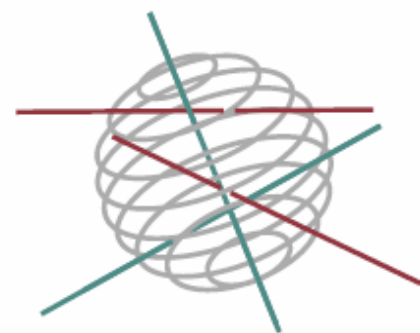


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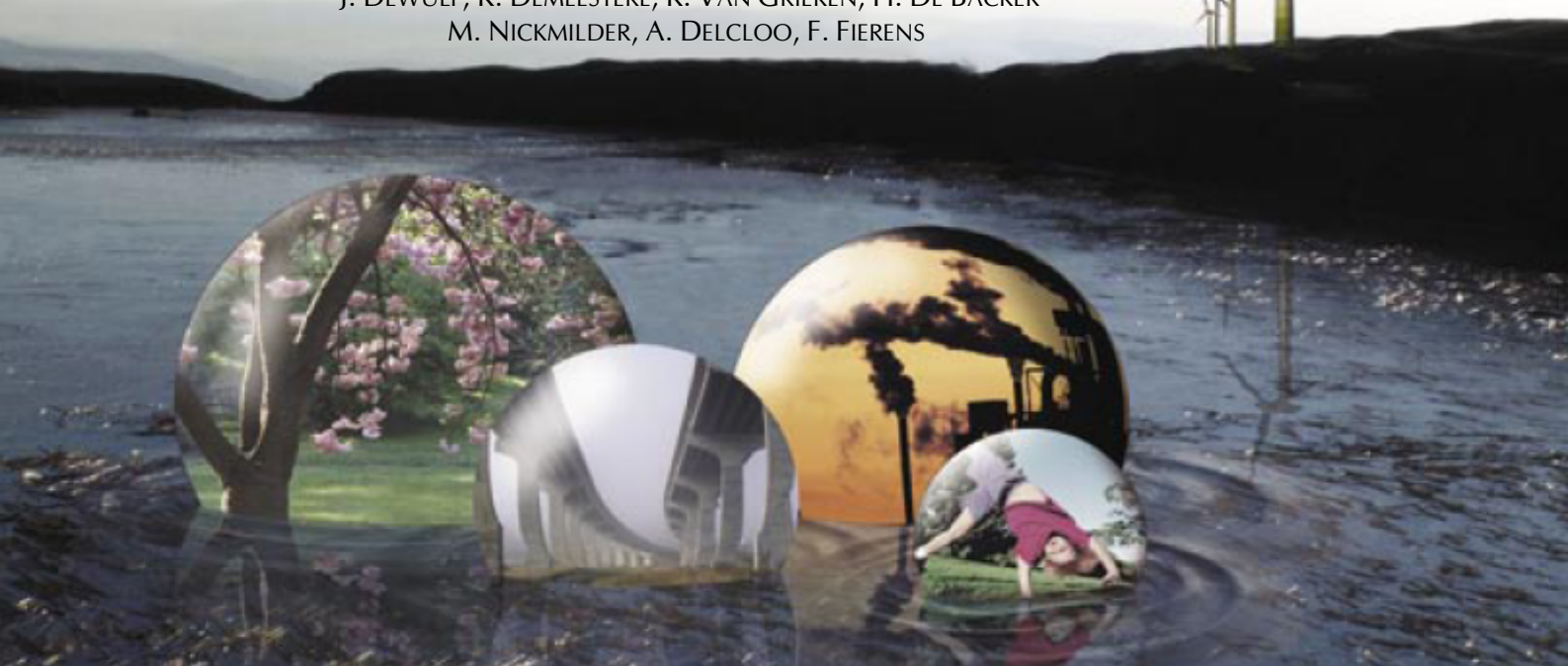
SCIENCE FOR A SUSTAINABLE DEVELOPMENT



## HEALTH EFFECTS OF PARTICULATE MATTER IN RELATION TO PHYSICAL-CHEMICAL CHARACTERISTICS AND METEOROLOGY

### “PARHEALTH”

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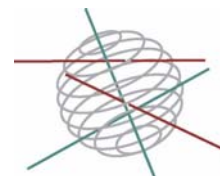
HEALTH AND ENVIRONMENT 

CLIMATE 

BIODIVERSITY   

ATMOSPHERE AND TERRESTRIAL AND MARINE ECOSYSTEMS   

TRANSVERSAL ACTIONS 



**Health & Environment**



FINAL REPORT PHASE 1

**HEALTH EFFECTS OF PARTICULATE MATTER  
IN RELATION TO PHYSICAL-CHEMICAL CHARACTERISTICS  
AND METEOROLOGY**

**“PARHEALTH”**

**SD/HE/01A**

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## **ACRONYMS, ABBREVIATIONS AND UNITS**

List of acronyms, abbreviations and units used in the report

APCI	Atmospheric Pressure Chemical Ionization
GC	Gas Chromatography
LC	Liquid Chromatography
LOD	Limit of Detection
LOQ	Limit of Quantification
MS	Mass Spectrometry
NO	nitric oxide
Oxy-PAHs	Oxygenated Polycyclic Aromatic Hydrocarbons
PFA	Platelet Function Analyzer
PM	particulate matter
ROS	Reactive Oxygen Species
US	UltraSound
VOCs	Volatile Organic Compounds

## 1. INTRODUCTION

### 1.1. Context

Solid and liquid phase material suspended in the atmosphere is referred to as 'particulate matter' (PM) or 'aerosols'. PM consists of a mixture of many species, including inorganic acids and salts, low volatility organic compounds, metallic agents and water all in highly variable concentrations. Our region has the highest annual mean mass PM concentrations of Europe (Aman 2004).

In this regard, within Europe, Belgium has the highest estimated loss in life expectancy that can be attributed to particulates, being estimated to 13.2 months compared with 9.0 months as the European average (Aman 2004). The research unit of Lung Toxicology (KULeuven) recently collected unique data for Belgium on the association of fine particulate air pollution and total, cardiovascular and respiratory mortality (Nawrot 2006). The novelty of these findings is that we show that the effects of air pollution are much stronger in summer months than in winter months, even in our temperate climate.

Until now, we can only speculate about the mechanisms underlying the much stronger association between mortality and particulates during warmer periods, even though the small particulate levels reach higher values in winter. One possibility is that the component-specific toxicity of small particulates may differ across the temperature range. In this context ozone, an oxidant of photochemical smog, is known to cause a variety of respiratory effects, and particulates and ozone may have an interactive role on acute effects of air pollution during warm days (Broeckaert 1999; Brunekreef 2002; Nickmilder 2003).

Animal toxicology and in vitro experimental studies support the hypothesis that chemical properties of inhaled particulates play a major role in toxic, genotoxic and carcinogenic mechanisms of particulates. However, the real impact of specific compounds associated with particulate matter on health outcomes and the possible interplay with ozone are not well understood yet. Whereas epidemiological investigations have opened the discussion on the effects of some inorganic species (especially metals such as Fe, V, Zn), data on the health impact of individual specific organic pollutants or groups of pollutants occurring in fine dust is very scarce (Welthagen 2003). One particular reason for this is the limited knowledge on the molecular composition of the organic fraction in particulates (Viana 2006). Whereas particulates consist for up to 50% of a complex mixture of organic compounds exhibiting a wide spectrum of physical-chemical properties (e.g. molecular weight and polarity), only approximately 20% of the organic fraction has been chemically identified and quantified (Mazurek 2002).

Airway inflammation from particulates likely involves inhalation of agents leading to the deposition or production in lung tissue of Reactive Oxygen Species (ROS). The ROS induce subsequent oxidant injury and inflammatory responses both in the lungs and systemically. Inhalation of particle-bound airborne transition metals (such as copper and iron) can also lead to the production of ROS in lung tissue (van Klaveren 1999).

Children and elderly represent two segments in the population which are susceptible for the short-term effects of air pollution (Brook 2004; Bernard 2005). In this regard the unit of Lung Toxicology (KULeuven) showed that ultrafine carbon particles may pass rapidly into the systemic circulation (Nemmar 2002) and showed significant effects of air pollution for the elderly on total and cardiovascular mortality (Nawrot 2006), while the unit of Occupational Medicine and Toxicology of the UCLouvain (Brussels) documented effects – mainly due to ozone – on lung inflammation in children (Bernard 2003, 2005; Broeckaert 1999; Lagerkvist 2004; Nickmilder 2003). In line with evidence gained from this previous work, this project investigates the effects of air pollution non-invasively with a focus on both the cardiovascular and respiratory systems.

Air quality measurements (Department of Organic Chemistry UGent and Environmental Chemistry of the UAntwerpen) focus on mass concentrations and composition of particulate matter at the sites of study. Especially, the component-specific toxicity of particulates is investigated in relation to meteorological conditions (RMI, Royal Meteorological Institute, Brussels) on different health endpoints. Also ozone and volatile organic compounds (VOCs) are measured in both a qualitative and quantitative way (Demeestere 2007; Demeestere 2008; Dewulf 1999; Dewulf 2006)..

In a cohort of children and a cohort of elderly, cardiovascular and respiratory parameters are measured in the same person on the same day (before and after ozone peak) and across seasons in association with component specific toxicity of fine particulate air pollution. This specific experimental design allows us to study the particulate induced effects independently of the direct meteorological effects on these endpoints.

### 1.2. Objectives

1. To investigate the short-term effects of particulates (both mass and physical-chemical characteristics) and ozone using sensitive endpoints of cardiovascular and respiratory responses in two susceptible segments of the population (children and elderly) in which the parameters will be measured in the same person within the same

day (before and after ozone peak) and across seasons on days with relatively high and low concentrations of fine particulates in the ambient air this will allow to determine possible threshold levels of PM and ozone in an integrated approach.

2. Sampling and analysis of VOCs in ambient air at the selected sites of study.  
Development and optimization of advanced analytical techniques for identification and quantification of specific organic compounds on particulates  
To explore the component-specific toxicity (chemical and physical) of particulates in association with meteorological conditions.

## 2. SCIENTIFIC METHODOLOGY

### 1. Health Studies

#### **Design and populations**

KULeuven studied residents living in facilities of two retirement communities in the centre of Antwerp and one outside the city centre. We started with a two to three week follow-up of 40 subjects. We collected data during a warmer period (June) and a colder period (February). Following a suggestion made by the referees of the interim evaluation, KULeuven & UCL collected data on infant mortality in Flanders (1998 – 2003), in order to investigate the association between exposure to elevated levels of PM and daily infant mortality (< 1 year of age). UCLouvain recruited scouts to study detrimental effects of PM constituents in combination with ozone in children. RMI/KMI & IRCEL provided forecasting on meteorological conditions and air pollution levels to plan our campaigns. Exclusion criteria included: being outside of the service flats for a longer period, psychiatric disorders, dementia, or medical conditions that would place the subject at risk from the blood donation. The ethical board of the KULeuven approved the study protocol.

#### **Blood collection and analysis**

KULeuven drew a venous peripheral blood sample at the same time of day to control for circadian rhythm. Blood was immediately analysed for platelet activity. For other planned analysis, blood was rapidly separated (less than 20 minutes after blood draw) into serum or plasma by using an onsite field laboratory. After centrifugation, each fraction was aliquoted, coded, transported frozen on dry ice from the field to our unit, and stored at -80°C.

#### **Exhaled breath condensate**

KULeuven & UCL collected exhaled breath condensate with the ECoscreen (Jaeger). The person was asked to breathe tidally in and out through a mouthpiece for 10 minutes. The collected volume was directly frozen on -20 °C and transported on dry ice to our unit and there stored at -80°C for further analysis

### 2. Forecasting of ambient air pollutants

Forecasting of the air pollutants concentrations was carried out with the CHIMERE model of KMI/RMI and IRCEL.

### 3. Ambient air monitoring

PM<sub>1</sub>, PM<sub>2.5</sub> and PM<sub>10</sub> mass concentrations were measured with an optic device (Aerocet 531, Met One). These measurements all were taken on the site (indoors and outdoors) (KULeuven). We further obtained PM<sub>2.5</sub> and PM<sub>10</sub> concentrations measured at a fixed monitoring station (from VMM) in Borgerhout, nearby serviceflat Cavell. From IRCEL we obtained interpolated PM<sub>10</sub> values.

Ambient ozone was continuously monitored using a UV photometric O<sub>3</sub> analyser Model 427 (Signal Instrument Company Limited, England) ((UCL). The equipment was calibrated before the start of the field study by an official laboratory of the national air monitoring network (lab of Mr Alain Derouane of IBGE-BIM). UGent and UA sampled gaseous inorganic and volatile organic compounds (VOC) as well as particulate matter (PM) in ambient air. Next, these research groups performed physical-chemical analysis of the collected air and PM samples, with focus on both organic (UGent) and inorganic (UA) pollutants. The KMI provided data on weather conditions.

#### **3.1. Development of a validated analytical method for identification and quantification of selected oxy-PAHs on particulates (UGent)**

In order to obtain a broad and multi-compound knowledge on the air quality on the selected sites of study, both organics sorbed on PM and gaseous VOCs are considered.

**3.1.1. Selection of target oxy-PAHs** A thorough scientific literature search was performed to reveal the state-of-the-art knowledge concerning the occurrence of oxy-PAHs on PM and analytical methods to determine these target compounds. This shows that the scientific knowledge in this area is relatively scarce, making clear the challenging character of the analytical method development and optimization (Albinet 2006, 2007a,b, 2008a,b; Lintelmann 2005, 2006; Schnelle-Kreis 2005a,b; Sklorz 2007a,b). A selection of target oxy-PAHs was made based on the literature search and the commercial availability of the standards. Some of the oxy-PAHs can be separated by use of gas chromatography (GC), while others are not thermally stable so that liquid chromatography (LC) is the method of choice for these compounds. In Table 1, an overview is given of the selected compounds and the separation technique that will be used.



**Table 1: Selection of target oxy-PAHs and the preferred separation technique.**

GC	LC
naphtalen-1-carboxaldehyde	benzo[a]pyren-1,6-dione
9H-fluoren-9-one	benzo[a]pyren-3,6-dione
phenanthren-9-carboxaldehyde	benzo[a]pyren-4,5-dione
9,10-anthraquinone	benzo[a]pyren-6,12-dione
naphtalen-1,4-dione	benzo[a]pyren-7,8-dione
7H-benzo[de]anthracen-7-one	benzo[a]pyren-11,12-dione
anthrone	chrysen-1,2-dione
benz[a]anthracen-7,12-dione	chrysen-5,6-dione
naphthacen-5,12-dione	phenanthren-9,10-dione
2-methylanthraquinone	6-oxo-7-oxa-benzo[a]pyrene
pyren-1-carboxaldehyde	
fluoren-2-carboxaldehyde	
1,8-naphthalic anhydride	

### 3.1.2. Sampling of particulate matter (WP 2.1)

In cooperation with the Flemish Environmental Agency (VMM) (Dr. E. Wauters, member of the following-up committee), PM<sub>10</sub> was sampled on glass or quartz filters by use of high-volume samplers (sampling time: 24h, sampling flow: ± 500 L/min). The loaded filters were deep frozen for storage before analysis. PM<sub>10</sub> was collected during the sampling campaigns at the elderly homes RVT Cavell (June 2007) and RVT Czagani (February 2008).

