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# Associations between ultrafine and fine particles and mortality in five central European cities – Results from the UFIRES study

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## ABSTRACT

**Background:** Evidence on health effects of ultrafine particles (UFP) is still limited as they are usually not monitored routinely. The few epidemiological studies on UFP and (cause-specific) mortality so far have reported inconsistent results.

**Objectives:** The main objective of the UFIRES project was to investigate the short-term associations between UFP and fine particulate matter (PM) < 2.5 µm (PM<sub>2.5</sub>) and daily (cause-specific) mortality in five European Cities. We also examined the effects of PM < 10 µm (PM<sub>10</sub>) and coarse particles (PM<sub>2.5–10</sub>).

**Methods:** UFP (20–100 nm), PM and meteorological data were measured in Dresden and Augsburg (Germany), Prague (Czech Republic), Ljubljana (Slovenia) and Chernivtsi (Ukraine). Daily counts of natural and cardio-respiratory mortality were collected for all five cities. Depending on data availability, the following study periods were chosen: Augsburg and Dresden 2011–2012, Ljubljana and Prague 2012–2013, Chernivtsi 2013–March 2014. The associations between air pollutants and health outcomes were assessed using confounder-adjusted Poisson regression models examining single (lag 0–lag 5) and cumulative lags (lag 0–1, lag 2–5, and lag 0–5). City-specific estimates were pooled using meta-analyses methods.

**Results:** Results indicated a delayed and prolonged association between UFP and respiratory mortality (9.9% [95%-confidence interval: –6.3%; 28.8%] increase in association with a 6-day average increase of 2750 particles/cm<sup>3</sup> (average interquartile range across all cities)). Cardiovascular mortality increased by 3.0% [–2.7%; 9.1%] and 4.1% [0.4%; 8.0%] in association with a 12.4 µg/m<sup>3</sup> and 4.7 µg/m<sup>3</sup> increase in the PM<sub>2.5</sub>- and PM<sub>2.5–10</sub>-averages of lag 2–5.

**Conclusions:** We observed positive but not statistically significant associations between prolonged exposures to UFP and respiratory mortality, which were independent of particle mass exposures. Further multi-centre studies are needed investigating several years to produce more precise estimates on health effects of UFP.

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## 1. Introduction

Epidemiological short-term studies on ultrafine particles (UFP) with a diameter < 100 nm (0.1 µm) and mortality are still rare, whereas a

large number of studies investigated the effects of particulate matter (PM) with an aerodynamic diameter < 10 µm (PM<sub>10</sub>) or < 2.5 µm (PM<sub>2.5</sub>, fine particles) (Atkinson et al., 2014; Rückerl et al., 2011). Most of the studies focused on the effects of fine particles on all-cause mortality and mortality due to cardiovascular and respiratory causes (Atkinson et al., 2014; Rückerl et al., 2011). A review by Atkinson et al. (2014) reported a 1.0% [95%-confidence interval: 0.5%; 1.6%] increase in all-cause mortality in association with a 10 µg/m<sup>3</sup> increase in PM<sub>2.5</sub> based on 23 estimates, but with substantial regional variation. The effect

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estimates associated with PM<sub>2.5</sub> on respiratory mortality were stronger (1.5% [1.0%; 2.0%]) than estimates for cardiovascular mortality (0.8% [0.4%; 1.3%]).

Due to their small size and little mass the deposition and clearance of UFP in the respiratory tract differ from larger particles (Kreyling et al., 2006). Because of the differences in deposition and the potential for translocation as well as their huge active surface, effects of UFP might be at least partly independent from those of larger particles such as PM<sub>10</sub> and PM<sub>2.5</sub> (Brook et al., 2004; HEI, 2013; Peters et al., 2011; Rückerl et al., 2011). So far, experimental studies do not provide sufficient evidence to confirm this hypothesis. Further, there is suggestive, but not consistent epidemiological evidence on the association between short-term exposures to UFP and cardiorespiratory health (HEI, 2013; WHO, 2013a). Moreover, hardly any epidemiological studies of long-term exposures to ambient UFP have been conducted yet (Ostro et al., 2015).

