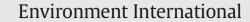
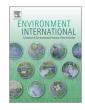
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## Airway oxidative stress and inflammation markers in exhaled breath from children are linked with exposure to black carbon



Sofie De Prins <sup>a,b</sup>, Evi Dons <sup>a</sup>, Martine Van Poppel <sup>a</sup>, Luc Int Panis <sup>a,c</sup>, Els Van de Mieroop <sup>d</sup>, Vera Nelen <sup>d</sup>, Bianca Cox <sup>e</sup>, Tim S. Nawrot <sup>e</sup>, Caroline Teughels <sup>f</sup>, Greet Schoeters <sup>a,b</sup>, Gudrun Koppen <sup>a,\*</sup>

<sup>a</sup> Environmental Risk and Health Unit, VITO (Flemish Institute for Technological Research), Boeretang 200, B-2400 Mol, Belgium

<sup>b</sup> Faculty of Pharmaceutical, Biomedical and Veterinary Sciences, University of Antwerp, Universiteitsplein 1, B-2610 Antwerp, Belgium

<sup>c</sup> Transportation Research Institute (IMOB), Hasselt University, Wetenschapspark 5 Bus 6, B-3590 Diepenbeek, Belgium

<sup>d</sup> Environment and Health Unit, Provincial Institute of Hygiene, Kronenburgstraat 45, B-2000 Antwerp, Belgium

<sup>e</sup> Centre for Environmental Sciences, Hasselt University, Agoralaan Gebouw D, B-3590 Diepenbeek, Belgium

<sup>f</sup> Environment & Health, Flemish Government, Department of Environment, Nature and Energy, Koning Albert II-laan 20 Bus 8, B-1000 Brussels, Belgium

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#### ABSTRACT

*Background:* The current study aimed at assessing the associations between black carbon (BC) exposure and markers for airway inflammation and oxidative stress in primary school children in a Western European urban area. *Methods:* In 130 children aged 6–12 years old, the fraction of exhaled nitric oxide (FeNO), exhaled breath condensate (EBC) pH, 8-isoprostane and interleukin (IL)–1 $\beta$  were measured in two seasons. BC concentrations on the sampling day (2-h average, 8:00–10:00 AM) and on the day before (24-h average) were assessed using measurements at a central monitoring site. Land use regression (LUR) models were applied to estimate weekly average BC exposure integrated for the time spent at home and at school, and seasonal average BC exposure at the home address. Associations between exposure and biomarkers were tested using linear mixed effect regression models. Next to single exposure models, models combining different BC exposure metrics were used.

*Results*: In single exposure models, an interquartile range (IQR) increase in 2-h BC (3.10 µg/m<sup>3</sup>) was linked with a 5.9% (95% CI: 0.1 to 12.0%) increase in 8-isoprostane. FeNO increased by 16.7% (95% CI: 2.2 to 33.2%) per IQR increase in 24-h average BC (4.50 µg/m<sup>3</sup>) and by 12.1% (95% CI: 2.5 to 22.8%) per IQR increase in weekly BC (1.73 µg/m<sup>3</sup>). IL-1 $\beta$  was associated with weekly and seasonal (IQR = 1.70 µg/m<sup>3</sup>) BC with respective changes of 38.4% (95% CI: 9.0 to 75.4%) and 61.8% (95% CI: 3.5 to 153.9%) per IQR increase in BC. An IQR increase in weekly BC was linked with a lowering in EBC pH of 0.05 (95% CI: -0.10 to -0.01). All associations were observed independent of sex, age, allergy status, parental education level and meteorological conditions on the sampling day. Most of the associations remained when different BC exposure metrics were combined in multiple exposure models, after additional correction for sampling period or after exclusion of children with airway allergies. In additional analyses, FeNO was linked with 24-h PM<sub>10</sub> levels, but the effect size was smaller than for BC. 8-Isoprostane was not linked with either 2-h or 24-h concentrations of PM<sub>2.5</sub> or PM<sub>10</sub>.

*Conclusion:* BC exposure on the morning of sampling was associated with airway oxidative stress while 24-h and weekly exposures were linked with airway inflammation.