### 3.1.3. Analytical method development for identification and quantification of selected oxy-PAHs on PM (WP 2.2-2.4)

#### (a) Analytical method for the oxy-PAHs to be separated by LC (see Table 1)

For this part of research, the UGent partner has collaborated with the Helmholtz Zentrum München.

For the sample preparation (WP 2.2), ultrasonic extraction (USE) with ethyl acetate was chosen as the extraction technique. USE (Bandelin, Berlin, Germany) of the filters was performed during 15 minutes. The mixture was ice cooled during sonication in order to avoid degradation of the oxy-PAHs. The extracts were concentrated using a rotary evaporator under reduced pressure until a volume of 3 ml was obtained. After filtration (0.2 µm Spartan 13/0.2 RC filter unit, Schleicher & Schuell, Dassel, Germany) and further reduction under nitrogen until dryness (Barkey vapotherm mobil S, Leopoldshöhe, Germany), the residue was redissolved in acetonitrile and filtered (0.2 µm Spartan 13/0.2 RC filter unit, Schleicher & Schuell, Dassel, Germany). The extracts were transferred into autosampler vials for further analysis.

Separation (WP 2.3) was done on a C18-column, and a gradient of methanol and ammonium acetate (10 mM) has been developed. For detection (WP 2.4), an API 3000 triple quadrupole mass spectrometer (MS) has been used. Ionization was done with Atmospheric Pressure Chemical Ionization in the positive mode (APCI+). Based on the individual fragmentation schemes observed during MS-MS detection, the multiple reaction monitoring (MRM) mode was used for qualitative identification and quantification leading to increased selectivity and sensitivity.

Before the analysis of collected PM<sub>10</sub> samples (WP 2.5), a calibration curve was made with injections (10 µl) of standard solutions of the selected oxy-PAHs in acetonitrile (8–325 pg/l).

#### (b) Analytical method for the oxy-PAHs to be separated by GC (see Table 1)

So far, the analytical method development for this class of oxy-PAHs focused on separation (WP 2.3) and detection (WP 2.4). A gas chromatographic separation technique was developed on a 30m x 0.25mm x 0.50µm VF-5ms column using helium as a carrier gas (1 ml/min). The GC oven temperature programme has been optimized in order to maximize chromatographic resolution for the selected compounds. Ionization and detection was done with electron ionization (70eV) and mass spectrometry on a Thermo Finnigan MAT95XP-Trap mass spectrometer.

## 3.2. Sampling and analysis of VOCs

### 3.2.1. Selection of the sampling sites

Two types of sampling sites were selected:

- Urban environment: service flats Cavell (Antwerp, June 2007) and Czagani (Broechem, February 2008). The ambient air in different apartments, the restaurant and the garden was sampled. In the same period, a selected group of inhabitants was medically investigated.
- Rural environment (Wallonia).

During summer 2007 (July-August), sampling of the ambient air at 3 different rural locations (Waismes, Torgny, Ramegnies-

Chin) was performed, while children participated in summer camp activities. The children were also medically investigated.

### **3.2.2. Analytical methodology: sampling**

Because of the expected low concentrations of VOCs in ambient air, analyte enrichment is necessary. Therefore, target compounds were pre-concentrated on 200 mg of the polymeric sorbent Tenax TA held in stainless steel sorbent tubes (Markes; outer diameter ¼ inch; length: 3 x ½ inch) Both active (sampling time: 30 min) and passive sampling (sampling time: 1 week) were done. Calibration of the sampling pump was performed before and after the sampling using a Gilibrator2 flow calibration system.

### **3.2.3. Analytical methodology: identification and quantification of VOCs**

The analysis was performed by thermal desorption gas chromatography-mass spectrometry (TD-GC-MS). A 30m FactorFour VF-1ms low bleed bounded phase capillary GC column (Varian, 100% polydimethylsiloxane, internal diameter 0.25mm, film thickness 1µm) was used for separation. Masses from m/z 29 to 300 were recorded in full scan mode on a Trace DSQ Quadrupole MS (Thermo Finnigan), hyphenated to the GC, and operating at an electron impact energy of 70 eV.

The TD-GC-MS method was developed and validated for a group of 69 VOCs. The calibration was optimized, resulting in the use of deuterated toluene as an internal standard.

For data assessment and interpretation, relative peak areas (relative to the internal standard) were calculated for both real samples and blanks. This allowed to define LOD (Limit of Detection) and LOQ (Limit of Quantification) values, and to decide whether obtained results have to be interpreted in a qualitative or quantitative way.

## **3.3 Passive sampling and analysis of gaseous inorganic and BTEX compounds (UAntwerpen)**

### **3.3.1 Sampling methodology**

Gaseous compounds (benzene, toluene, ethylbenzene, xylenes (BTEX), NO<sub>2</sub>, SO<sub>2</sub> and O<sub>3</sub>) were collected by means of Radiello® passive samplers (Fondazione Salvatore Maugeri, Padova, Italy). This sampler comprises a compound-specific adsorbing cartridge, surrounded by a cylindrical microporous diffusive body; mounted on a supporting plate. Sampling was done both outside and inside of Cavell (Antwerp, June 2007) and Czagani (Broechem, February 2008), the exposure time of a single cartridge was 7 days; the mean concentrations of pollutants were calculated for these periods of time.

### **3.3.2 Analytical procedure**

#### **NO<sub>2</sub>, SO<sub>2</sub>**

The analytical procedure involved the recovery of NO<sub>2</sub><sup>-</sup>, SO<sub>3</sub><sup>2-</sup> and SO<sub>4</sub><sup>2-</sup> from the chemiadsorbed cartridges by means of water extraction (5 mL of milli-Q water) followed by two 1-minute mechanical stirring steps, between which the extracts were left to settle for one hour. NO<sub>2</sub><sup>-</sup>, SO<sub>3</sub><sup>2-</sup> and SO<sub>4</sub><sup>2-</sup> were determined by means of ion chromatography (IC).

#### **O<sub>3</sub>**

The analytical procedure involves ozonolysis of 4,4'-dipyridylethylene (during exposure), while sample preparation involves the formation of a yellow-coloured azide, which intensity is proportional to the amount of sampled ozone. Subsequently analysis is performed by means of UV-VIS spectrophotometry.

#### **BTEX**

The VOCs were recovered from a cartridge by chemical desorption, using 2 mL of carbon disulfide (CS<sub>2</sub>), followed by two 1-minute mechanical stirring steps, between which the extracts were left to settle one hour. Analysis was performed by gas chromatography coupled to mass spectrometry (GC-MS), using 2-fluorotoluene as internal standard.

## **3.4 Active sampling and inorganic and single particle analysis of particulate matter (Partner 4 UA)**

### **3.4.1 Sampling methodology**

#### **Bulk aerosol samples**

Harvard-type impactors (MS&T Area Samplers, Air Diagnostics and Engineering, Inc. Harrison, ME, USA), equipped with very quiet oil-free pumping units (Air Diagnostics and Engineering, air sampling pump, model SP-280E) were used to collect PM<sub>2.5</sub> in: service flats Cavell, June 2007, children camps July-August 2007 (Waimes-Malmedy, Torgny-Virton and Ramegnies-Chin) and service flats Czagani, February 2008). PM<sub>1</sub> was sampled in service flats (Czagani, February 2008). Total suspended matter (TSP) was sampled in Cavell with the use of stacked filter units (Polycarbonate Open Filter Holder type SM 16509B, produced by Sartorius®). Samples were collected indoors and outdoors; additionally in Cavell, the samples were taken in two apartments as well. Aerosols were collected on Nuclepore polycarbonate filters (pore size 0.4 µm, Nuclepore Whatman International Ltd., England) or teflon filters (PALL Life Sciences, USA). Sampling time was approximately 24 hours, each filter was exchanged just before the medical test performed by UA, and stored in the freezer upon analysis.

#### **Single particles**

Berner cascade impactor (Type LPI 30/0,06; Hauke GmbH, Gmunden, Austria) connected to a Becker pump was used to sample single particles. The particle size ranges (cut-off aerodynamic diameters) for the used stages (# 3, 4, 5, 6, 7 and 8) were equal to 0.25, 0.5, 1, 2, 4 and 8 µm, respectively. Particles were impacted on silicon (diameters 0.25 and 0.5 µm) and aluminium substrates (diameters 1, 2, 4 and 8 µm). Samples were taken in selected days during the whole sampling period in Cavell and Czagani, as well as in Waimes-Malmedy, Torgny-Virton and Ramegnies-Chin. The total time of

sampling was up to 6 hours per day.

#### **Elemental carbon (EC)**

The soot (or elemental carbon) concentration in air was determined with a dual wavelength aethalometer (Magee Scientific Company, USA) in Czagani (February 2008) indoors and outdoors during the whole sampling period.

The aethalometer is an optical method that measures continuously the attenuation of a beam of light at a near-infrared wavelength (880 nm) that is transmitted through the sample when collected on a fibrous filter. Another wavelength of 370 nm was used simultaneously to determine so-called UV-absorbing Particulate Material (UVPM). This is a sum of EC and a mixture of organic compounds that absorb UV photons. The UVPM is expressed in units of "EC Equivalent". In case this value is higher than the EC concentration, it shows that there is an additional optical absorption that is of the same magnitude as if it was produced by the presence of black carbon in the amount that is equal to the difference between them.

#### **3.4.2 Analysis procedure**

##### **Bulk aerosol samples**

The mass concentration of dust was determined gravimetrically by weighing the filters before and after the sampling. Both times the filters were conditioned for 24 hours before weighing in controlled temperature and relative humidity, in order to minimise possible influences on the determined mass concentrations, due to adsorbed H<sub>2</sub>O on the aerosols. Each time weighing was performed in three repetitions. Afterwards, the elemental composition of aerosols was determined by Energy Dispersive X-ray Fluorescence (EDXRF; Epsilon 5, PANalytical, Almelo, The Netherlands). Subsequently, the contribution of the water soluble fraction (concentrations of ions such as sulphates, nitrates, chlorides plus sodium, potassium, ammonium, magnesium and calcium cations) was measured by ion chromatography (IC Dionex, USA). For anion separation, the eluent was composed of 3.5 mM Na<sub>2</sub>CO<sub>3</sub> / 1.0 mM NaHCO<sub>3</sub> and the flow rate was set at 1.2 mL.min<sup>-1</sup>. For cation separation, the eluent was 20 mM H<sub>2</sub>SO<sub>4</sub>, with a flow rate of 1.0 mL.min<sup>-1</sup>. Calibration was performed using certified standard multi-ion solutions (Combined Seven Anion Standard II, Combined Six Cation Standard II, Dionex, USA). The gravimetric analysis and ED-XRF analysis of aerosol filters are so-called "non-destructive" analysis steps, for they don't require any special sample treatment; however determination of their water-soluble ion content demands leaching out in a minimal quantity of Milli-Q water through ultrasonic leaching before introducing the sample to ion chromatography.

##### **Single particles**

The elemental composition of single particles was determined with the use of electron probe X-ray micro analysis (EPMA) (JEOL 733, Tokyo, Japan) coupled with EDX detector (Oxford Instruments, CA). The combination of computer-controlled single-particle analysis using thin-window (TW) EPMA and a reverse Monte Carlo quantification procedure proved to be very beneficial for providing elemental concentrations within 15 % relative accuracy, also for low-Z elements. Beam-sensitive particles such as ammonium sulphate and nitrate were analysed by using a liquid-nitrogen cooled stage.

### 3. INTERMEDIARY RESULTS

#### **Study 1: Acute mortality and modelled chemical composition of PM [KMI/IRM and IRCEL]**

KMI used a chemical transport model (CTM) to model the composition of particulate matter for our region of interest with a coarse resolution of 50 km<sup>2</sup> in function of the available input data (meteofields, emissions).

The meteorological input fields are gathered from the ECMWF (European Centre for Medium-Range Forecast) operational forecasts. Since the horizontal- and vertical resolution of this operational weather forecast model changed significantly over the years, the input data used is interpolated at a horizontal resolution of about 50 km<sup>2</sup>. The emission database we used is the European Monitoring and Evaluation Program (Verstreng 2003) database. We have been using the EMEP emissions for the year 2002.

The modelled composition was linked to the mortality data for Flanders for the period 1997-2004 by KULeuven. Although, the modelling of daily variation in PM composition was done for whole Belgium, mortality data for a long period were only available for Flanders. We hope that mortality data of the Walloon area will become available in the course of the next two years of the project, so that we will be able to provide data for the whole country.

#### **Statistical analysis**

Database management and statistical analyses were done with SAS software (V.9.1). First, we tested for an interaction between modelled PM<sub>10</sub> (and measured PM<sub>10</sub>) and outdoor temperature (mean daily temperature above and below 16°C). Because of a significant interaction, we studied the dose-response relationship between the mass concentration of PM<sub>10</sub> and its modelled composition by the two temperature strata. The reason of testing these two specific strata was based by evidence from the literature of a U-shape mortality curve with outdoor temperature with the lowest mortality observed at a mean daily temperature of 16°C (Huynen, 2001). We identified covariates by a stepwise regression procedure with the *p*-values for variables to enter and to stay in the model set at 0.10. Covariates considered for entry in the model were ammonia, carbon, nitric acid, sodium, secondary organic aerosols and sulphuric acid while PM<sub>2.5</sub> mass concentration modelled or measured PM<sub>10</sub> were forced into the regression model. We additionally tested whether the addition of a squared term of temperature within the temperature strata significantly added to the prediction of mortality.

#### **Results**

The total number of non-traumatic deaths during the 7-year period was 354 357. Daily mean outdoor temperature and modelled PM<sub>10</sub> concentrations averaged 11.7°C (standard deviation (SD) 6.1) and 19.9 µg/m<sup>3</sup> (SD 11.7), respectively. There was an interaction between modelled PM<sub>10</sub> on days above and below an average daily temperature of 16°C (*p*<0.001). The distribution of the modelled composition differed also between the ambient temperature strata (table 2).

**Table 2: Modelled composition of PM<sub>10</sub> according to temperature strata**

		Mean (SD), µg/m <sup>3</sup>	P value
Sodium	< 16°	1.32 (1.73)	< 0.0001
	> 16°	0.52 (0.58)	
Secondary organic aerosols	< 16°	0.67 (0.98)	< 0.0001
	> 16°	2.28 (2.02)	
Nitric Acid	< 16°	6.02 (5.61)	< 0.0001
	> 16°	2.66 (3.02)	
Ammonia	< 16°	2.93 (2.08)	< 0.0001
	> 16°	2.12 (1.28)	
Carbon	< 16°	0.57 (0.41)	< 0.0001
	> 16°	0.66 (0.34)	
Sulphuric acid	< 16°	3.55 (2.28)	0.0625
	> 16°	3.73 (1.72)	
PM <sub>10</sub>	< 16°	13.36 (9.59)	< 0.0001
	> 16°	11.78 (6.63)	

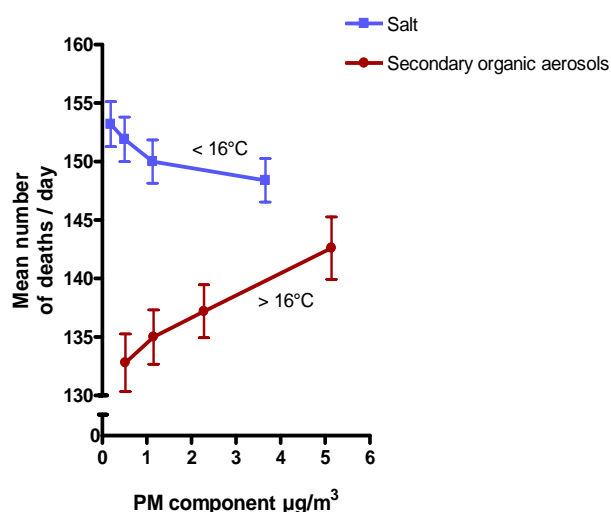


Figure 1: Total daily non-traumatic mortality means (95% CI) plotted against quartiles of PM constituents stratified according to outdoor temperature (< and > 16°C)

Both before (Figure 1) and after (Table 3) cumulative adjustment for temperature and mass PM<sub>10</sub>, secondary organic aerosols were positively associated with mortality on days above 16°C while the mass concentration of sodium was inversely associated with mortality on days below 16°C. The other studied components did not enter the regression models.

