.5%	0.11
Traffic variables, number of participants ^a		22,430	22,428	21,551		
Traffic intensity on nearest road	5,000 veh/day	1.06 (0.98,1.14)	1.05 (0.98,1.13)	1.05 (0.98,1.13)	56.4%	0.04
Traffic load in a 100m buffer	4,000,000 veh x m/day/	1.11 (0.94,1.31)	1.09 (0.94,1.27)	1.10 (0.93,1.30)	57.4%	0.04

OR: odds ratio; CI: confidence interval.

Model 1: unadjusted ; Model 2: adjusted for age and sex; Model 3 : adjusted for age, sex, smoking, overweight and education level.

The logistic regression models were conducted with random effects per city/area for each study except for SALIA where there was only one area and EGEA where family structure was taken into account.

The OR corresponds to the fixed effect when the p-value for heterogeneity was >0.1, when the p-value for heterogeneity was <0.1 the random effect is stated.

I-square : variation of estimate effect attributable to heterogeneity.

^aFor traffic intensity on the nearest road. ^bFor PM₁₀.

Table 3. Results from random-effects meta-analyses for adjusted association between asthma incidence per 10 $\mu\text{g}/\text{m}^3$ increase for NO_2 and PM_{10} - Sensitivity and stratified analyses.

Analysis	Number of subjects		OR (95%CI) from model 3 ^a		Heterogeneity between cohorts			
	NO_2	PM_{10}	NO_2	PM_{10}	I-squared	p	I-squared	p
					NO_2	PM_{10}		
Main analyses	22814	17098	1.10 (0.99,1.21)	1.04 (0.88,1.23)	46.20%	0.10	0.00%	0.44
Stratified analyses								
By age*								
Restricted to age<50	14875	10499	1.08 (0.96,1.21)	1.07 (0.86,1.32)	56.60%	0.06	10.60%	0.35
Restricted to age \geq 50	7909	6287	1.02 (0.94,1.12)	1.05 (0.78,1.42)	0.00%	0.54	0.00%	0.72
By sex**								
Men only	4098	2264	1.06 (0.92,1.24)	1.00 (0.63,1.59)	0.00%	0.45	0.00%	0.61
Women only	18725	14751	1.07 (0.97,1.19)	1.07 (0.91,1.26)	0.45	0.11	0.00%	0.51
By smoking status [#]								
Ever smokers only	11664 ^b	8576	1.13 (0.99,1.29)	1.17 (0.79,1.74)	49.80%	0.08	40.30%	0.14
Never smokers only	11159 ^b	8433	1.01 (0.88,1.16)	1.10 (0.87,1.39)	50.00%	0.08	0.00%	0.52
Sensitivity analyses								
Using asthma incidence definition with coherent age of onset (NSHD excluded)	19935	14585	1.09 (0.93,1.28)	1.07 (0.59,1.93)	65.40%	0.02	64.10%	0.03
Among non movers	15289	11780	1.04 (0.98,1.11)	1.12 (0.91,1.37)	0.00%	0.50	0.00%	0.88
Excluding E3N	10715	7185	1.15 (1.03,1.27)	1.17 (0.82,1.66)	11.90%	0.34	8.30%	0.36
Excluding ECRHS	19014	15151	1.12 (0.98,1.29)	1.13 (0.83,1.55)	56.50%	0.06	15.40%	0.32
Excluding EGEA	22317	16790	1.03 (0.98,1.09)	1.02 (0.86,1.20)	3.50%	0.39	0.00%	0.79
Excluding NSHD	20624	15121	1.08 (0.98,1.20)	1.06 (0.84,1.32)	49.10%	0.10	13.80%	0.33
Excluding SALIA	20768	15052	1.09 (0.98,1.21)	1.03 (0.85,1.26)	50.10%	0.09	5.90%	0.37
Excluding SAPALDIA	20632	16191	1.11 (1.00,1.24)	1.05 (0.89,1.24)	55.20%	0.06	0.00%	0.42
Excluding 5% upper and lower extreme values	20642	15412	1.03 (0.97,1.10)	1.11 (0.89,1.37)	0.00%	0.92	0.00%	0.84
Fixed effect between cities/areas within the same study	22814	17098	1.14 (1.01,1.29)	1.05 (0.86,1.29)	59.20%	0.03	2.00%	0.40
Restricted to cities/areas with both NO_2 and PM_{10}	17097 ^c	17097 ^c	1.11 (0.99,1.24)	1.04 (0.88,1.23)	39.40%	0.14	0.00%	0.44
Restricted to cities/areas with high goodness of fit for NO_2 exposure models ($R^2\geq 0.6$)	21048	NA	1.09 (0.98,1.21)	NA	47.40%	0.09	NA	
Two pollutants model (NO_2 PM_{10})	17097	17097	1.17 (0.99,1.38)	0.98 (0.79,1.21)	46.20%	0.10	0.00%	0.42

NA: Not applicable.

^aMeta-analysis from the study-specific adjusted logistic regression models. The logistic regression models were adjusted for: age (except for the model stratified by age), sex (except for the model stratified by sex), smoking (except for the model stratified by smoking), overweight and education level (model 3) with random effects per city/area (except for the model considering city/area as fixed effect) for each study except for SALIA where there is only one area and EGEA where family structure was taken into account. ^bInconsistent N due to NSHD : 11664+11159=22823 \neq 22814. ^cInconsistent N due to ECRHS.

