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Blood Pressure and Same-Day Exposure to Air Pollution at School: Associations with Nano-Sized to Coarse PM in Children

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Short running title: Children's blood pressure and UFP exposure

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Abstract

Background: Ultrafine particles (UFP) may contribute to the cardiovascular effects of particulate air pollution, partly because of their relatively efficient alveolar deposition. In this study, we assessed associations between blood pressure and short-term exposure to air pollution in a population of school children.

Methods and Results: In 130 children (aged 6-12 years) blood pressure was determined during two periods (spring and fall 2011). We used mixed models to study the association between blood pressure and ambient concentrations of particulate matter and ultrafine particles measured in the schools' playground. Independent of gender, age, height and weight of the child, parental education, neighborhood socio-economic status, fish consumption, heart rate, school, day of the week, season, wind speed, relative humidity and temperature on the morning of examination, an interquartile range (860 particles/cm^3) increase in nano UFP fraction (20-30 nm) was associated with a 6.35 mmHg (95% CI: 1.56, 11.14; p=0.01) increase in systolic blood pressure. For the total UFP fraction, systolic blood pressure was 0.79 mmHg (95% CI: 0.07, 1.51; p=0.03) higher, while no effects on systolic blood pressure were found for the nano-sized fractions with a diameter larger than 100 nm, nor $PM_{2.5}$, PM_{coarse} and PM_{10}. Diastolic blood pressure was not associated with any of the studied particulate mass fractions.

Conclusion: Children attending school on days with higher UFP concentrations (diameter smaller than 100 nm) had higher systolic blood pressure. The association was dependent on UFP size, and there was no association with the $PM_{2.5}$ mass concentration.

Introduction

Air pollution is a complex mixture of solid particles and gases. Different size distribution modes can be identified for airborne particles. Coarse mode particles, with diameters larger than 2.5 μm, are generally produced by mechanical processes. The formation of ultrafine particles (UFP, particles with a diameter less than 100 nm) is often related to combustion or gas-to-particulate conversion. In contrast to $PM_{2.5}$ (particles with a diameter less than 2.5 μm), long-range transport is usually not a major source of UFP in urban areas because of the short lifetime of UFP (Sioutas et al. 2005). Relative to larger particles, UFP demonstrate greater cytotoxicity and inflammatory capacity per mass basis. UFP produce a significant inflammatory response in lung cells. The pulmonary inflammatory response induced by UFP may trigger or enhance systemic effects including those of the cardiovascular system (Donaldson et al. 2001; Oberdorster 2001).

Short-term elevation in particulate air pollution has been associated with an increased risk for acute myocardial infarction and stroke (Peters et al. 2001). One important biological mechanism that may contribute to these short-term associations is an air pollution-mediated pro-hypertensive response (Brook et al. 2009). Jacobs et al. reported that PM concentrations were positively associated with blood pressure measured on the same day in an elderly study population (Jacobs et al. 2012). A French study in pregnant women showed that a interquartile range increase in PM_{10} was associated with a 1.1% increase in diastolic blood pressure during the first trimester of pregnancy (Hampel et al. 2011). Human exposure studies show that blood pressure increases within only hours of exposure and that blood pressure can remain high until 24h post-exposure (Cosselman et al. 2012). Chuang et al. found increases in systolic and diastolic blood pressure in association with 1-3h moving averages of submicrometer particles with a size range of 0.02 to 1 μm in patients with lung function impairment (Chuang et al. 2005). An interquartile range

increase of 20.8 μg/m³ in 24h mean outdoor $PM_{2.5}$ was associated with an increase in pulse pressure of 4.0 mmHg in elderly taking anti-hypertensive medication (Jacobs et al. 2012). In a population of adults, present day levels of PM_{10} and nitrogen dioxide were associated with an increase in blood pressure (Choi et al. 2007). So far, no acute changes in blood pressure in association with PM_{10}, $PM_{2.5}$, nitrogen dioxide and ozone have been found in school children (Bilenko et al. 2013; Liu et al. 2013; Sughis et al. 2012).

Studies of the effects of air pollution in children have mainly investigated neonatal or infant mortality (Scheers et al. 2011), birth weight (Pedersen et al. 2013), prematurity (Stieb et al. 2012) and respiratory endpoints, such as the incidence of asthma or impaired lung development (Braback and Forsberg 2009). Limited research has been done to evaluate cardiovascular parameters, including peripheral blood pressure, in relation to acute changes in urban pollution in children. In this study we investigate differences in blood pressure of children in association with their exposure at school to a broad span of particles ranging from the nano to the coarse size. Furthermore, to investigate possible underlying mechanisms, an inflammatory marker was measured in exhaled breath condensate (EBC), a relevant matrix to study immediate or short-term responses to air pollution.