Independent of temperature and PM<sub>10</sub> mass concentrations, a 1.6 µg/m<sup>3</sup> (IQR-difference) higher sodium concentration on PM<sub>10</sub> was associated with a 0.8 % decrease in mortality during days with a temperature below 16°C. The corresponding analysis, on days with an average temperature above 16°C showed that a 3.2 µg/m<sup>3</sup> (IQR-difference) increase in secondary organic aerosols is associated with 3.4% increase in mortality, Table 3.

Table 3: Differences in daily all cause mortality (%) per IQR of concentrations or temperature

	Estimates*	95% CI	P
≥ 16°C			
PM <sub>10</sub> , + 14 µg/m <sup>3</sup>	+ 3.4%	0.1 to 9.0	0.043
Sec. org. aerosols, + 3.2 µg/m <sup>3</sup>	+ 3.4	0.6 to 8.8	0.025
Temperature, + 3.2°C	+ 1.4%	-0.2 to 4.2	0.075
< 16°C			
PM <sub>10</sub> , + 14 µg/m <sup>3</sup>	+ 0.8%	-1.0 to 3.4	0.29
Sodium, + 1.6 µg/m <sup>3</sup>	- 0.8 %	-2.0 to -0.2	0.014
Temperature, + 6.4°C†	- 4.9	-11.3 to -3.5	0.0002

\*estimates calculated for a IQR difference, †including both a linear and quadratic term. Significant covariates were selected by stepwise regression analysis in which PM<sub>2.5</sub> was forced to the model.

In this time-series analysis of modelled PM concentrations of several constituents, we showed that secondary organic aerosols were the most potent trigger for mortality during warm days (> 16°C) while with an average temperature below 16°C there is a marginal decrease in mortality on days with higher fraction of sodium on PM<sub>10</sub>. These effects could not be explained by differences in temperature or total mass. These novel results will merit future research on acute effects and composition of PM on mortality. The use of regression coefficients based only on PM<sub>10</sub> mass constituents only may underestimate the effects of some its components. KMI and KULeuven will extend the analysis with PM<sub>10</sub> modelled components and will include mortality data from the Walloon region when they will become available.

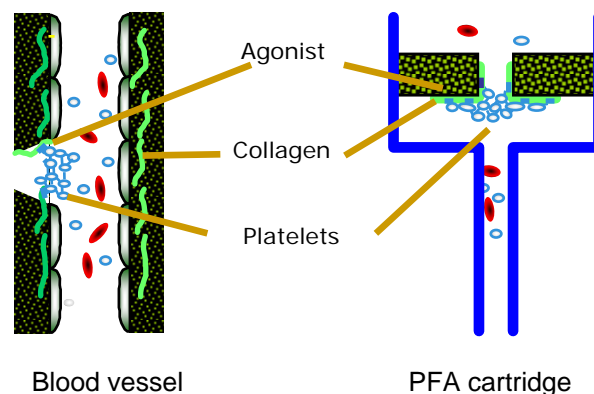
## Study 2: Acute health effects of particulate air pollution in elderly [KULeuven, UCLouvain, UGent, UAntwerpen, KMI/IRM, IRCEL]

In a cohort of elderly, KULeuven measured cardiovascular and respiratory parameters in the same person within the day and across seasons and evaluate their relationship with both physical properties and specific inorganic and organic components associated with particulates. This specific experimental design allows us to study the particulate induced effects, in association with ozone peaks, independently of the direct meteorological effects.

So far, KULeuven organised a field laboratory in two of the selected service flats (Cavell, Antwerp and Czagani, Broechem). We measured the participants' sitting blood pressure, platelet function (PFA-100 closure time), and total number of platelets at

baseline and at a follow-up clinical visit 1 to 2 week(s) apart. At the two clinical visits blood was taken and immediately deep-frozen for future analyses and exhaled breath condensate was collected.

Platelet function was measured with the platelet function analyzer (PFA-100). The PFA-100 test cartridge consists of a capillary, a blood sample reservoir, and a membrane coated with collagen/epinephrine with a central opening (Figure 2). Blood is aspirated through the capillary and flows through the opening in the collagen-coated membrane thus exposing platelets to high shear rates, causing platelet activation. A platelet thrombus forms at the opening, thus gradually diminishing and finally arresting blood flow. The time from the start of aspiration until the opening completely closes, i.e., the closure time, reflects platelet aggregation in a shear stress-dependent way (Nemmar 2003).



**Figure 2: Principle of the PFA-100. Platelet activation is measured as the closure time (CT). This is the time from the start of blood flow until the platelets form a thrombus and blood flow stops ex-vivo.**

KULeuven gathered information on smoking, drinking, social class, energy spent in physical activity and use of medication by the use of a standardized questionnaire.

Air pollution measurements included collecting TSP, PM<sub>2.5</sub> and PM<sub>1</sub> on polycarbonate filters with low volume samplers UA and PM<sub>10</sub> collection with high volume samplers UGent for analysis of oxy-PAHs (for details see previous chapter on implementation of the methodology).

### Statistical analysis

We performed pollutant-specific, exposure-response analysis using mixed models (Verbeke and Molenberghs 2000) as implemented in the SAS system. A random effect for individual participants accounted for similarities across the two clinical examinations for each person. In other words this method allows each subject to serve as his or her control over time and controls for potential confounding from between-subject covariates that do not change over time. Furthermore, associations can be studied in a relatively small sample size.

By use of such models we regressed the percent change from baseline in each measurement of platelet function against pollutant concentration averaged over each exposure session (1 hour, 5, 12, 24, 36 and 48 hours before blood draw) within each person.

## Preliminary results of the study in elderly

### I. Characteristics of the population

The study population consisted mainly of women. Mean age of the participants was 83 years (range 68 to 91 years). Medication variables were stable during the two to three week follow-up. Table 4 summarizes their characteristics.

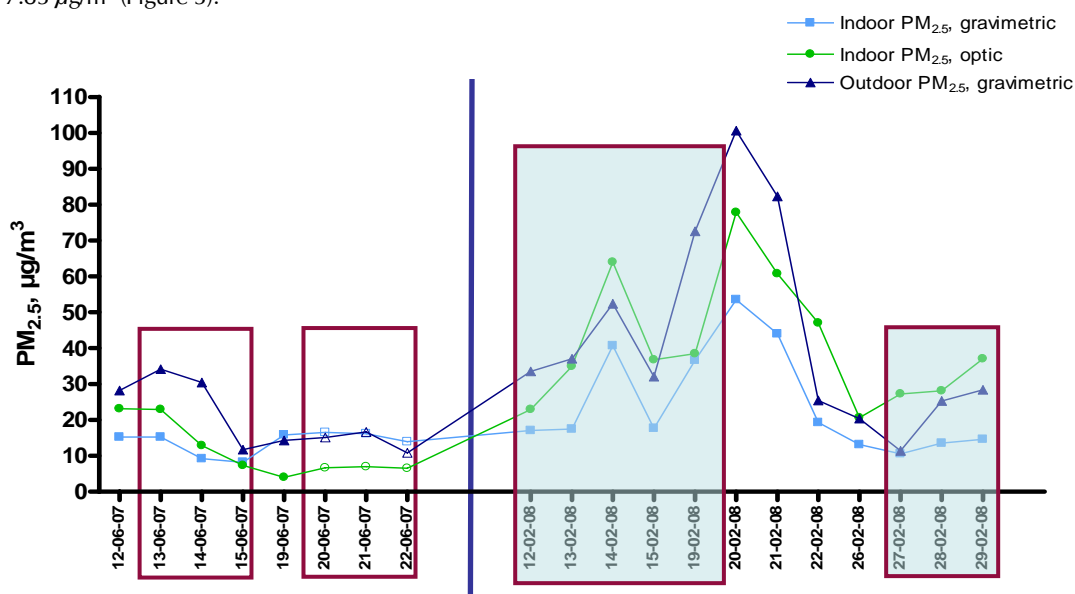
**Table 4: Subject characteristics (at baseline)**

Subjects' characteristics* (n = 39)	Mean or %	SD
Age, years	82.8	6.3
Women, %	77 %	
BMI, kg/m <sup>3</sup>	27.6	4.8
Exhaled NO, ppb	27	16.7
PFA closure time, sec	146.3	60.7
Systolic blood pressure, mmHg	140.6	21.4
Diastolic blood pressure, mmHg	75.3	14.7
Clara cell protein (CC-16), $\mu\text{g/l}$	13.3	6.1
Surfactant associated protein D (SPD), $\mu\text{g/l}$	138.1	78.5

## II. Exposure levels

### Total mass concentration

The 24 hour outdoor PM<sub>2.5</sub> concentration averaged 34.14 (SD: 24.72) and ranged from 10.85 to 100.6  $\mu\text{g/m}^3$  using the gravimetric methods at the site of the study. The 24 hour PM<sub>2.5</sub> concentration averaged 29.31 (SD: 20.79) and ranged from 4.05 to 77.83  $\mu\text{g/m}^3$  (Figure 3).

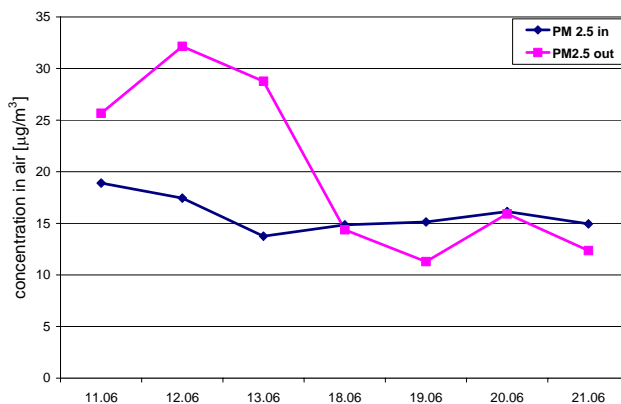


**Figure 3: Outdoor 24 hour averages of PM<sub>2.5</sub> concentrations over the study period**

### Determination of the mass concentration of particulate matter

**a) Cavell (June 2007)**

TSP outdoor varied from 16 to 34  $\mu\text{g}/\text{m}^3$  during the first week and 17 to 25  $\mu\text{g}/\text{m}^3$  during the second week. The indoor TSP concentrations were in the range 13-21  $\mu\text{g}/\text{m}^3$  and 17-18  $\mu\text{g}/\text{m}^3$  for the first and second week, respectively.

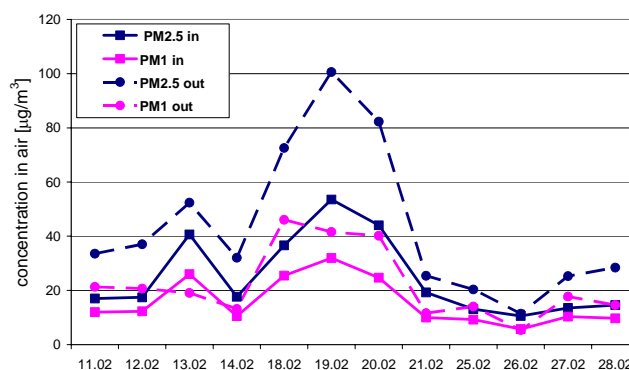


**Figure 4: Daily mass concentration of particulate matter PM2.5 during the sampling period**

PM2.5 outdoor concentrations ranged from 26 to 32  $\mu\text{g}/\text{m}^3$  during the first week and 11 to 16  $\mu\text{g}/\text{m}^3$  during the second; whilst indoor ones were in the range of 9-19  $\mu\text{g}/\text{m}^3$  and 15-16  $\mu\text{g}/\text{m}^3$  for the first and second week, respectively (Figure 4).

**b) Czagani (February 2008)**

During the first week of sampling, the outdoor concentration of PM1 and PM2.5 did not exceed the value of 26  $\mu\text{g}/\text{m}^3$  and 53  $\mu\text{g}/\text{m}^3$ , respectively. In the second week, however, the results were significantly higher with a value of 46  $\mu\text{g}/\text{m}^3$  for PM1 and 100  $\mu\text{g}/\text{m}^3$  for PM2.5, which reached already smog concentrations.



**Figure 5: Daily mass concentration of particulate matter PM2.5 during the sampling period**

The maximum PM concentration was observed on 19<sup>th</sup> February (the peak day). It should be however stressed that in the second week the level of 50  $\mu\text{g}/\text{m}^3$  of PM2.5 was exceeded indoors. The contribution of PM1 in PM2.5 fraction ranged from 60% to 70% and was significantly higher during the first week. During the third week the PM concentration in both fractions diminished fairly and did not exceed 30  $\mu\text{g}/\text{m}^3$  (limit value for PM2.5 outside)

**Determination of gaseous organic and inorganic pollutants**

**a) Cavell**

Gaseous pollutants, both inorganic ( $\text{SO}_2$ ,  $\text{NO}_2$ ,  $\text{O}_3$ ) and organic (BTEX, i.e. benzene, toluene, ethylbenzene and xylenes), were collected.

In case of inorganic pollutants,  $\text{NO}_2$  content was ca. 10-fold higher than  $\text{SO}_2$ , and this ratio was observed both indoors and outdoors during the whole sampling campaign.



The elevated content of toluene indoors ( $10 \mu\text{g}/\text{m}^3$  first week and  $12 \mu\text{g}/\text{m}^3$  second week, while outdoors it was 2 and  $3 \mu\text{g}/\text{m}^3$ , respectively) is a phenomenon reported in open literature (Cocheo et al., 2000; Ilgen et al., 2001). The ratio of toluene/benzene is very helpful in tracing the source of pollutants the value around 3 or lower proves the source of both BT as vehicle emission (petrol evaporation and traffic). If it increases above this value (and this is the case of the service flats indoor air), there must be an additional source of toluene (paints, wallpapers, furniture, etc). The results are presented in the form of graphs below (Figure 6).