*p-value for interaction between participants <50 and \geq 50 years old for NO_2 : 0.88 and for PM_{10} : 0.99

**p-value for interaction between males and females for NO_2 : 0.66 and for PM_{10} : 0.80.

#p-value for interaction between smokers and non-smokers for NO_2 : 0.35 and for PM_{10} : 0.69.

Figure Legends

Figure 1. NO₂ and PM₁₀ in µg/m³ concentrations by study. Boxes extend from the 25th to the 75th percentile, bars inside the boxes represent the median, whiskers indicate the minimum and maximum values.

Figure 2. Associations of NO₂ and NO₂ back-extrapolated (per 10 µg/m³) on asthma incidence. Meta-analyses. Meta-analysis from the study-specific adjusted random-effect logistic regression models. The logistic regression models were adjusted for: age, sex, smoking, overweight and education level (model 3) with random effects per city/area for each study except for SALIA where there is only one area and EGEA where family structure was taken into account. I-V: Inverse-Variance weighted (fixed effect) pooled estimate of all studies. I-square: variation in estimate effect attributable to heterogeneity. D+L: DerSimonian and Laird (random effect) pooled estimate of all studies. Study-specific odds ratio are shown as black diamonds with horizontal lines representing 95% CIs. The size of the grey squares reflects the statistical weight of the study in the meta-analyses. The meta-analytic odds ratios are shown as blue diamonds, the middle of the diamond corresponds to the odds ratio value, and the width of the diamond represents the 95% CI.

Figure 1.

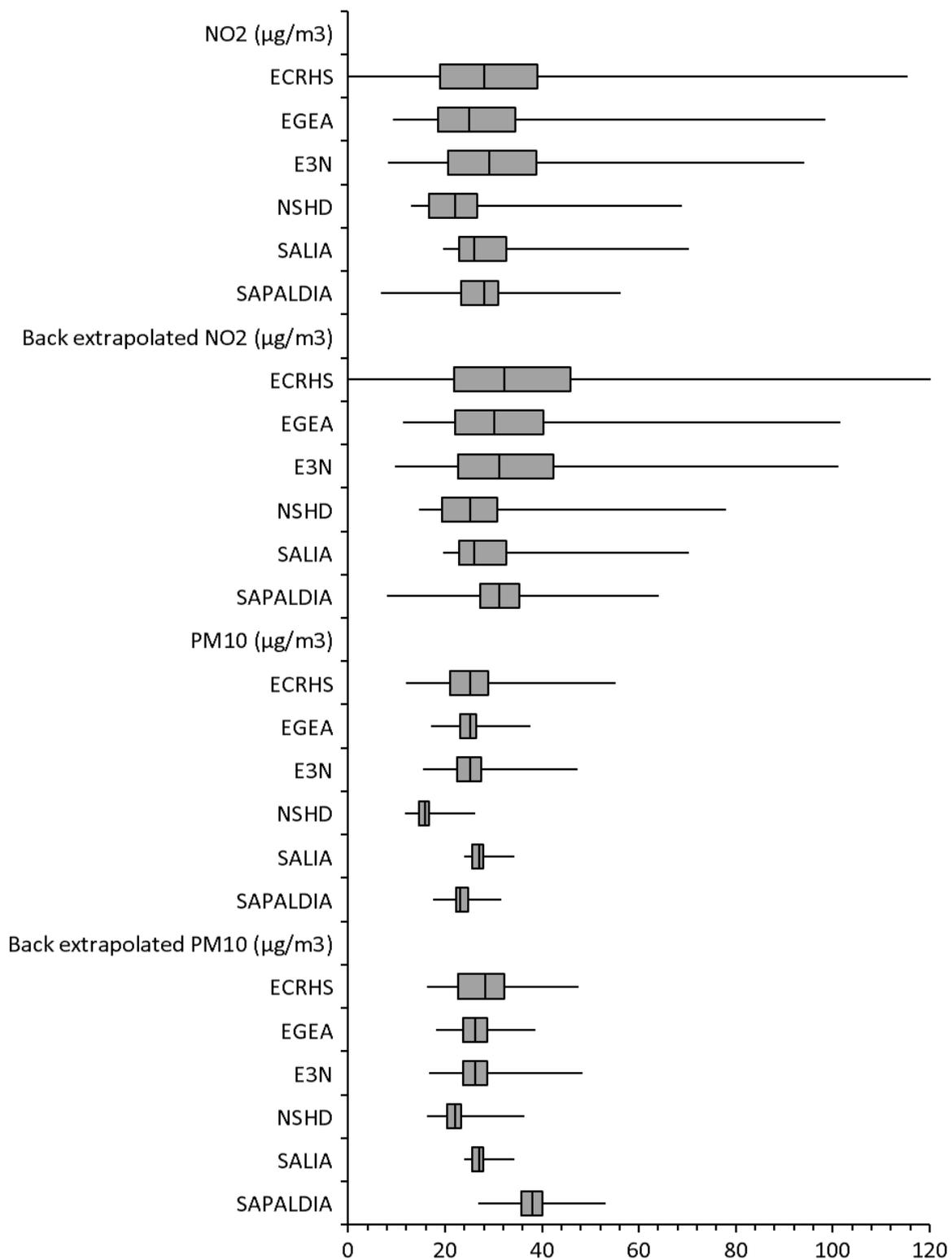


Figure 2.