Methods

Study population

Children aged 6-12 years were recruited at two primary schools in Antwerp, Belgium, within the framework of the HEAPS (Health effects of air pollution in Antwerp schools) study (Dons et al. 2014). Children were eligible for inclusion if 1) they were living for at least one year at their current address, 2) they were not planning on moving during the next year, 3) there was no indoor smoking in their houses and 4) their parents were able to complete a questionnaire in

Dutch. A subset of 130 children was selected to participate in a study measuring blood pressure. Written informed consent was requested from the parents. Each child was examined twice in periods of ca. 26 weeks apart: the first sampling period fell within the spring season 2011 (May 17th- June 20th) whereas the second period fell within fall 2011 (November 10th- December 13th). Six children no longer participated in the second campaign due to change of school or parental refusal. On three study days no data on UFP were obtained. This resulted in repeated measurements for 90 children and single measurements for 40 children. Additional information such as the child's address, age, parental educational status, fish consumption and travel time from home to school was obtained via questionnaires filled out by the parents, the day before the clinical examination. The individual socio-economic status (SES) was defined as the highest level of education of the mother or the father and was categorized as low (high school not finished), middle (high school finished) and high (higher education or university). In addition, neighborhood SES was assessed using median household income of for the year 2011, provided at the statistical sector level by the Belgian National Institute for Statistics (FGOV). Fish consumption was coded as a categorical variable: never/rarely, one or two times/week and three or more times/week. Since only a small number of children (4%) had a fish consumption of 3 or more times/week, this category was taken together with one or two times/week. Data on travel time were missing for nine children. The study was approved by the Medical Ethics Committee of Antwerp University.

Clinical measurements

The sampling was organized on weekdays between 09.00 AM and 01.00 PM in the school. Height and weight were measured, while children were not wearing shoes but were fully clothed. Underweight, normal weight, overweight and obesity were determined based on 'Vlaamse

groeicurven 2004' (VUB 2004), which take age and gender into account. After the children had rested for five minutes in a sitting position, a study nurse measured blood pressure by making five to seven consecutive readings using an automated blood pressure instrument (Stabilo Graph, Germany) with a pediatric cuff. The guidelines of the European Society of Hypertension were followed for the measurement of blood pressure (O'Brien et al. 2003). The first blood pressure measurement was excluded. The mean of the remaining blood pressure measurements was used for analysis.

Markers of inflammation

Interleukin (IL) 1β was measured in EBC. EBC was collected using an RTube™ sampling device (Respiratory Research, Inc., Austin, TX, USA). The RTube™ was mounted with an aluminum sleeve that was cooled on dry ice for at least 10 minutes before collection. Participants were asked to breath tidally through a mouthpiece connected to the tube during 15 minutes, yielding approximately 1 mL of EBC. No food was taken 1 h prior to collection. After collection the EBC was immediately divided in aliquots of 250 µL, using 1.5 mL protein LoBind tubes (Eppendorf, Hamburg, Germany). Samples were kept on dry ice and stored at -80 °C until further analysis. IL-1β was analyzed using a Meso Scale Discovery Ultra-Sensitive Kit (Meso Scale Discovery, Rockville, MD, USA) which had a detection limit of 50 fg/mL. Samples below the detection limit (21% of all samples) were given a value of 25 fg/mL. Plates were read using a SECTOR® Imager 6000 instrument (Meso Scale Discovery).

Air pollution monitoring

Ambient concentrations of UFP, $PM_{2.5}$ and PM_{10} were measured in the playground of both schools during the study period; data from 08.00 to 10.00 AM were used to assess exposure. Air

pollution monitoring devices were placed on ground level of the playground. Air was sampled at a height between 1.5 and 2.5 m.

UFP was measured with a Scanning Mobility Sizer (SMPS, 3080, TSI, USA) and UFP monitor (3031,TSI, USA). This latter device allows to determine the number of particles per size fraction (20-30 nm, 30-50 nm, 50-70 nm, 70-100 nm, 100-200 nm and >200 nm). Total UFP fraction was defined as the sum of all separate fractions. The SMPS has a higher size resolution of 64 size bins per decade. Both instruments were compared and the SMPS size distribution was recalculated to the UFP 3031 size bins.

$PM_{2.5}$ and PM_{10} concentrations were measured with an optical counter (1.108, Grimm, USA), with a sensitivity of 1 particle count/L, a mass resolution of 0.1 µg/L and a reproducibility of 2%. This device has 15 different size channels for particles with a size between 0.3 and 20 µm. During the monitoring campaign the data were validated to the reference (filter) method using a low volume sampler (Partisol 2025, Thermo Scientific, USA), equipped with a $PM_{2.5}$ sampling inlet at a flow rate of 16.7L/min. PM_{coarse} was defined as the PM_{10} fraction minus the $PM_{2.5}$ fraction (Peng et al. 2008).

UFP data in the playground of their own school were missing for 30 children in the first sampling period and 26 children in the second sampling period. These missing values were imputed using UFP measurements at the playground of the other school (at a distance of 2800 m). Comparison of simultaneous measurements on both locations showed no significant difference. Temperature, relative humidity and wind speed from 08.00 to 10.00 AM were obtained from a local fixed and validated measuring station (42R801) located 2 and 3 km from the examination locations.