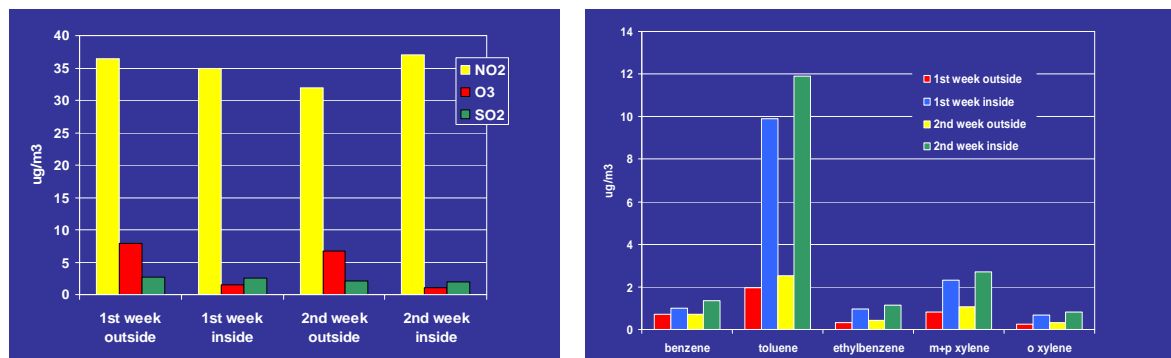


Figure 6: Concentration of gaseous pollutants indoors and outdoors during the sampling period

a) Czagani (February 2008)

Pollutants sampled in Czagani were generally of a higher concentration than the pollutants collected in Cavell. This is especially pronounced for outdoor ozone concentrations that reached  $27 \mu\text{g}/\text{m}^3$  and  $28 \mu\text{g}/\text{m}^3$  in the second and third week of sampling, respectively. However the indoor concentration of ozone was very low, below the detection limit in the first and second week.  $\text{SO}_2$  concentration never exceeded the value of  $5 \mu\text{g}/\text{m}^3$ . The content of  $\text{NO}_2$  in the air was slightly higher reaching the value of  $43 \mu\text{g}/\text{m}^3$ .

Benzene concentration approximated the limit value of  $5 \mu\text{g}/\text{m}^3$  in the first and the second week of sampling. Toluene concentrations were close to those of benzene, and except for the third week, no significant difference was noticed between indoor and outdoor concentration. This is most probably to the fact of dilution of the indoor sources input, as the hall in Czagani had increased outdoor air influence compared to Cavell. The results are shown in the graphs below (Figure 7).

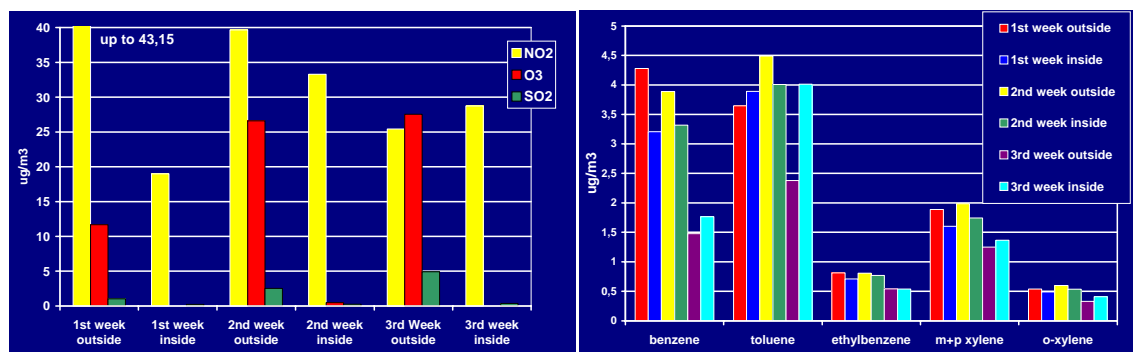


Figure 7: Concentration of gaseous pollutants indoors and outdoors during the sampling period

Determination of the water soluble fraction of particulate matter

b) Cavell

During the first week of sampling, the content of sulphates increased to a large extent in comparison to the other anions. It varied from 2 to  $10 \mu\text{g}/\text{m}^3$  both indoors and outdoors. Nitrates reached a maximum of  $4 \mu\text{g}/\text{m}^3$  outside ( $0.3 \mu\text{g}/\text{m}^3$  inside). Their content in TSP outside and inside differed by one order of magnitude, while in  $\text{PM}_{2.5}$  the difference was less significant. The chloride amount was in most cases at a very low level, reaching the highest value of  $0.04 \mu\text{g}/\text{m}^3$  in TSP outside.

Among cations, only the ammonium concentration was considerably high, varying from 0.4 to  $3 \mu\text{g}/\text{m}^3$  and no special pattern of changes between outdoor and indoor was found, both in TSP and  $\text{PM}_{2.5}$ . The other cations were present in low amounts, whereas Ca was most concentrated (max.  $0.9 \mu\text{g}/\text{m}^3$ ) and Mg – the least (max.  $0.06 \mu\text{g}/\text{m}^3$ ).

The second week of sampling was characterized by lower sulphate levels, ranging from 2 to  $4 \mu\text{g}/\text{m}^3$  and both inside and

outside contents were comparable. Nitrates were present at a comparable level as previously ( $0.1\text{-}3\ \mu\text{g}/\text{m}^3$ ) with also an analogous indoor/outdoor ratio. Chlorides, similarly to the previous week, were negligible. The ammonium level was decreased and did not exceed  $0.8\ \mu\text{g}/\text{m}^3$ . The concentration of the other cations didn't differ much from their values of the first week, although Mg level was somewhat higher ( $0.1\ \mu\text{g}/\text{m}^3$ ).

### c) Czagani

During winter period (February 2008) the composition of the water soluble fraction was fairly dominated by nitrates. It refers to outdoor PM in both size ranges (Figures 8 and 9). However, the sulphate level was elevated according to the general PM fluctuation (Figure 5) reaching the maximum on the peak day (both in PM1 and in PM2.5). Chloride concentrations were nearly negligible in both PM size fractions, although slightly higher in PM1 indoors.

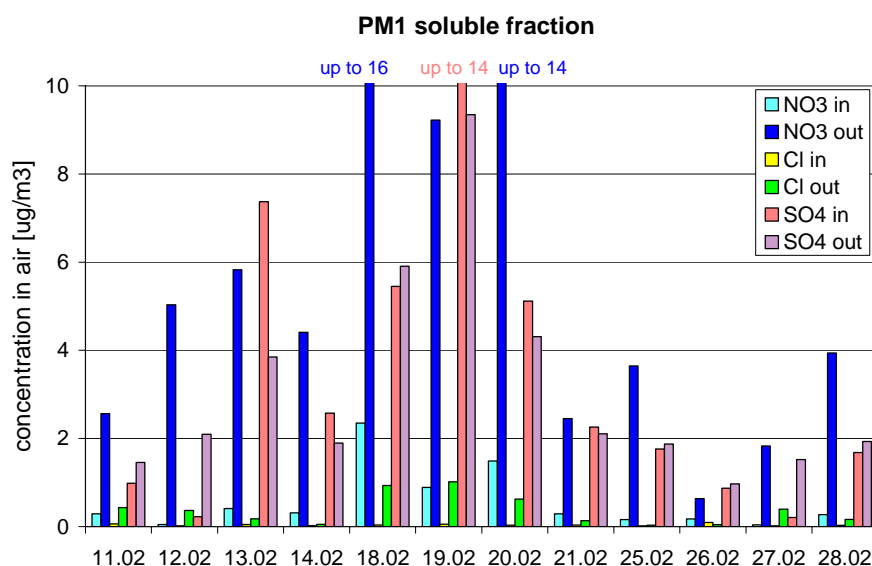


Figure 8: Concentration of water soluble ions in PM1 (indoors and outdoors) during the sampling period

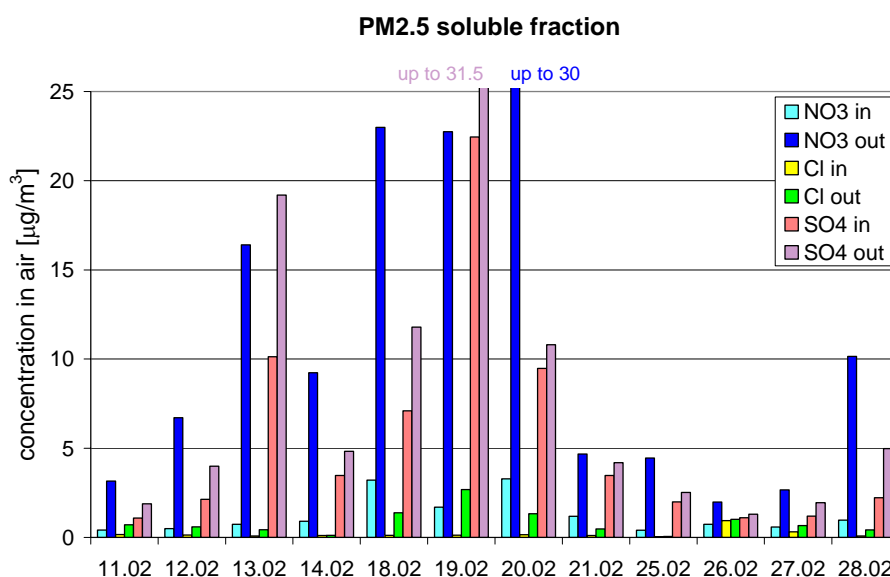


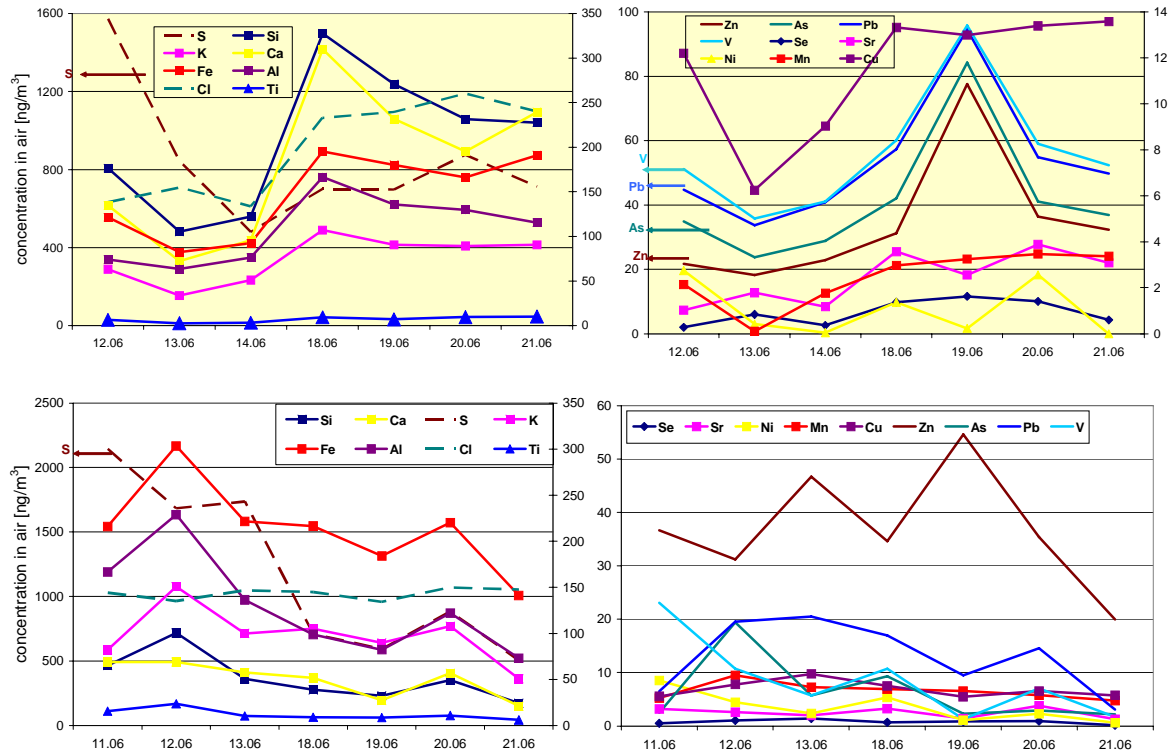
Figure 9: Concentration of water soluble ions in PM2.5 (indoors and outdoors) during the sampling period

### Determination of the total elemental composition in the bulk samples

#### a) Cavell

Concentrations of both light (from Z=12) to heavy (to Z=82) elements were measured. Among light elements, the highest contents were found for S, Si and Ca – both outside and inside the service flats. Fe and Al levels were comparable, pointing a crust source of these elements. In case of hazardous elements, As level was elevated (one

episode) up to 20 ng/m<sup>3</sup> inside and 17 ng/m<sup>3</sup> outside (the same day). Generally, the level of As was in the range of 2-11 ng/m<sup>3</sup> indoors and 2-10 ng/m<sup>3</sup> outdoors; both outside and inside levels were comparable. The Pb content was always higher in the outdoor environment, reaching up to 30 ng/m<sup>3</sup>. The difference in concentration was more pronounced, being often twice higher at the outside than inside the building. Cd content was always below the detection limit.



**Figure 10: Daily fluctuation of PM2.5 elemental concentrations indoors (upper) and outdoors (lower) during the sampling period**

The pictures to the left (Figure 10) show the contribution of naturally originated elements. Sulphur concentration is assigned to the primary axis; all the other elements are assigned to the secondary axis. All elements, which are of crust origin follow the same pattern of time distribution. There are two different sources of sulphur and chlorine, although only sulphur level is correlated with elevated PM2.5 concentration. The plot to the right presents the time distribution of elements of probable anthropogenic origin. The four elements: V, Pb, Zn and As gained maximum concentration during the second week

The sulphur content outdoors is changing similarly to the general PM2.5 concentration, unlike chlorine which is justified considering their different origin. The so-called anthropogenic elements (heavy elements) outdoors have various patterns of time fluctuations than indoors.

### b) Czagani

Figures 11 and 12 present daily variation of the elements concentration during the whole sampling period (three weeks). The left sided plots refer to light elements, of natural origin, whilst the right sided ones show the variation of heavier elements, which are attributed mainly to anthropogenic sources.

The elemental concentrations reflect the changes of PM mass concentrations, which is especially pronounced for sulphur, which is also the most abundant element in both size fractions (up to 3 115 ng/m<sup>3</sup> on 19<sup>th</sup> February in PM1 indoors, while 2015 ng/m<sup>3</sup> outdoors). The other elements presented in the picture below (Figure 11, to the left) had elevated concentrations during the dust peak. Among toxic (heavy) elements (see the picture below to the right), zinc reached the exceptionally high level (90 ng/m<sup>3</sup>) in the outdoor environment. Moreover, the same pattern of daily fluctuations is observed for Pb, Cu and Mn, although not as much concentrated (for Pb it is 32 and 52 ng/m<sup>3</sup> indoors and outdoors, respectively).