The few epidemiological short-term studies on UFP and (cause-specific) mortality so far have reported inconsistent results (HEI, 2013). One of the first studies on health effects of UFP reported 1-day delayed increases in respiratory mortality (15.5% [5.5%; 26.4%]) and 4-days delayed increases in cardiovascular mortality (5.1% [−1.0%; 11.5%]) in association with an interquartile range (IQR) increase in UFP (12,680 particles/cm<sup>3</sup>) (Wichmann et al., 2000). Increases in natural and cardiorespiratory mortality with a delay of at least two days in association with UFP increases were also found in other analyses (Breitner et al., 2009; Breitner et al., 2011; Stolzel et al., 2007). However, shorter time lags were also reported (Atkinson et al., 2010; Forastiere et al., 2005). In a study conducted in London an IQR increase of 10,166 particles/cm<sup>3</sup> in total particle number concentration (PNC) was associated with increases in all-cause mortality (1.4% [0.5%; 2.4%]), cardiovascular mortality (2.2% [0.6%; 3.8%]) and respiratory mortality (2.3% [−0.1%; 4.8%]) with a 1-day delay, while no associations were found for other time lags (Atkinson et al., 2010). Moreover, two studies conducted in Helsinki and Prague studying the association between PNC in different size ranges and (cause-specific) mortality found only weak or no associations (Branis et al., 2010; Halonen et al., 2009).

The project “Ultrafine particles – an evidence based contribution to the development of regional and European environmental and health policy” (UFIREG) had the goal to monitor UFP with the same instrumentation and assess the short-term health effects of ultrafine and fine particles on daily (cause-specific) mortality in time-series analyses. So far, European studies on short-term associations between UFP and mortality were primarily focused on Western European countries (HEI, 2013). However, the UFIREG project involved cities from Central and Eastern European countries using harmonised exposure and epidemiological methodology in all cities. Five cities in four Central and Eastern European countries participated in the study: Augsburg and Dresden (Germany), Chernivtsi (Ukraine), Ljubljana (Slovenia) and Prague (Czech Republic). The UFIREG project started in July 2011 and ended in December 2014. We hypothesised that we would be able to observe independent associations of ultrafine PNC and fine particle mass concentrations on (cause-specific) mortality. Moreover, we also investigated PNC, PM<sub>10</sub>, coarse particles with an aerodynamic diameter > 2.5 µm and < 10 µm (PM<sub>2.5–10</sub>) and nitrogen dioxide (NO<sub>2</sub>) as pollutants of secondary interest.

## 2. Methods

The study population comprised residents of Augsburg, Chernivtsi, Dresden, Ljubljana and Prague. Daily counts of (cause-specific) deaths were obtained from official statistics for each of the five cities. Only residents of a city who died in that city were considered. Infants younger than one year were excluded from the analyses. The causes of death are based on the International Statistical Classification of Diseases and Related Health Problems (ICD-10). Deaths due to natural causes (ICD-10: A00–R99) and deaths due to cardiovascular (ICD-10: I00–I99) and

respiratory diseases (ICD-10: J00–J99) were considered. Mortality data for Augsburg and Dresden were obtained from the Research Data Centres of the Federal Statistical Office and the Statistical Offices of the Free States of Bavaria and Saxony, respectively. For Ljubljana, mortality data were obtained from the National Institute of Public Health in Slovenia. All data for Prague were provided by the Institute of Health Information and Statistics of the Czech Republic. For Chernivtsi, mortality data were provided by the Main Department of Statistics in Chernivtsi Region.