Residential distance to major roads

The children's home addresses were geocoded by address; the accuracy was visually inspected using Google Maps and coordinates were manually adapted when they differed from the actual position of the residence. Residential distance to major roads (based on road classification, not intensity) was calculated in ArcGIS 9.3 using the Tele Atlas MultiNet (TeleAtlasMultinet).

Statistical analysis

Statistical analyses were conducted using the SAS statistical package, version 9.3 (SAS Institute, Cary, NC, USA) and GraphPad Prism version 5.00 (GraphPad Software, San Diego, CA, USA). Mann-Whitney U and Fisher exact tests were used to assess differences between the two schools for continuous and categorical data, respectively. The association between blood pressure and air pollution was examined by treating the pollutants as continuous variables. We used mixed models with random subject effects accounting for repeated measures, assuming a compound symmetry covariance structure. Models were adjusted for the following fixed effects: gender, age, height and weight of the child, parental education, neighborhood socio-economic status, fish consumption, heart rate, school, day of the week, season, wind speed, relative humidity and temperature on the morning of examination. Time-invariant subject characteristics (such as gender and parental education) were included to permit the assumption of a normally distributed random subject intercept. The shape of the association between blood pressure and air pollution and temperature was explored by using natural cubic splines with different numbers of degrees of freedom. Model fit was assessed by using the Akaike Information Criterion (AIC). The interaction term between season and temperature was explored. For the UFP variables, the single-pollutant analyses described above were repeated with additional adjustment for $PM_{2.5}$ concentrations in the model. P-values ≤ 0.05 were considered to indicate statistical significance.

In a series of sensitivity analysis, the model was additionally adjusted for travel time from home to school and for residential distance to major roads. Further the analyses were repeated excluding imputed UFP data, excluding days with low UFP concentration (total UFP <5000 particles/cm^3) and excluding days with high UFP concentrations (total UFP >10000 particles/cm^3).

The association between IL-1β, a marker of inflammation, and air pollution was examined by mixed models adjusted for the same confounders as before except heart rate. Estimates (with 95% confidence intervals) are presented for interquartile range (IQR) increases in pollutant concentrations.

Results

The study population consisted of 130 children aged 6-12 years (50% girls). Mean (SD) height was 135.8 (10.1) cm and mean (SD) weight amounted 30.9 (7.9) kg (Table 1). Systolic and diastolic blood pressure averaged (SD) 107.1 (8.8) and 60.8 (7.1) mmHg respectively. Children of school 1 had higher systolic and diastolic blood pressure, shorter travel time to school and a slightly lower average household income (Table 1).

The mean accumulated concentration for UFP, $PM_{2.5}$, PM_{coarse} and PM_{10} fractions, measured on the different examination days between 8.00 and 10.00 AM and the corresponding temperature from 08.00 to 10.00 AM are given in Figure 1 and 2. The mean relative humidity was 70.3% and 84.0% for the first and second sampling period, respectively. The daily variation in relative humidity is shown in Supplemental Material, Figure S1. The distribution for the different UFP fractions and PM is given in Table 2. Correlations between the different size fractions of UFP and the coarse size fractions are shown in Supplemental Material, Table S1. The largest UFP

fractions (100-200 nm and >200 nm) were significantly correlated with $PM_{2.5}$ and PM_{10}, in contrast the smallest UFP fractions (20-30 nm) were not correlated with $PM_{2.5}$ or PM_{10}.

Comparing the fit of the models with a different number of degrees of freedom for air pollution, the association between blood pressure and air pollution showed linearity (data not shown). Temperature, however, was inversely associated with blood pressure at temperatures above approximately 12°C, but not at lower temperatures (data not shown). Models with an interaction term between temperature and season (corresponding to a piecewise linear model with a breakpoint at 12°C) provided the best model fit and were used in further analyses. In spring (temperatures above 12°C) systolic blood pressure for a 1°C increase in temperature is -1.35 mmHg (95% CI: -2.08, -0.63, p=0.0003) lower, whereas in autumn (temperatures below 12°C) the effect of temperature was not significant (-0.09 mmHg; 95% CI: -0.58, 0.40, p=0.72).

Adjusting for gender, age, height and weight of the child, parental education, neighborhood socio-economic status, fish consumption, heart rate, school, day of the week, season, wind speed, relative humidity, temperature and the interaction between season and temperature, systolic blood pressure was significantly associated with UFP fractions up to 100 nm, measured during the morning of clinical examination.