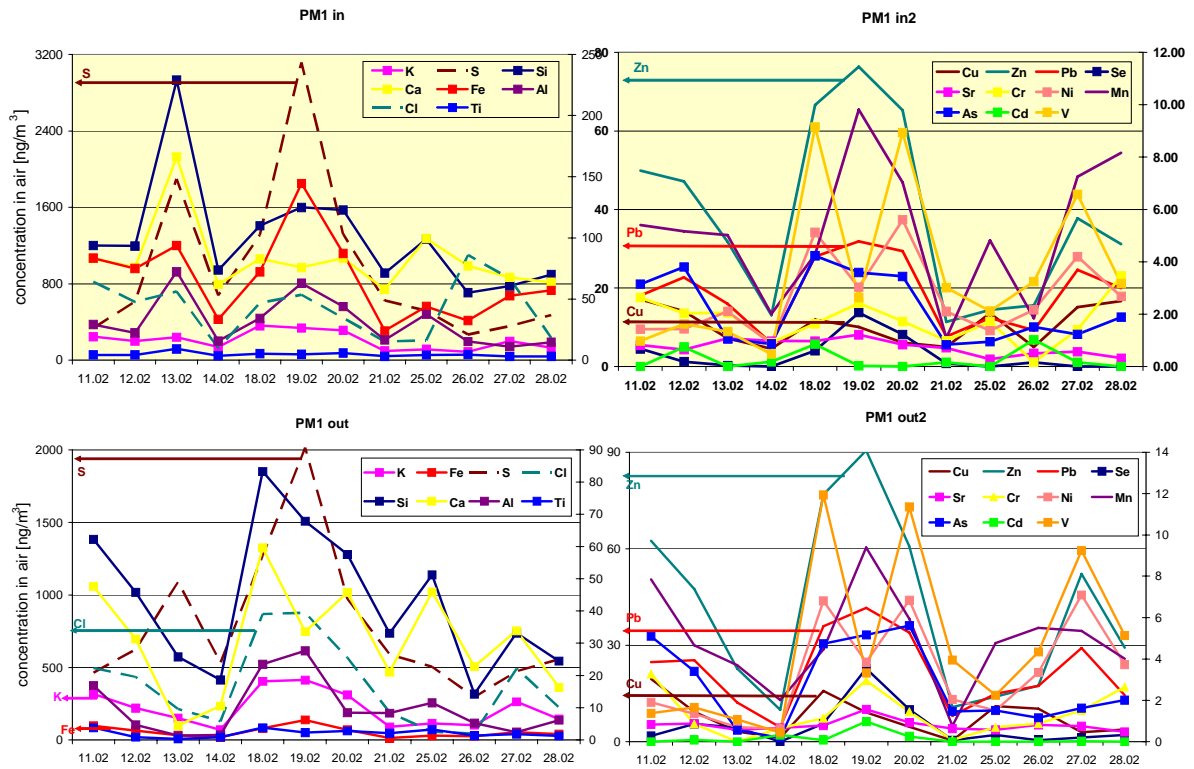


Figure 11: Daily fluctuation of elemental concentrations of PM1 during the sampling period

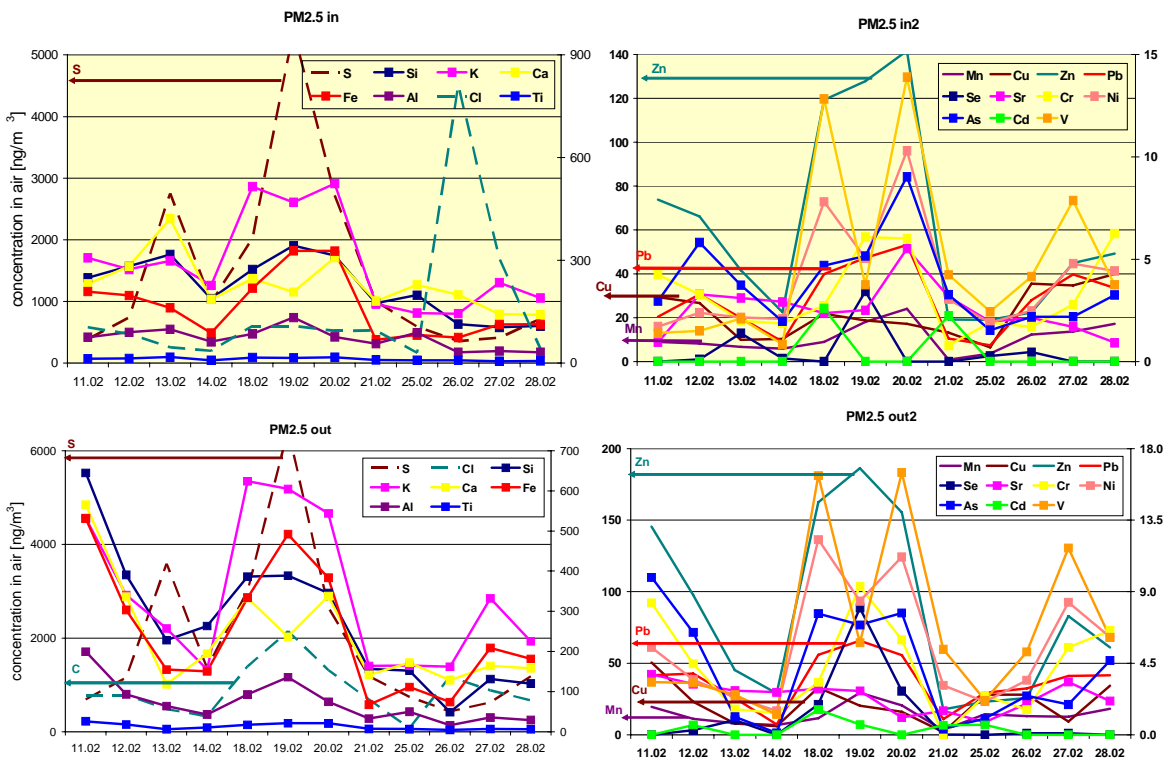


Figure 12: Daily fluctuation of elemental concentrations of PM2.5 during the sampling period

There is also a correlation between the concentration of Ni and V, both inside and outside. These two elements show actually a minimum in concentration on the peak day. Arsenic amount did not correlate with any other element and also didn't reach extremely high concentration.

In the PM2.5 fraction, the sulphur concentration was also the highest on the dust peak day (19<sup>th</sup> February), reaching 5400 ng/m<sup>3</sup> indoors and 6450 ng/m<sup>3</sup> outdoors). Chlorine level varied differently, because its concentration was exceptionally high

during the third week (26<sup>th</sup> February) on the day of minimum PM concentration. The heavy (toxic) elements reached also elevated levels during the peak day, and again, four of them (Zn, Pb, Cu and Mn) follow the same pattern of temporal changes (common source possible). This concerns both the outdoor and indoor environment. The arsenic amounts both indoors (12<sup>th</sup> and 20<sup>th</sup> February) and outdoors (11<sup>th</sup>, 12<sup>th</sup>, 18<sup>th</sup>, 19<sup>th</sup> and 20<sup>th</sup> February) exceeded the level of 6 ng/m<sup>3</sup> which is a threshold value for ambient air according the fourth daughter directive (2004/107/EG). All other elements show similar variation patterns in PM2.5 as it was in PM1.

### Determination of the elemental carbon (EC) concentration

#### Czagani

The concentration of elemental carbon was measured continuously during the whole sampling period (11-29 February 2008) both outdoors and indoors, together with the concentration of UVPM (expressed in units of "BC Equivalent"). The time interval was 5 minutes. The values are the arithmetic mean from these periods of time.

The highest concentration noted was equal to 27 µg/m<sup>3</sup> (20<sup>th</sup> February at 20<sup>45</sup>). The concentration indoors reflected the outdoor concentration of EC, with a time-lag of 1-2 hours (Figures 13 and 14), what indicates clearly that the main source of indoor air EC concentration was the outdoor ambient air.

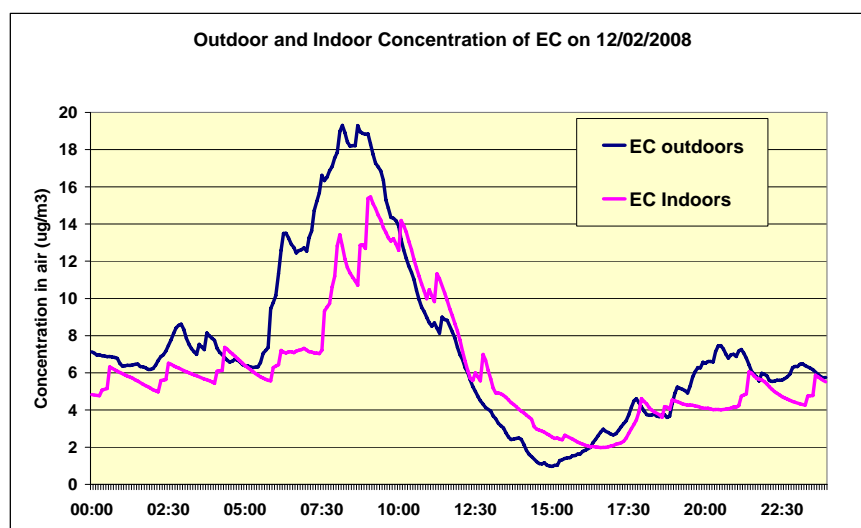


Figure 13: Outdoor and Indoor concentration of elemental carbon (EC) on the 12/02/2008

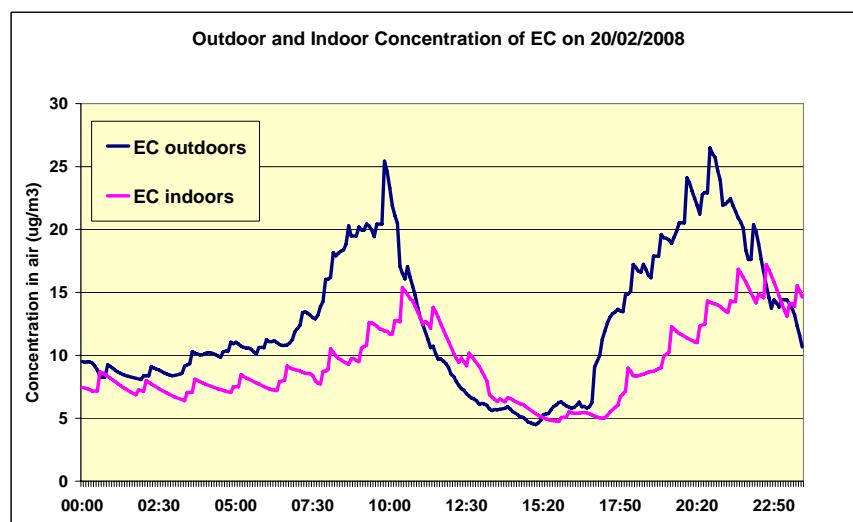


Figure 14: Outdoor and Indoor concentration of elemental carbon (EC) on the 20/02/2008

The only exception from this rule was on the day of 24 February (Figure 15) where a sharp peak occurs only indoors around midday, pointing out the indoor source of EC.

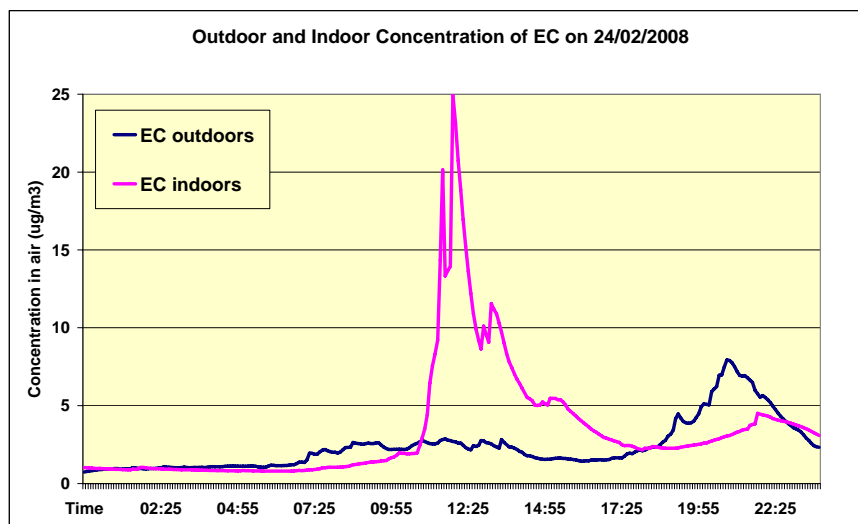


Figure 15: Outdoor and Indoor concentration of elemental carbon (EC) on the 24/02/2008

Generally the concentrations were elevated in the evening hours (Figures 14 and 15), except for only few days. This might be ascribed to either transport from urban centers (Allen et al., 1999) or a local influence, i.e. emission from the heating of nearby houses as the sampling was done in a winter period. Another fact that confirms the latter source on at least some of the days is a higher UVPM level than EC (frequently observed) as presented in Figure 16. It is related to additional emission of aromatic organic compounds from the same source.

Furthermore, the elevated concentrations can be seen during morning hours 7-10 a.m. (Figures 13, 14, 16), which can be a result of increased traffic and subsequently increased emissions (Allen et al., 1999). However this is not an as frequent phenomenon as the evening peak, since Czagani is located at a relatively remote location.

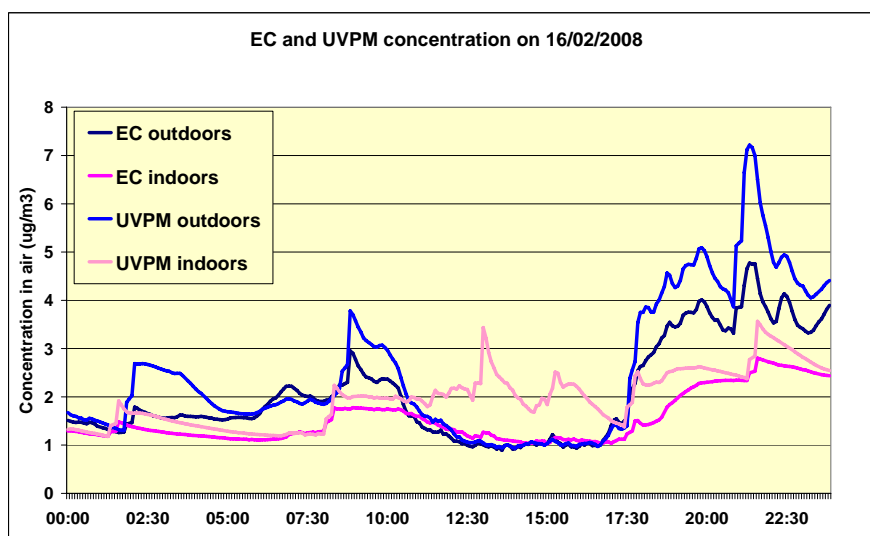


Figure 16: EC and UVPM concentration both indoors and outdoors on the day of 16/02/2008