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### 1. Introduction

Particulate matter (PM) is a heterogeneous mixture highly variable in particle size and content. Generally, the small-sized

*E-mail addresses:* sofie.deprins@vito.be (S. De Prins), evi.dons@vito.be (E. Dons), martine.vanpoppel@vito.be (M. Van Poppel), luc.intpanis@vito.be (L. Int Panis), els.vandemieroop@pih.provant.be (E. Van de Mieroop), vera.nelen@pih.provant.be (V. Nelen), bianca.cox@uhasselt.be (B. Cox), tim.nawrot@uhasselt.be (T.S. Nawrot), caroline.teughels@lne.vlaanderen.be (C. Teughels), greet.schoeters@vito.be (G. Schoeters), gudrun.koppen@vito.be (G. Koppen).

n.be (C. Teughels), greet.schoeters@vito.be (G. Schoeters), ben). dominated p

combustion-derived particles ( $<PM_{2.5}$ ) are thought to be more harmful to health than PM from other sources (Janssen et al., 2012). Combustion-derived particles such as black carbon (BC, mainly in size range  $<2.5 \,\mu$ m) have a larger surface area per unit mass and a greater capacity to reach deep into the airways (Seaton et al., 1995). Moreover, since these particles are enriched in organic carbon content and pro-oxidative polycyclic aromatic hydrocarbons (PAHs) they have a high oxidative potential (Miyata and van Eeden, 2011). Janssen et al. (2011) showed that BC – formed by incomplete combustion of fossil fuels, biofuels, and biomass – is a valuable additional air quality indicator to evaluate the health risks of air pollution dominated primarily by combustion particles. The study showed that

<sup>\*</sup> Corresponding author at: VITO (Flemish Institute for Technological Research), Boeretang 200, B-2400 Mol, Belgium. Tel.: +32 14 33 51 65; fax: +32 14 58 05 23.

the estimated increase in life expectancy associated with a hypothetical traffic abatement measure was four to nine times higher for BC compared with an equivalent change in PM<sub>2.5</sub> mass (Janssen et al., 2011).

In epidemiological studies, short-term exposure to BC has been associated with respiratory symptoms in asthmatic (Patel et al., 2010; Spira-Cohen et al., 2011) and non-asthmatic (Patel et al., 2010) children. BC exposure was furthermore linked with airway inflammation markers in asthmatics (McCreanor et al., 2007; Patel et al., 2013) and nonasthmatics (Patel et al., 2013). The health effects of PM exposure are likely mediated by oxidative stress and the activation of inflammatory cells (Knol et al., 2009; Miller et al., 2012). Moreover, diesel exhaust particles (DEPs), largely consisting of BC, are thought to induce and enhance allergic inflammation (Inoue and Takano, 2011).

The aim of the current study was to assess the effects of BC exposure during different time windows on airway oxidative stress and inflammation markers in a general population of primary school children in a Western European urban area. In Antwerp, the current study region, BC levels are expected to be at the higher end of the distribution of annual average concentrations in developed countries (EEA, 2011; U.S.EPA, 2012) because of the dense road network with heavy traffic and residential heating (Dons et al., 2014). BC exposure was estimated using air quality measurements at a central monitoring site for shorter time windows (2-h and 24-h average), and land use regression (LUR) modeling for longer term exposure (weekly and seasonal average). In recent years, LUR has gained attention and proved to be a useful technique for assessing medium or long term outdoor air pollution exposure (Brauer et al., 2003; Dons et al., 2013; Hoek et al., 2008; Ryan and LeMasters, 2007). In the children, we measured the fraction of exhaled nitric oxide (FeNO) as a marker of lower airway inflammation (Ricciardolo et al., 2004). Additional inflammation markers such as pH and interleukin (IL)-1B were analyzed in exhaled breath condensate (EBC). EBC is a non-invasive way to study the respiratory system and it contains non-volatile compounds that originate from the airway lining fluid (Effros et al., 2012). The acidification of the airways, reflected by a lowering of EBC pH, is associated with airway inflammation (Kuban and Foret, 2013). IL-1 $\beta$  is a pro-inflammatory cytokine involved in host defense and local and systemic inflammation (Dinarello, 2009). In the airways, IL-1 $\beta$  is produced by epithelial cells (Hirota et al., 2012; Sousa et al., 1996) and macrophages (Ishii et al., 2004). Oxidative stress in EBC was assessed by means of 8isoprostane, a lipid peroxidation product of arachidonic acid reflecting oxidation of cell membrane phospholipids (Kuban and Foret, 2013).