Systolic blood pressure was 6.35 mmHg higher (95% CI: 1.56, 11.47, p=0.01) with an IQR increase in the smallest UFP fraction (20-30 nm, IQR = 860/cm^3, Figure 3). The corresponding associations with UFP fractions of 30-50 nm, 50-70 nm and 70-100 nm were 1.18 mmHg (95%CI: 0.05,2.31; p=0.04, IQR = 712/cm^3), 0.92 mmHg (95%CI: -0.05, 1.89; p=0.07, IQR = 540/cm^3) and 0.86 mmHg (95%CI: 0.05, 1.68; p=0.04, IQR = 358/cm^3), respectively, while no significant associations effects were estimated for UFP ≥ 100 nm, or for $PM_{2.5}$, PM_c and PM_{10}.

(Figure 3) An IQR increase in the total UFP fraction (1,666/cm^3), was associated with a 0.79 mmHg increase (95%CI: 0.07 to 1.51; p=0.03) in systolic blood pressure. When the results were additionally adjusted for $PM_{2.5,}$ results were similar (Figure 3). Diastolic blood pressure was not significantly associated with either ultrafine particles or larger particulates (see Supplemental Material ,Table S2).

Additional adjustment for travel time to school or for residential distance to major roads gave similar results (Table 3). Associations remained positive and significant when imputed UFP data and days with high UFP concentrations were excluded. (Table 3). However, exclusion of days with low UFP concentrations from the analysis, the associations were no longer significant.

Finally, we investigated associations with a marker of lung inflammation. An IQR increase in the smallest UFP fraction was associated with a 24.2% increase in IL-1β (95%CI: 4.83,47.16, p=0.02) but no association appeared with $PM_{2.5}$ or PM_{10} (Table 4).

Discussion

In this study, children's systolic blood pressure was positively associated with ambient UFP measured in their school's playground on the same morning. Associations were statistically significant for particles < 100 nm in diameter, while larger particles, $PM_{2.5,}$ and PM_{10} were not significantly associated with blood pressure. To our knowledge, this is the first study of differences in children's blood pressure in association with different size fractions of PM on the same day. In general, children might be more sensitive to air pollution due to their relatively higher ventilation rate and metabolic turnover, as well as by the fact that some of the organ systems including the immune system are still in development (Kim 2004). Furthermore, their physical behavior, such as a greater physical activity, spending more time outdoors and their

closer proximity to traffic exhaust emission sources compared with adults, might add to their vulnerability towards hypertensive effects of airborne particles (Kim 2004).

Because of their small size, UFP make up only a small fraction of the total $PM_{2.5}$ mass, even though they represent the largest actual number of particles within fine PM. Since UFP have a higher particle number concentration, the surface area is much higher and as such, carry large amounts of adsorbed or condensed toxic air pollutants which results in a different surface chemistry in comparison with larger particles (Delfino et al. 2005). Therefore, reductions in $PM_{2.5}$ mass may not necessarily reduce the risk of cardiovascular events (Brook et al. 2010). This was also demonstrated in our study. We found that particle size is a determining factor in the effectiveness of particulate pollutants to cause rapid changes in blood pressure of 6 to 12-year old children, systolic blood pressure was significantly associated with exposure to UFP < 100 nm in diameter only, and estimated differences in systolic blood pressure with an IQR increase in exposure decreased with increasing particle size. UFP and PM measurements were performed from 08.00 to 10.00 AM, while the clinical examination was organized between 09.00 AM and 01.00 PM. The mean UFP concentrations for the different fractions were highly correlated between the time frame of 08.00-08.30 AM and 09.30-10.00 AM (correlations >0.70). Therefore, the reported changes between 08.00-10.00 AM might also reflect the exposure later in the morning.

In contrast to some studies in adults (Wu et al. 2013) and pregnant women (Hampel et al. 2011) or specific patients groups including adults with diabetes (Hoffmann et al. 2012; Jacobs et al. 2012) and elderly (Chuang et al. 2005), blood pressure was not associated with current $PM_{2.5}$ or PM_{10} concentrations in our study population of children..

In contrast with some previous studies (Choi et al. 2007; Chuang et al. 2005), diastolic blood pressure was not associated with UFP fractions in our study population. It is uncertain why associations with systolic and diastolic blood pressure differ. A possible reason is that systolic and diastolic blood pressures have different regulation pathways and can respond to environmental stimuli in a different way. While the main physiological role of systolic pressure is to force blood through the arteries during a heartbeat, which is responsive to the sympathetic nervous system and stress stimuli, the role of diastolic blood pressure is to provide perfusion of peripheral tissues during heart relaxation (Paunović et al. 2011).

The clinical significance of particulate-induced increases in blood pressure could be considerable. Childhood blood pressure is an important predictor of hypertension and cardiovascular disease later in life (Hartiala et al. 2012; Juhola et al. 2012; Lurbe et al. 2005; Lurbe and Torro 2010; Raitakari et al. 2003). Although blood pressure is believed to be a complex trait, determined by numerous genetic, biological, behavioral, social and environmental factors, avoiding or removing potentially irreversible adverse factors as early as possible seems reasonable (Simonetti et al. 2011).