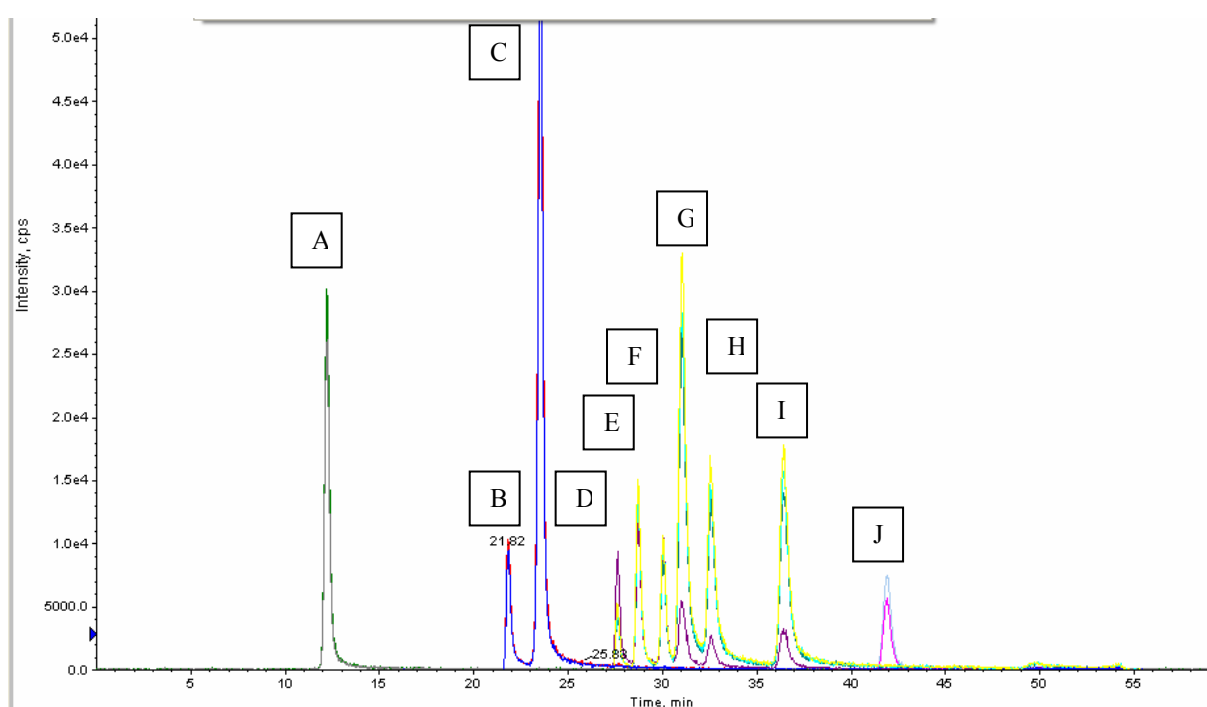
#### Analytical method development for identification and quantification of selected oxy-PAHs on PM (WP 2.2-2.4) (UGent)

##### (a) Analytical method for the oxy-PAHs to be separated by LC (see Table 1)

To evaluate the US extraction technique, recoveries of the selected oxy-PAHs from both glass and quartz fiber filters were determined (Table 6). The filters were spotted with 10  $\mu$ l of a standard solution of the target oxy-PAHs and analysed with the LC-MS-MS method. The same standard solution (10  $\mu$ l) was analysed after direct injection. The ratio of the peak intensities gives the matrix independent recovery. A typical chromatogram obtained from injection (10  $\mu$ l) of a standard solution of oxy-PAHs is given in Figure 17 and shows the good chromatographic resolving power.

**Table 6: Recoveries (%) for US extraction of oxy-PAHs from quartz (n = 4) and glass fiber**

	Quartz fiber filter	Glass fiber filter
Benzo[a]pyren-1,6-dione	70.59	64.27
Benzo[a]pyren-3,6-dione	73.53	64.08
Benzo[a]pyren-4,5-dione	69.11	68.48
Benzo[a]pyren-6,12-dione	69.89	<b>67.77</b>
Chrysen-5,6-dione	61.40	67.24
6-oxo-7-oxa-benzo[a]pyrene	77.00	73.08
Phenanthren-9,10-dione	69.20	62.54



**Figure 17: Separation and detection of selected oxy-PAHs by LC-MS-MS.**

(A) Phenanthren-9,10-dione; (B) Chrysen-1,2-dione; (C) Chrysen-5,6-dione; (D) Benzo[a]pyren-7,8-dione; (E) Benzo[a]pyren-4,5-dione; (F) Benzo[a]pyren-11,12-dione; (G) Benzo[a]pyren-1,6-dione; (H) Benzo[a]pyren-3,6-dione; (I) Benzo[a]pyren-6,12-dione; (J) 6-oxo-7-oxa-benzo[a]pyrene. The different lines represent the chromatograms according to the different MRM transitions for both the quantifier and qualifier ions.

**(b) Analytical method for the oxy-PAHs to be separated by GC (see Table 1)**

As is clear from Figure 18 representing a typical chromatogram obtained after the injection (1  $\mu$ l) of a standard solution of the selected oxy-PAHs (60-137 ng/ $\mu$ l) in dichloromethane, the developed GC-MS method enabled good separation and detection. Only 7H-benzo[de]anthracen-7-one en pyren-1-carboxaldehyde were not baseline separated and further research is necessary to improve their separation. Next, a conversion of phenanthren-9,10-dione to fluoren-9-one was observed during injection (270°C). This observation made it clear that the analysis of phenanthren-9,10-dione is not suitable for GC analysis. Therefore, this compound will be determined by LC-MS-MS in further analysis. Further research is also necessary to develop a sample preparation technique compatible with the GC-MS analytical method

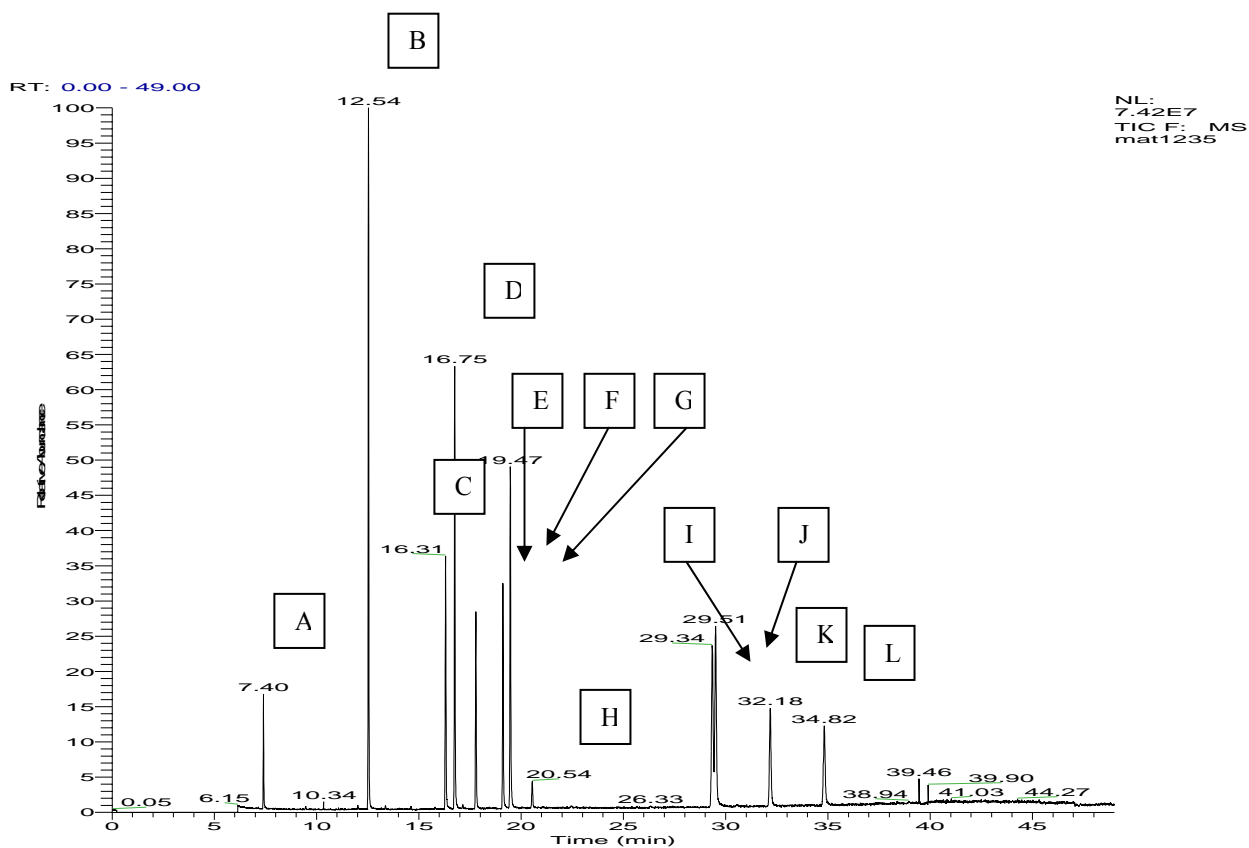


Figure 18: Separation and detection of selected oxy-PAHs by GC-MS.

(A) Naphthalen-1,4-dione; (B) 9*H*-Fluoren-9-one; (C) Fluoren-2-carboxaldehyde; (D) 9,10-anthraquinone; (E) 1,8-naphthalic anhydride; (F) 2-methylanthraquinone; (G) Phenanthren-9-carboxaldehyde; (H) phenanthren-9,10-dione; (I) 7*H*-benzo[de]anthracen-7-one; (J) pyren-1-carboxaldehyde; (K) Benz[a]anthracen-7,12-dione; (L) Naphacen-5,12-dione.

(c) Explorative results: concentrations of oxy-PAHs on PM<sub>10</sub> sampled at the service flats Cavell and Czagani (WP 2.5)

Although only foreseen for phase 2 of the project, the developed LC-MS-MS method has already been tested for a first time on the PM<sub>10</sub> samples collected during the sampling campaigns in the service flats Cavell and Czagani. Concentration ranges of the selected oxy-PAHs are represented in Tables 7 and 8. They are expressed in mass per volume of sampled air (pg/m<sup>3</sup>) and mass per mass of collected PM<sub>10</sub> (pg/mg); the latter being calculated with the PM<sub>10</sub> mass concentrations, ranging from 16 to 96 µg/m<sup>3</sup>.

Table 7: Oxy-PAHs concentrations sampled at the service flat Cavell (n = 16)

	Concentration in air (pg/m <sup>3</sup> )			Concentration on PM10 (pg/mg)		
	min	max	median	min	max	median
Benzo[a]pyren-1,6-dione	1.1	3.6	2.6	24.5	162.2	83.4
Benzo[a]pyren-3,6-dione	0.9	7.1	3.9	19.6	280.8	125.7
Benzo[a]pyren-6,12-dione	0.7	5.2	1.6	23.3	130.4	63.3
Chrysen-5,6-dione	0.9	2.5	2.1	18.7	97.4	67.1
Phenanthren-9,10-dione	20.6	120.5	73.2	441.6	5254.8	2591.5

Benzo[a]pyren-4,5-dione, Benzo[a]pyren-7,8-dione, Benzo[a]pyren-11,12-dione, Chrysen-1,2-dione and 6-oxo-7-oxa-benzo[a]pyrene were not detected in the samples of RVT Cavell.



**Table 8: Oxy-PAHs concentrations in PM<sub>10</sub> sampled at the service flat Czagani (n=36)**

	Concentration in air (pg/m <sup>3</sup> )			Concentration on PM10 (pg/mg)		
	min	max	median	min	max	median
Benzo[a]pyren-1,6-dione	17	91	37	667	2297	980
Benzo[a]pyren-3,6-dione	19	116	42	779	3024	1213
Benzo[a]pyren-4,5-dione	2	53	11	100	851	341
Benzo[a]pyren-6,12-dione	29	303	71	1162	5318	2310
Chrysen-5,6-dione	6	42	17	212	1871	451
6-oxo-7-oxa-benzo[a]pyrene	2	50	9	109	712	224
Phenanthren-9,10-dione	47	823	199	1179	22485	4683

Benzo[a]pyren-7,8-dione, Benzo[a]pyren-11,12-dione, Chrysen-1,2-dione were not detected in the samples of RVT Czagani.

### **VOCs concentrations in the ambient air of the service flats Cavell and Czagani**

Using active sampling and TD-GC-MS analysis, 69 VOCs were identified in indoor and outdoor air of the service flat Cavell. Seventeen compounds of which the standards were available were quantified. The concentration range of the quantified VOCs was between 1 and 400 µg/m<sup>3</sup>. In the ambient air of the service flat of Czagani, 27 VOCs were quantified. Their concentrations ranged between 0.15 and 1212 µg/m<sup>3</sup>. Using passive sampling at the same locations and TD-GC-MS analysis, 62 VOCs were identified. Quantification of these compounds is foreseen after determination and/or calculation of the uptake rates.

### **III. Health effects**

#### **Effect on platelet function**

At baseline, the closure time of the PFA-100 averaged 146.3 (SD: 60.7) seconds. Between two clinical examinations in the same person, a significant association emerged between the changes in indoor 12, 24, 36 and 48 averaged PM<sub>2.5</sub> levels and in closure time of PFA-100. This association was also found for outdoor PM<sub>2.5</sub> concentrations averaged over 24 and 48 hours. Figure 19 shows the data for those not receiving antiplatelet medication.

In mixed models accounting further for the number of platelets, treatment and outdoor temperature, a 10 µg/m<sup>3</sup> increase in the mean 24 hour outdoor PM<sub>2.5</sub> concentration was associated with a mean decrease of -17.3 seconds (95% confidence interval: -29.5 to -5.2; p=0.010) in the closure time of the PFA-100, while in the same model higher mass concentrations of mean 24 hour chloride concentration on the PM<sub>2.5</sub> fraction was associated with a mean increase of 0.76 seconds (95% confidence interval: 0.12 to 1.4 p=0.02). The other trace metals were not significantly (p>0.13) associated with the platelet function.

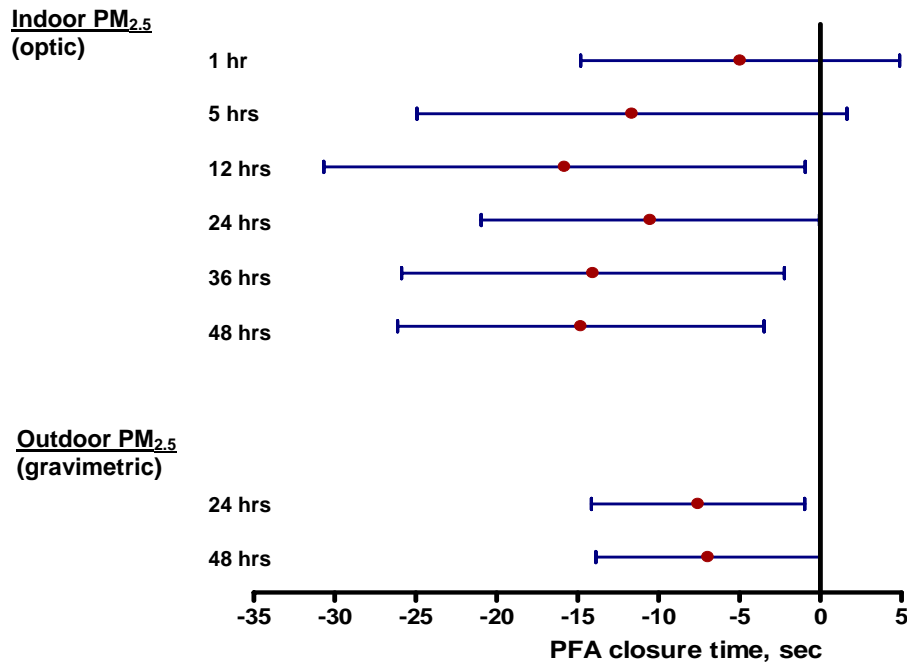


Figure 19: Point Estimates and 95% CI of the absolute change in platelet function (measured as closure time, sec) per 10  $\mu\text{g}/\text{m}^3$  increase in PM<sub>2.5</sub> indoor or outdoor. The inter quartile range of PM<sub>2.5</sub> for 24 hours averages indoor and outdoor were respectively 27.68 and 19.72  $\mu\text{g}/\text{m}^3$ . Hours indicate average concentration x hours before blood sampling.