The main focus in this paper is on BC exposure and the link with airway inflammation and oxidative stress markers. We studied whether those associations were also observed with other exposure parameters such as  $PM_{2.5}$  and  $PM_{10}$  and if these associations were influenced by airway allergy.

#### 2. Materials and methods

#### 2.1. Study population

Children aged 6–12 years old were recruited from two primary schools (circa 2 km apart) in Antwerp, a city of approximately 500,000 inhabitants located in the northern part of Belgium (Europe). Invitations were sent to 744 children of which 242 were willing to participate. Subjects were eligible for participation when they had attended the current school for at least one year, had no plans to change school or home residence within the next year, had lived for at least one year in the Antwerp agglomeration, were not exposed to tobacco smoke inside their house (although children with smoking parents were not excluded) and were willing to donate biological samples. After the exclusion of 68 children for not meeting these criteria, 130 subjects were selected so that the population was equally distributed over both schools, grades, age and sex.

Both the biomonitoring and simultaneous air quality measurements were performed in two seasons (May–June and November– December 2011), each campaign lasting six weeks. In the first period, 130 children participated. In the second period six children dropped out of the study because they changed schools or the permission to participate was withdrawn. Parents were asked to fill in a questionnaire providing information on the child's allergy and asthma status, parental education and parental smoking behavior. Biomonitoring was performed at the schools on ten different days in the first period and on ten different days in the second period. All children were sampled between 9:00–13:00 h.

The study was approved by the ethical committee of the University of Antwerp and subjects' parents gave written informed consent to participate in the study.

#### 2.2. Air quality assessment

# 2.2.1. Continuous BC, $PM_{2.5}$ and $PM_{10}$ measurements at a central monitoring site

As part of the official monitoring network, BC, PM<sub>2.5</sub> and PM<sub>10</sub> measurements were available at a central site in the city of Antwerp (monitoring station 42R801). BC was measured by a multi-angle absorption photometer (without size-selective inlet), PM<sub>2.5</sub> and PM<sub>10</sub> were measured by a beta attenuation monitor (ESM FH62I-R). The monitoring site was located approximately 2 and 3 km away from the two schools which the children attended. The average distance  $(\pm SD)$ from the children's homes to the central monitor was 2.8  $(\pm 2.2)$  km. Overall, 90% of the children's home residences were located within a 4.6 km buffer around the monitoring station. 2-h BC exposure of the children was defined as the average BC concentration between 8:00 and 10:00 h at the day of biomonitoring. Exposure during morning rush hour is important since children are then traveling to school and pollution levels are elevated. The 24-h average on the day before sampling and the weekly average before sampling were also calculated.

During the study, a micro-aethalometer (type AE51, Aethlabs, San Francisco, CA, USA) was temporarily installed at the central monitoring site; the same devices were used to measure BC on several home locations. The correlation between BC measurements by MAAP and by the micro-aethalometer at the central site was very high ( $R^2 = 0.78$  in period 1,  $R^2 = 0.89$  in period 2).

#### 2.2.2. BC measurements at home and LUR modeling

Weeklong BC measurements, using micro-aethalometers (type AE51, Aethlabs), were performed simultaneously at several home locations of children participating in the biomonitoring. In five consecutive weeks, 42 different locations were monitored. This was repeated in the second period, measuring at exactly the same locations. The central monitoring site served as a reference location. Based on these measurements, independent LUR models were built for the warmer (period 1) and the colder (period 2) season, but the explained variance was similar ( $R^2_{warm} = 0.70$ ;  $R^2_{cold} = 0.69$ ). The performance of the models was evaluated by calculating  $R^2$  and RMSE from leave-one-out cross-validation (LOOCV  $R^2_{warm} = 0.39$ ;  $R^2_{cold} = 0.51$ ; impacted by one and two influential observations respectively). A detailed description of the LUR models can be found in Dons et al. (2014).

Residential addresses of the children were geocoded and seasonal BC concentrations were calculated for each study subject using the LUR models. Seasonal LUR-estimates for home and school locations were recalculated into daily concentrations by using the temporal trend observed at the central monitoring site. Average BC exposure during seven days before medical examination was assessed, taking into account exposures at home and at school and the time spent in each of these microenvironments. Download English Version:

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