Indeed, repeated particle induced elevations in blood pressure also leads to repeated increases in arterial wall stress and may on the long-term result in chronically elevated pressures. Epidemiological evidence for a chronic increase in arterial stiffness in children due to traffic related air pollution, as exemplified by residential traffic related indicators, exists (Iannuzzi et al. 2010).

Our current epidemiological observations in children are in line with human exposure studies. In a cross-over study, where participants were exposed 2h to diesel exhaust, increases in systolic blood pressure were reported until 24h post-exposure. No effects on diastolic blood pressure were reported (Cosselman et al. 2012). Further, a controlled experiment in healthy adults (aged 18-35 years) inhaling UFP for two hours showed changes in heart rate variability and loss of sympathovagal balance (Samet et al. 2009). Existing evidence suggests that air pollution is able to trigger an acute autonomic imbalance, favoring sympathetic nerve activity causing smooth muscle contraction and thus vasoconstriction (Pieters et al. 2012). In a crossover experiment, systolic blood pressure was significantly lower during a 2h walk in Beijing, China, in participants wearing a particulate-filter face mask than in participants that were not protected by a face mask. Wearing the face mask was also associated with increased heart rate variability, which suggests that the rapid increase in blood pressure due to particle inhalation can be mediated through the autonomic nervous system (Langrish et al. 2009). In other controlled studies, ultrafine carbon particles did not change blood pressure or heart rate variability but altered endothelial dysfunction or caused retinal vasoconstriction (Louwies et al. 2013; Routledge et al. 2006; Shah et al. 2008).

Experimental evidence of intratracheally instilled UFP in hamsters showed that UFP can pass from the lungs into the blood circulation within minutes (Nemmar et al. 2001). Due to specific characteristics (high surface area, particle number, metal and organic carbon content) of UFP, they may be transferred directly into the circulation and cause systemic inflammation and peripheral vascular oxidative stress resulting in reductions of nitric oxide, enhancing vasoconstriction and as such change blood pressure. Further, excess production of endothelin-1, a potent vasoconstrictor, after exposure to air pollution, can cause changes in blood pressure

(Bouthillier et al. 1998). In animal models, plasma endothelin was up regulated after exposure to diesel exhaust and concentrated air particles (Vincent et al. 2001). These results were confirmed in an epidemiological setting where patients with metabolic syndrome and healthy volunteers showed an increase in plasma endothelin-1 concentrations three hours after diesel exhaust exposure (Peretz et al. 2008).

An association between PM and the development of pulmonary inflammation through pro-inflammatory cytokine induction, has been well documented (Pope and Dockery 2006). IL-1β was measured in EBC, which is useful for detecting early lung inflammation (Garey et al. 2004). In our study population, some UFP fractions were associated with higher IL-1β in EBC. IL-1β can be switched on by activated NF-ĸB as an early event of acute inflammatory response, which can subsequently lead to the production of other inflammatory cytokines (Jimenez et al. 2002). Furthermore, the secretion of IL-1β could be considered as an early event in cardiovascular and respiratory illness due to its capacity to induce apoptosis, inhibit myofibroblasts differentiation and repress cell proliferation in rat lung fibroblasts (Zhang and Phan 1999).

Our study has both strengths and limitations. Our study was limited in amount of repeated measurements and participants because it was part of a larger biomonitoring program with a fixed design. The UFP concentrations did not differ significantly between the two periods (varied in both periods; see Figure 1) consequently adaptation towards the blood pressure measurements cannot explain our findings as variation in exposure was random and independent of the first or second blood pressure reading. To account for diurnal variation in blood pressure, all children were examined on the same moment of the day. To reduce the effect of remaining variability, at least five blood pressure readings were taken after five minutes of rest in the sitting position and the first blood pressure measurement was excluded. Simonetti et al. (Simonetti et al. 2011)

reported that parental smoking is an independent risk factor for children's blood pressure. In this regard, indoor smoking was an exclusion criteria, although this does not account for exposure to passive smoke elsewhere. As reported by Bilenko et al. and Liu et al., noise exposure might be a confounding factor in the association between air pollution and blood pressure (Bilenko et al. 2013; Liu et al. 2013). As we used a repeated measure design and the child was in both sampling periods examined on the same location and living at the same residential address at the different examinations, noise exposure is unlikely to be a time-varying factor and therefore unlikely to bias our estimates on acute exposure. Additional adjustment for residential proximity to a major road, as a proxy for night time noise exposure, did not alter our association between systolic blood pressure and acute UFP exposure (Table 3).

The major strength of the current study is the measurement of the different sized UFP and PM fractions in school playgrounds to reflect exposure as accurately as possible.

Conclusion

Children attending school on days with higher ultrafine particulate concentrations (diameter smaller than 100 nm) had a higher systolic blood pressure. This association was largely dependent on particle size and was not confounded by the $PM_{2.5}$ mass concentration.

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Table 1. Characteristics of the study population.