#### Effects on blood pressure

Increases in systolic blood pressure (Figure 20) and pulse pressure were associated with PM<sub>2.5</sub> exposure at most time points while diastolic blood pressure was not changed.

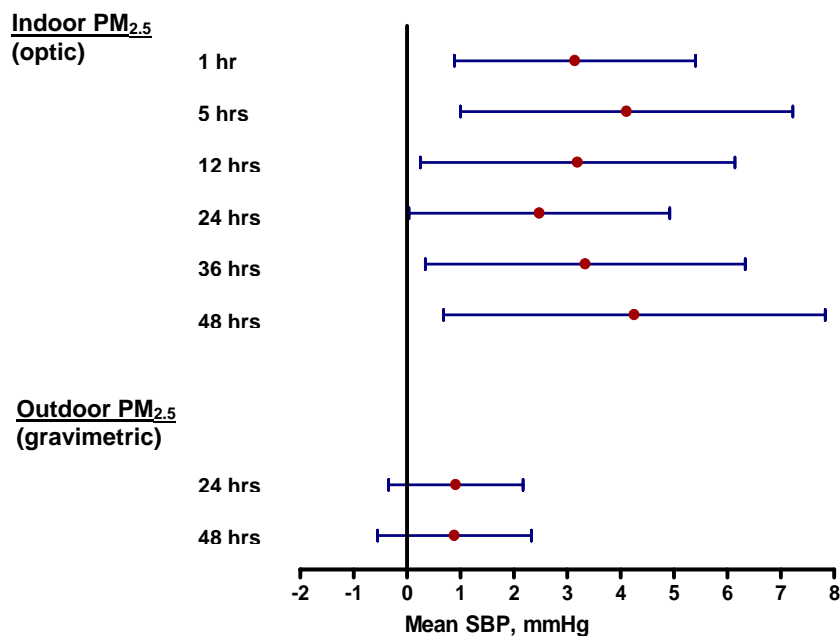


Figure 20: Point Estimates and 95% CI of the absolute change in systolic blood pressure (mmHg) per 10  $\mu\text{g}/\text{m}^3$  increase in PM<sub>2.5</sub> indoor or outdoor. The inter quartile range of PM<sub>2.5</sub> for 24 hours averages indoor and outdoor were respectively 27.68 and 19.72  $\mu\text{g}/\text{m}^3$ . Hours indicate average concentration x hours before blood sampling.

### Effects on exhaled NO

PM<sub>2.5</sub> exposure was associated with increases in exhaled NO, but only reached statistical significance with outdoor concentrations (Figure 21). All PAH species (24 hour average PM) correlated positively with exhaled NO independent of the PM mass concentration; a 36.8 pg/m<sup>3</sup> increase (inter quartile range increase) in 24 hour mean benzo(a)pyren1,6-dione was associated with a 7.0 ppb increase in exhaled NO.

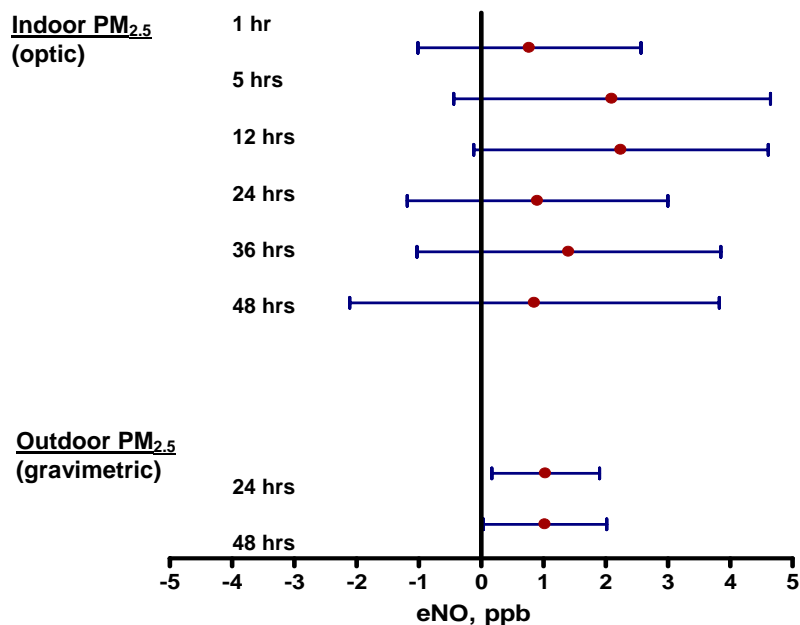


Figure 21: Point Estimates and 95% CI of the absolute change in exhaled NO (ppb) per 10 µg/m<sup>3</sup> increase in PM<sub>2.5</sub> indoor or outdoor. The interquartile range of PM<sub>2.5</sub> for 24 hours averages indoor and outdoor were respectively 27.7 and 19.7 µg/m<sup>3</sup>. Hours indicate average concentration x hours before blood sampling.

### Study 3: Acute health effects of particulate air pollution and ozone in children (UCLouvain, UGent, UAntwerpen, KMI/IRM and IRCEL)

The objective of this study was to assess the short-term effects of ambient air pollutants and their interactions on the health of children. All measurements are performed on summer days with elevated concentrations of ozone in ambient air or on winter days characterized by high concentrations of particulate matter.

During the summer of 2007, from the 16 scouting companies who had accepted to participate to the study, we could perform experiments only on three of them because of poor weather conditions (there was no ozone peak last summer). The three camps that were studied will thus be control groups. These camps were located at Waismes (July 06), Torgny (July 25) and Ramegnies-Chin (August 05).

During the winter of 2008, although 8 scouting companies had accepted to participate to second phase of the study for February and March, no campaign could be performed because of absence of pollution by particulate matter on the days the scouts were available.

### Preliminary results of the study in children

#### 1. Characteristics of the population

Almost all children are Caucasians. Mean age of the participants was 10.2 years (range: 7.5-12.5). Table 9 summarizes their characteristics.

**Table 9: Subjects characteristics**

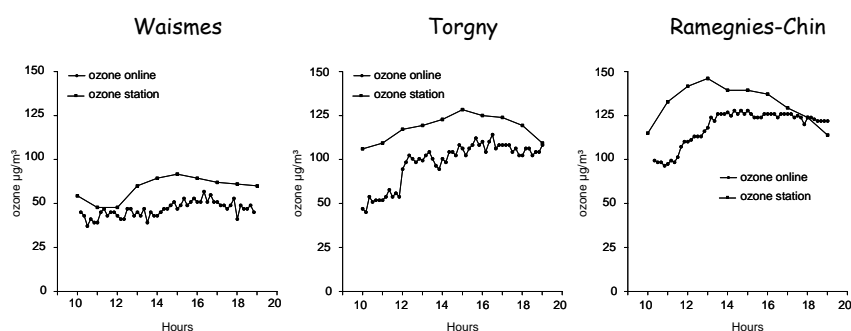
	Waismes	Torgny	Ramegnies-Chin
n	13	16	20
Sex (boys, %)	69.2	50	85
Caucasian (%)	92.3	100	100
Age (mean, years)	10.2	10.1	10.3
Height (mean, m)	1.40	1.41	1.41
Weight (mean, kg)	35.5	36.7	34.5
BMI (mean, kg/m <sup>2</sup> )	16.8	18.2	17.1
Variation of eNO (ppb)	-0.8	0.6	-2.6
Variation of FEV1 (%)	-0.7	-2.3	2.9

Parameters directly measured during the medical examination are also shown in table 9 and display no significant variations between the three groups of children.

## II. Exposure levels

### Ambient air pollutants monitoring summer 2007

During these three days, we measured online the ambient ozone concentration and we checked the measurements by comparison with the nearest IRCEL monitoring stations. As shown in Figure 22 the online measure was systematically lower (24 %) than the stations observations. This systematic difference persisted when comparing our measures with that of nearest German and French monitoring stations, indicating indeed that the online measurements of ozone will have to be corrected.



**Figure 22: Ambient ozone monitoring**

Figure 23 displays the ambient NO<sub>x</sub> measured by IRCEL stations and of PM concentrations measured online and by IRCEL stations. The concentrations of these pollutants are low during the three studied days.

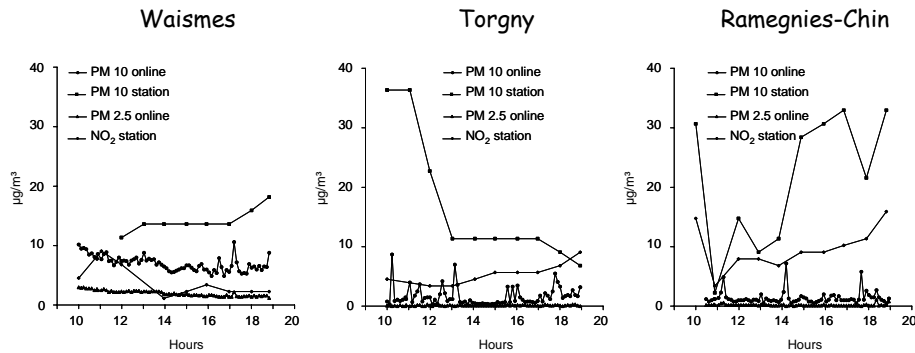


Figure 23: Ambient  $\text{NO}_x$  and PM monitoring

**Determination of the total mass concentration of particulate matter (UAntwerp)**

In the case of summer camps TSP concentrations were the following: 11, 10 and 37  $\mu\text{g}/\text{m}^3$  in Waimes-Malmedy, Torgny-Virton and Ramegnies-Chin, respectively.  $\text{PM}_{2.5}$  levels were 5-7, 6-7 and 26-30  $\mu\text{g}/\text{m}^3$  at the same sites.

**Determination of the inorganic water soluble fraction of particulate matter**

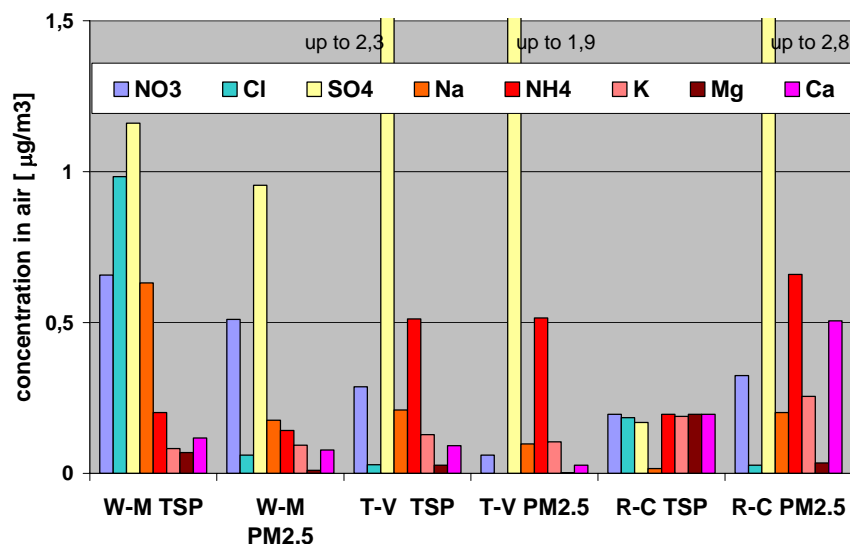


Figure 24: Concentration of water soluble ions in TSP and  $\text{PM}_{2.5}$  collected in the summer camp sites

The composition of water soluble fraction in TSP and  $\text{PM}_{2.5}$  collected in 3 sites of children summer camps is presented in Figure 24. Most ions were detected in low amounts, only sulphates were slightly elevated.

**Determination of the total elemental composition in the bulk samples (UAntwerp)**

Elemental composition of TSP and  $\text{PM}_{2.5}$  implied mostly crustal elements such as Si, K, Al, Ca, Fe. Sulphur was present at relatively high concentrations: 0.3, 0.5 and 0.7  $\mu\text{g}/\text{m}^3$  ( $\text{PM}_{2.5}$ ) in Waimes-Malmedy, Torgny-Virton and Ramegnies-Chin, respectively. Among hazardous elements, As level was relatively low (2-3  $\text{ng}/\text{m}^3$ ) at all sites, while the Pb amount varied significantly among the sites – in the first and second campaign, the Pb content did not exceed 3  $\text{ng}/\text{m}^3$ , but was elevated at the third site (15  $\text{ng}/\text{m}^3$  in TSP, 10  $\text{ng}/\text{m}^3$  in  $\text{PM}_{2.5}$ ).

### Determination of VOC (UGent)

During the sample campaigns in Waimes-Malmedy, Torgny-Virton and Ramegnies-Chin, VOCs in the outdoor air were sampled in an active way. Overall, 44 VOCs were detected and 9 compounds of which the standards were available were quantified, showing a concentration range between 0.2 to 18  $\mu\text{g}/\text{m}^3$ .

Results of a selected group of these compounds (benzene, toluene and propylbenzene) are given in Figure 20, and compared to the concentrations measured at the service flats RVT Cavell and Czagani.

In general, VOCs concentrations measured in the indoor air of the service flats are a factor 1 to 6 higher than those measured in the outdoor air around the same flats. Next, VOCs concentrations in the urban environment (outdoor) are 3 to 30 times higher compared to those measured in the rural environment (outdoor).

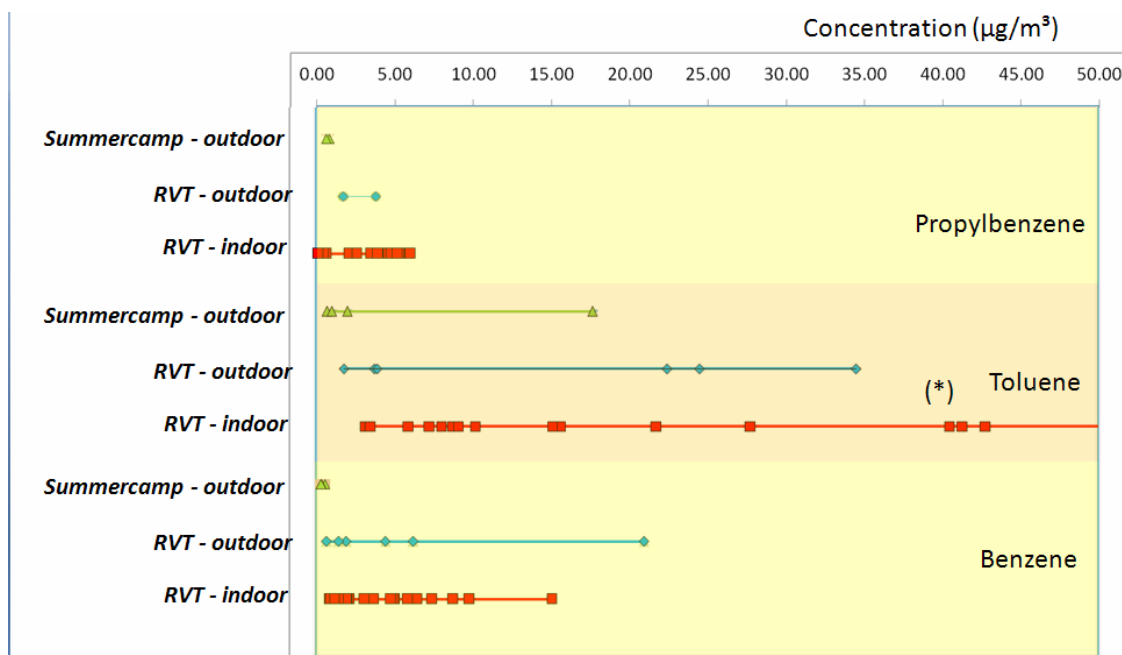


Figure 25: Concentrations of benzene, toluene and propylbenzene in the selected outdoor (summer camps and service flats) and indoor (service flats) environment (n= 48).