Characteristics	All	School 1	School 2
Number of participants	130	63	67
Girls	65 (50%)	31 (49%)	34 (51%)
Age, years	9.0 ± 1.4	9.0 ± 1.5	8.9 ± 1.4
Height, cm	135.8 ± 10.1	135.8 ± 10.7	135.8 ± 9.6
Weight, kg	30.9 ± 7.9	31.8 ± 8.8	30.1 ± 7.0
BMI, kg/m^3			
Underweight	18 (14%)	7 (11%)	11 (6%)
Normal weight	97 (75%)	45 (72%)	52 (78%)
Overweight	11 (8%)	9 (14%)	2 (3%)
Obese	4 (3%)	2 (3%)	2 (3%)
SES indicators			
Parental education (individual level)			
Low	4 (3%)	2 (3%)	2 (3%)
Middle	28 (22%)	15 (24%)	13 (19%)
High	98 (75%)	46 (73%)	52 (78%)
Income households (aggregated statistical sector, in euro)	21347.8 ± 2578.2	20 407.3 ± 1600.5	22 195.9 ± 2977.3*
Fish consumption			
Never/rarely	27 (21%)	16 (25%)	11 (16%)
1 or more times a week	103 (79%)	47 (75%)	56 (84%)
Travel time from home to school, min	11.1 ± 6.8	8.9 ± 4.3	13.3 ± 9.2*
Systolic blood pressure, mmHg	107.1 ± 8.8	110.4 ± 8.2	104 ± 8.1*
Diastolic blood pressure, mmHg	60.8 ± 7.1	63.1 ± 6.7	58.6 ± 6.8*
Heart rate, beats/min	83.7 ± 10.3	83.6 ± 10.1	83.8 ± 10.7

Data are given as mean ± SD or number (%).

*Significant difference between the two schools.

Table 2. Exposure characteristics.

PM Fraction	Min	25th P	75th P	Max	IQR
20-30 nm, #/cm^3	582	1018	1878	2084	860
30-50 nm, #/cm^3	603	1637	2349	4116	712
50-70 nm, #/cm^3	358	947	1486	2886	540
70-100 nm, #/cm^3	203	673	1031	3035	358
100-200 nm, #/cm^3	240	666	908	4601	242
>200 nm, #/cm^3	33	143	279	1205	136
Total UFP, #/cm^3	2020	5538	7204	17701	1666
$PM_{2.5}$, μg/m^3	2	8	43	53	35
PM_{coarse}, μg/m^3	1	5	14	34	10
PM_{10}, μg/m^3	5	21	45	64	24

UFP: ultrafine particles, PM: particulate matter, PM_{coarse}: PM_{10} fraction minus the $PM_{2.5}$ fraction

Table 3. Sensitivity analysis. Estimates in systolic blood pressure (mmHg) are given for an IQR increase in total UFP concentrations.

Analysis	N	β (95%CI)	p-value
Model 1	220	0.79 (0.07,1.51)	0.03
Model 1+ travel time	211	0.78 (0.03, 1.53)	0.05
Model 1 + log distance to major roads	220	0.81 (0.09, 1.53)	0.03
Exclusion of imputed UFP measurements	164	1.27 (0.47, 2.07)	0.004
Exclusion of days with low UFP	182	0.42 (-0.24, 1.07)	0.22
Exclusion of days with high UFP	193	1.96 (0.32, 3.61)	0.02

Data were imputed on four days, exclusion of days with low total UFP (< 5000 particles /cm³) on two days, exclusion of days with high total UFP (> 10 000 particles/cm³) on two days

Table 4. Estimated % difference in interleukin 1β (95% CI) per IQR increase in the corresponding UFP/PM fraction.

UFP/PM Fraction	IQR	β (95%CI)	p-value
20-30 nm, #/cm^3	860	24.20 (4.83, 47.16)	0.02
30-50 nm, #/cm^3	712	4.27 (-0.56, 9.35)	0.09
50-70 nm, #/cm^3	540	3.79 (-0.30, 8.05)	0.08
70-100 nm, #/cm^3	358	3.28 (0.33, 6.31)	0.03
100-200 nm, #/cm^3	242	1.40 (0.13, 2.68)	0.03
>200 nm, #/cm^3	136	1.98 (-0.48, 4.49)	0.12
Total UFP, #/cm^3	1666	2.92 (0.30, 5.61)	0.03
$PM_{2.5}$, μg/m^3	35	-6.28 (-18.54, 7.83)	0.37
PM_{coarse}, μg/m^3	10	9.89 (0.17, 20.56)	0.05
PM_{10}, μg/m^3	24	-1.33 (-8.91, 6.88)	0.74

Regression coefficients calculated for a IQR increase in exposure. Models are adjusted for gender, age, height and weight of the child, parental education, neighborhood socio-economic status, fish consumption, school, day of the week, season, wind speed, relative humidity and temperature.

Regression coefficients calculated for a IQR increase in exposure. Models are adjusted for gender, age, height and weight of the child, parental education, neighborhood socio-economic status, fish consumption, school, day of the week, season, wind speed, relative humidity and temperature.

UFP: ultrafine particles, PM: particulate matter, PM_{coarse}: PM_{10} fraction minus the $PM_{2.5}$ fraction.

Figure Legends

Figure 1. Concentration range of the accumulated UFP-fractions (left y-axis) and temperature (right y-axis) from 08.00 to 10.00 AM on the day of clinical examination.

Figure 2. Concentration range for $PM_{2.5}$, PMcoarse and PM_{10} from 08.00 to 10.00 AM on the day of clinical examination.

Figure 3. Association between systolic blood pressure and different UFP and PM fractions. Estimates (with 95% CI) represent the difference in systolic blood pressure for an interquartile range (IQR, Table 2)) increase in the corresponding UFP or PM fraction. Dots show results adjusted for gender, age, height and weight of the child, parental education, neighborhood socio-economic status, fish consumption, heart rate, school, day of the week, season, wind speed, relative humidity, temperature on the morning of examination and the interaction between season and temperature. Triangles are additionally adjusted for $PM_{2.5}$ (only for the different UFP fractions).

Figure 1.

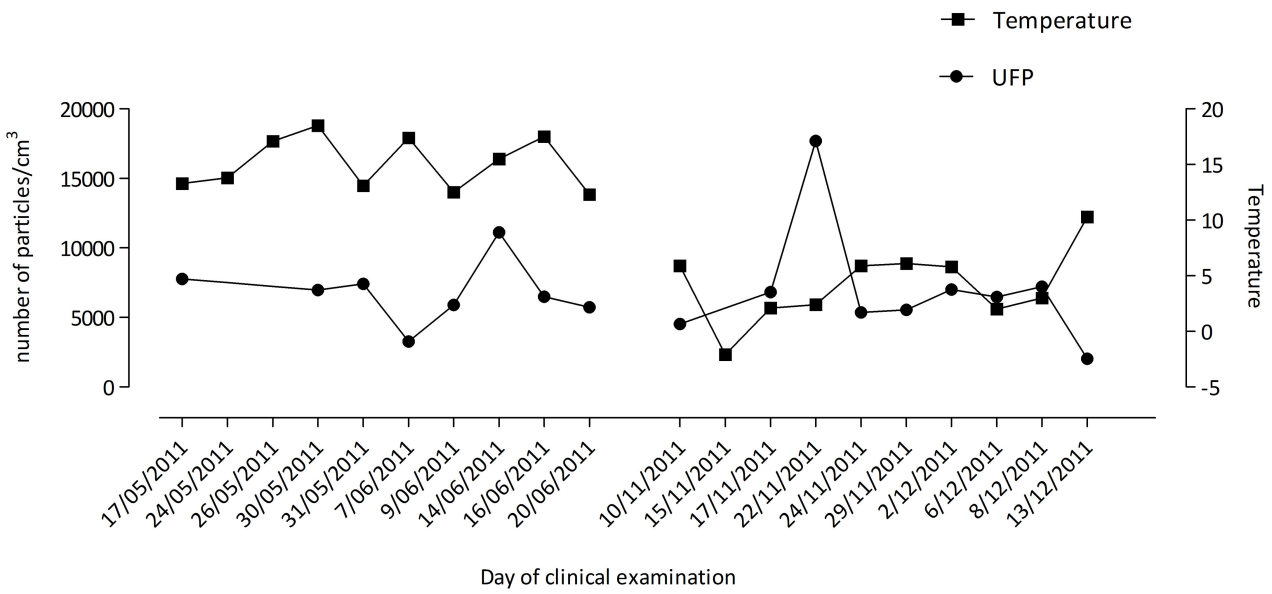

Temperature
UFP
number of particles/cm3
20000
15000
10000
5000
0
20
15
10
5
0
-5
Temperature
17/05/2011
24/05/2011
26/05/2011
30/05/2011
31/05/2011
7/06/2011
9/06/2011
14/06/2011
16/06/2011
20/06/2011
10/11/2011
15/11/2011
17/11/2011
22/11/2011
24/11/2011
29/11/2011
2/12/2011
6/12/2011
8/12/2011
13/12/2011
Day of clinical examination

Figure 2.