(\*) Toluene concentrations in the indoor air (service flat) are in the range from 3 to 318  $\mu\text{g}/\text{m}^3$ . For reasons of clarity, the scale of the concentration axis is limited to 50  $\mu\text{g}/\text{m}^3$ .

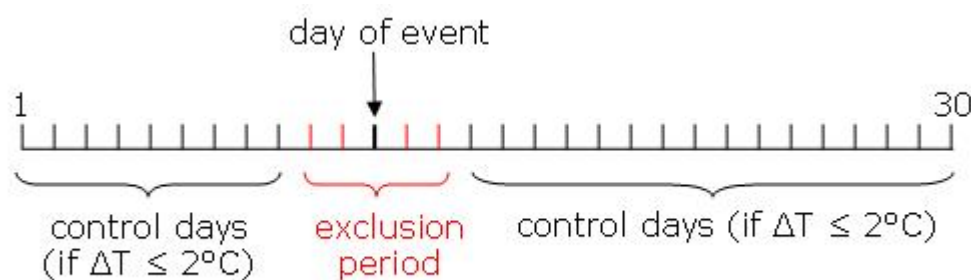
### **Study 4: Associations between infant mortality and air pollution in an affluent society (KULeuven)**

Remark: This analysis was not included in the initial proposal, but was suggested by the referees of the interim evaluation.

Recently, there has been growing concern about a possible link between exposure to air pollution and infant mortality. Infants may be particularly vulnerable to  $\text{PM}_{10}$ , since alveoli of human lungs mainly develop postnatally. In this study, we use the case-crossover design in order to investigate short term effects of elevated  $\text{PM}_{10}$  levels on infant mortality in Flanders.

Data on mortality in infants (< 1 year of age, N = 1655) and daily temperatures were collected during the period 1998-2003. Exposure to ambient PM was determined as daily average  $\text{PM}_{10}$  concentration at the infant's residence (municipality level), obtained by a land use interpolation model (Janssen et al. 2008)

In the case-crossover design, each subject serves as its own control. Control days for each case were generated by applying a time-stratified design in order to minimise statistical bias (Janes et al. 2005), and they were matched on temperature in order to control for confounding by variation in daily temperature (Figure 26). Conditional logistic regression was used for parameter estimation and calculation of odds ratios and confidence intervals (CI).



**Figure 26: Time-stratified case-crossover design. Control days were taken from the same month as the event day, with a  $\pm 2$  days exclusion period in order to correct for short term autocorrelation and a match on temperature between event and control.**

## Preliminary results of the infant mortality study

### I. Exploratory results

We collected 1655 infant mortality cases in the period 1998-2003. This corresponds with a mortality rate of 4.51 per 1000 births and a portion of 4.82 infant deaths per 1000 deaths in total. Statistics on most prominent causes of death and age class are provided in Table 10. Traumatic events (N = 71) were excluded from further analyses.

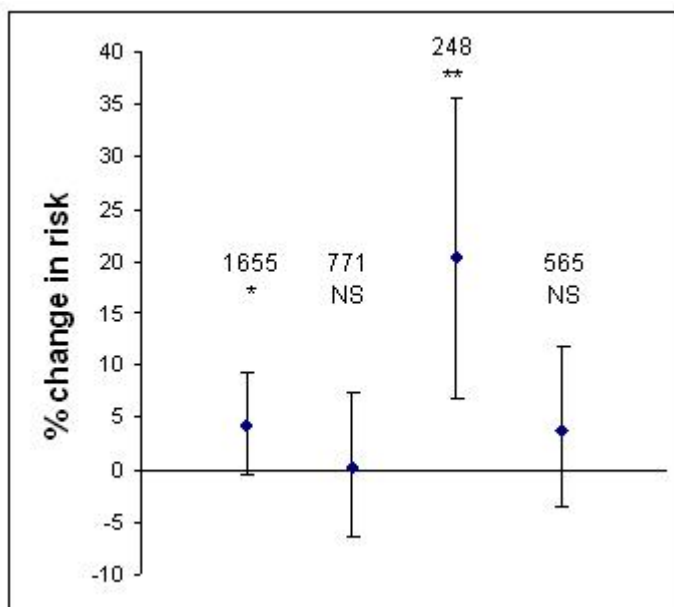
Mean daily average PM<sub>10</sub> concentration during the study period, for each day averaged along all measurement stations, was 32.2  $\mu\text{g}/\text{m}^3$ . The median was 29.0  $\mu\text{g}/\text{m}^3$  (IQR 22.5 – 38.5  $\mu\text{g}/\text{m}^3$ )

**Table 10. Most prominent causes of death in neonates in Flanders 1998-2003, by age class.**

Cause of death with ICD code	Early neonatal ( $\leq 1$ week of age)	Late neonatal (2–4 weeks of age)	Postneonatal (2–12 months of age)	Total
Perinatal circumstances (P00 – P96)	480	121	83	684
Congenital and chromosomal abnormalities (Q00 – Q99)	270	106	143	519
SIDS (R95)	0	7	194	201
Traumatic events (V00 – Y98)	6	1	64	71
Others	21	14	145	180
<b>Total</b>	<b>777</b>	<b>249</b>	<b>629</b>	<b>1655</b>

### II. Results of case-crossover analyses

Overall, we established a trend towards elevated infant mortality associated with higher levels of PM<sub>10</sub>. A 10  $\mu\text{g}/\text{m}^3$  in mean daily PM<sub>10</sub> increase was associated with a 4.3% increase (95% CI: -0.3 to 9.2%, P = 0.068) in the risk of death. The association between infant mortality and daily air pollution was most striking and highly significant in neonates from 2 to 4 weeks of age (N = 248). The corresponding increase in risk of death per 10  $\mu\text{g}/\text{m}^3$  increase in PM<sub>10</sub> concentration was 20.4% (95% CI 6.8 – 35.7%, P = 0.002). In other age categories, no significant relationship between PM<sub>10</sub> and daily mortality was found ( $\leq 1$  week of age: N = 771, 0.3% increase in risk of death per 10  $\mu\text{g}/\text{m}^3$  increase in PM<sub>10</sub>, 95% CI -6.3 to 7.3%, P = 0.93; > 4 weeks of age: N = 565, 3.8% increase in risk of death per 10  $\mu\text{g}/\text{m}^3$  increase in PM<sub>10</sub>, 95% CI -3.5 to 11.7%, P = 0.31) (Figure 27). Exclusion from analysis of cases with congenital or perinatal causes of death (3/4 of all cases) led to a loss of power but did not substantially alter results, neither overall or by age class.



**Figure 27: % change in risk of death per 10  $\mu\text{g}/\text{m}^3$  increase of  $\text{PM}_{10}$  concentration. Numbers indicate sample size. NS non-significant at  $\alpha=0.1$ ; \*  $P<0.1$ ; \*\*  $P<0.01$**

In addition, we conducted analyses with  $\text{PM}_{10}$  concentrations at lag day 1 to lag day 3 in order to detect a possible short term delay in the effects of exposure on infant mortality. However, we made a mistake in programming the SAS macro which generates temperature-matched control days for each case. At present, the macros are being rerun and the corrected results are not available yet.



#### 4. PRELIMINARY CONCLUSIONS AND RECOMMENDATIONS

We showed by combining 7-year mortality data with modeled PM<sub>10</sub> constituents lower mortality on colder days with higher PM sodium concentrations, while on warmer days the most important identified component was secondary organic aerosols. The associations were independent of outdoor temperature. Further analysis with use of PM<sub>10</sub> modeled constituents should be looked into to see whether our data are consistent. Due to a lack of computerized mortality data for the French speaking area of Belgium we had to restrict our analysis to Flanders. Computerized daily mortality data will become available during the next study phase. Combining regions will lead to different compositions and a higher contrast which will increase the power of our database.

Daily variation in measured PM constituents both metals, carbon and oxy-PAHs at the site of service flats (elderly) is considerable. Our studies in elderly indicate an association of PM with platelet activation (PFA-100) and with systolic blood pressure. The latter association must be cautiously interpreted and must be further elucidated in the next phase of the campaign, because we cannot completely rule out an effect of adaptation to the blood pressure measurements. Our data on platelets is supported by experimental data in animals (Nemmar, 2003). Furthermore, we found evidence that on days with higher chloride concentrations the effect of PM on platelet activity was diminished. Exhaled NO, a marker of airway inflammation, correlated with the variation in oxy-PAHs species.

Depending on confirmation in the bigger elderly cohort and by studies in children, our preliminary results show increased platelet activation and airway inflammation with preliminary evidence for a prooxidant reaction with oxy-PAHs in the lungs and a protection of PM chloride load on platelet function; Furthermore modeled PM constituents suggest lower mortality during days with higher sodium load and that secondary formed aerosols were positively associated with non-traumatic mortality during warmer days. In the next two years the role of these specific compounds must be further elucidated.

## 5. FUTURE PROSPECTS AND PLANNING

### Study in elderly

In the first year of the program a lot of efforts were put in contacting and organizing the first field campaign. In 2009 we plan to increase the cohort of service flat residents to 70 with clinical measures and blood sampling at least on 2 different occasions, one to three weeks apart. We will rerun the preliminary analysis and look in more detail to the role of chemical characteristics of the PM exposure on the measured endpoints. Furthermore, we are running a case-crossover analysis as suggested by the interim evaluation on preterm mortality (preliminary results presented).

### Study in children

Cardiopulmonary morbidity and mortality is associated with several environmental exposures. Mechanistically, pathophysiological changes in the cardiopulmonary system may lead to the induction of inflammatory responses. In 800 primary school children we explore associations between environmental exposures and serum concentrations of lung Clara cell protein 16kDa, a biomarker that has recently been used to assess the integrity of the lung epithelium. We explore the associations further with adjustment for temperature and take both long-term and short-term exposure into account.

### Air quality measurements - organic compounds

1. Development and optimization of advanced analytical techniques for identification and quantification of oxy-PAHs on particulates (WP 2.2-2.4) (UGent)
  - Optimization and validation of the LC-based analytical method that is developed to determine a first set of oxy-PAHs will be considered, e.g. by investigating the need of clean-up between extraction and LC injection and/or the need for internal standard calibration.
  - The GC-based method to determine a second set of oxy-PAHs has to be further developed and optimized. Special attention has to be paid to improve the chromatographic separation of peaks that are not resolved so far, and to develop an appropriate extraction and sample preparation methodology that is compatible with the GC-MS analysis.
2. Analysis of selected oxy-PAHs sorbed on PM<sub>10</sub> collected in urban environment during the sampling campaigns that are planned for September/October 2008 (WP 2.5) (UGent)
3. Sampling and analysis of Volatile Organic Compounds (Partner 3) (UGent)
  - A third and fourth sampling campaign is planned for respectively July/August and September/October 2008. Again, both urban (service flats) and rural areas (summer camps) will be selected for sampling VOCs.
  - Data of the VOCs concentrations in both indoor and outdoor environment will be further assessed and interpreted, more particularly with respect to the calibration of the TD-GC-MS methodology for a larger number of identified compounds. Next, a methodology will be developed to determine unknown uptake rates for quantifying VOCs collected by passive sampling.

### Air quality measurements – Anorganic compounds

1. Analysis of single particles from sampling campaign in elderly (Czagani, February 2008) is planned as well as completing the analysis from sampling in Cavell and summer sampling campaigns in 2007 from children camps (UAntwerpen).
2. Analysis of samples collected in Faulx-Les-Tombes (children camp, summer 2008) what involves, the gravimetric analysis of PM (PM<sub>1</sub>, PM<sub>2.5</sub>, PM<sub>10</sub>), XRF analysis of elemental composition of the PM fractions, IC analysis for determination of ionic species, analysis of single particles with the use of EPMA (Electron Probe Microanalysis) and determination of EC (elemental carbon) concentration (UA).
3. Further sampling campaigns in children camps (summer 2008) and elderly (September/October 2008) are planned, followed by the sample analysis (see chapter 2), additionally the concentration of PM<sub>10</sub> and inorganic (O<sub>3</sub>, NO<sub>2</sub>, SO<sub>2</sub>) and organic gases (BTEX) is to be determined (in elderly).

### Modelled air quality

Partner 5 (RMI/KMI) in collaboration with IRCEL will provide air quality data to organise our planned field work in the course of this year. Modelled PM<sub>2.5</sub> composition will be used in time-series analysis of mortality during a 7-year period (upon the PM<sub>10</sub> data).

## 6. FOLLOW-UP COMMITTEE

The follow-up committee contributes at different stages in project. First, Flemish Environment Agency provided high volume sampler to collect dust at the site of the nursing homes for the determinations of organic compounds. Second, IRCEL provides interpolated PM values, predicts air pollution levels together with the KMI/RMI to organize our field campaigns. Further IRCEL and VMM help to validate our on site measures by providing the concentrations at fixed monitoring sites. All members of the committee interact actively on the interpretation of our preliminary results.

Myriam Bossuyt	Vlaamse Milieumaatschappij (MIRA)
Jérôme de Roubaix	Ministère de la Communauté française
Yseult Navez	SPF Santé publique, Sécurité de la Chaîne alimentaire et Environnement
Catherine Bouland	Institut Bruxellois pour la Gestion de l'Environnement (IBGE)
Maja Mampaey	Vlaamse Gemeenschap AMINAL
Suzanne Remy	Institut Scientifique de Service Public (ISSeP)
Edward Roekens	Vlaamse Milieu Maatschappij - Afdeling Meetnetten en Onderzoek
Rudi Torfs	Vlaamse Instelling voor Technologisch Onderzoek (VITO)
Peter Vanderstraeten	Institut Bruxellois pour la Gestion de l'Environnement (IBGE)

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## 8. PUBLICATIONS

### 8.1. Publications of the teams

#### *8.1.1. Peer review*

A. Delcloo, T. Nawrot, L. Jacobs, B. Nemery and H. De Backer, Association between daily mortality and the composition of fine particulate matter by the use of the CTM CHIMERE, European Aerosol Conference, Thessaloniki, 24-29 August, 2008. [abstract]

H. Scheers, L. Jacobs, B. Nemery, F. Fierens, S. M. Mwalili, T. Nawrot (2008) Associations between air pollution and infant mortality in an affluent society: a case-crossover study. Poster at the International Conference on "Health Aspects of Indoor and Outdoor Air Pollution", Luxembourg (LUX), 12/11/2008. [abstract]

Nawrot T, Kunzli N, Sunyer J, Tingming S, Moreno T, Mar V, Heinrich J, Forsberg B, Kelly F, Muhamed SM, Nemery B, Borm P. The association between oxidative properties of ambient pm2.5 and elemental composition: heterogeneous associations in 19 European cities. [submitted]