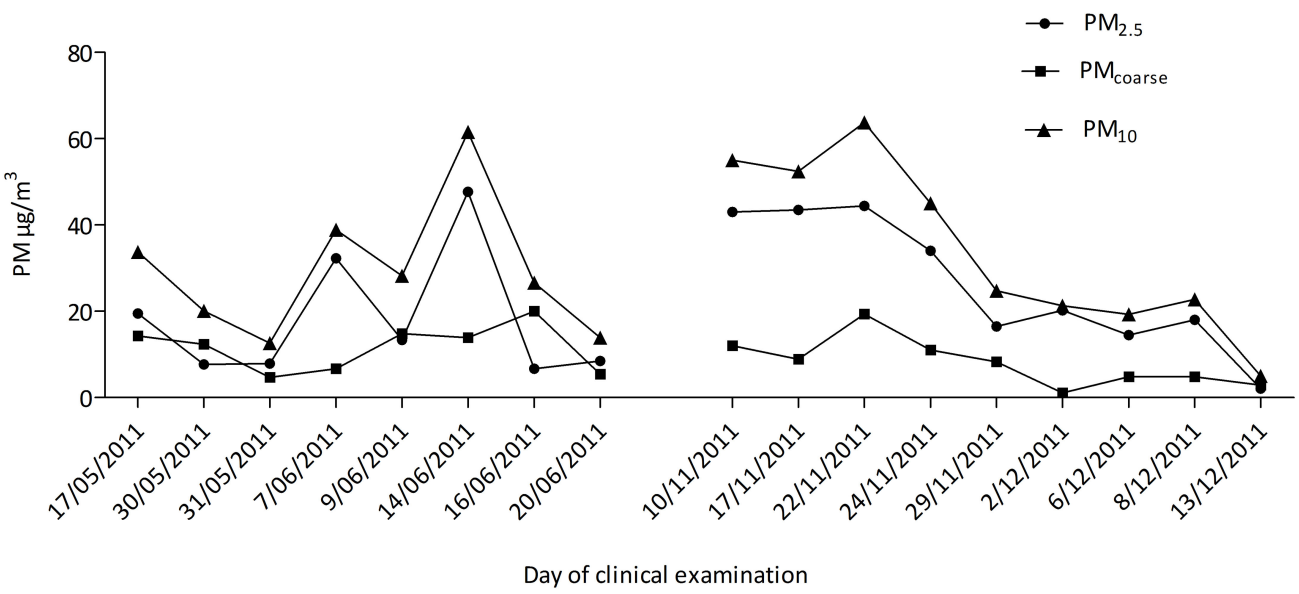
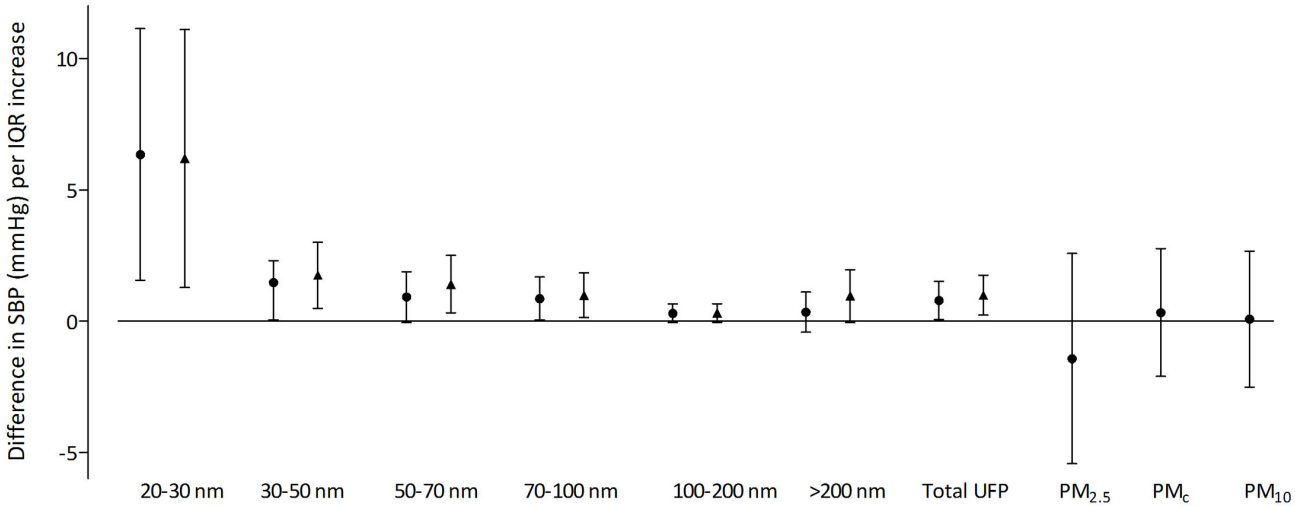

PM2.5
PMcoarse
PM10
80
60
40
20
0
PM µg/m3
17/05/2011
30/05/2011
31/05/2011
7/06/2011
9/06/2011
14/06/2011
16/06/2011
20/06/2011
10/11/2011
17/11/2011
22/11/2011
24/11/2011
29/11/2011
2/12/2011
6/12/2011
8/12/2011
13/12/2011
Day of clinical examination

Figure 3.


Difference in SBP (mmHg) per IQR increase
10
5
0
-5
20-30 nm
30-50 nm
50-70 nm
70-100 nm
100-200 nm
>200 nm
Total UFP
PM2.5
PMc
PM10