We also obtained information on additional variables for confounding adjustment, including indicator variables for weekdays and holidays, meteorological parameters (air temperature, relative humidity, barometric pressure), and – if available – influenza epidemics. Information on influenza epidemics in Augsburg and Dresden were provided by the German Influenza Working Group of the Robert Koch Institute (<https://influenza.rki.de/Default.aspx>). Data on influenza epidemics in Prague were obtained from the National Institute of Public Health in Prague and the Hygiene Station of the City of Prague. In Ljubljana, these data were provided by the National Institute of Public Health in Slovenia. No information on influenza epidemics was available in Chernivtsi. Sociodemographic data such as number of inhabitants (per age-group and sex), estimated percentage of smokers, population density or number of newborns and deceased persons was used to describe the population in the cities involved in the project. Data for Augsburg derived from the Statistical Yearbook of Augsburg. For Dresden, data were obtained from the census in 2011 and the Statistical Office of the Free State of Saxony. The Statistical Office of the Republic of Slovenia provided sociodemographic data for Ljubljana. Data for Prague were obtained from the Institute of Health Information and Statistics of the Czech Republic and the Czech statistical office. For Chernivtsi data derived from the Main Statistic Department in Chernivtsi Region.

Air pollution and meteorological parameters were measured on an hourly basis at local fixed measurement sites. The providers of air pollution and meteorological data are described elsewhere (UFIREG-report 2014). The measurement stations in Augsburg, Chernivtsi, Dresden and Ljubljana were located at urban background sites. The monitoring station in Prague was located at a suburban background site. Meteorological parameters included air temperature, relative humidity and barometric pressure. PM<sub>10</sub>, PM<sub>2.5</sub>, and NO<sub>2</sub> were measured in Augsburg, Dresden, Ljubljana and Prague. However, these parameters were not available in Chernivtsi. In Augsburg, Dresden, Ljubljana and Prague PM<sub>2.5</sub> and PM<sub>10</sub> were measured at the same measurement site. PM<sub>2.5–10</sub> was calculated as the difference between site specific PM<sub>10</sub> and PM<sub>2.5</sub>. PNC were measured using custom-made mobility particle size spectrometers, either Differential or Scanning Mobility Particle Sizers. They enabled highly size-resolved PNC measurements in the range from 10 to 800 nm, except for Prague, where PNC were measured from 10 to 500 nm. The mobility particle size spectrometers delivered data in a 5- to 20-minute time-resolution. Hourly averages were calculated with a threshold of 75% data availability. The overall availability of PNC data reached more than 75% at all stations (UFIREG-report 2014). Imputation of hourly missing PM data was only possible for Augsburg and Prague where an additional urban background measurement station was available. Imputation was performed using a modified APHEA (Air Pollution and Health: A European Approach) procedure (Berglind et al., 2009; Katsouyanni et al., 1996). Missing hours of one monitor were imputed by a weighted average of the other monitor. If the respective hourly mean value was not available at both monitors, the average of the preceding and the following hourly means was used. Daily 24-hour averages of all air pollutants and meteorological parameters were only calculated if 75% of the hourly values were available.

An extensive quality assurance programme was an essential part of the high standards for data collection. It comprised staff training, an initial comparison of spectrometers in a laboratory, frequent on-site comparisons against reference instruments, remote monitoring, and automated function control units at two sites (Dresden and Chernivtsi).

The quality assurance programme showed that the deviation for particles smaller than 15 nm was between 20% and 60%. Therefore, the size class 10 to 20 nm was excluded from the epidemiological analysis. We investigated UFP in the size range 20 to 100 nm and PNC in the size range 20 to 800 (for Prague 20 to 500 nm) in all cities.

Depending on the start of the measurements and the availability of epidemiological data, the following study periods were chosen for the epidemiological analyses: Augsburg and Dresden: January 2011 to December 2012; Ljubljana and Prague: January 2012 to December 2013; Chernivtsi: January 2013 until March 2014. The period 2011 to 2012 for the German cities was chosen due to German data protection rules; data on (cause-specific) mortality of 2013 was not available by the end of the project period.

### 2.1. Statistical analysis

Spearman's rank correlation coefficient was used to calculate correlations between air pollution and meteorological parameters. The association between air pollutants and mortality was investigated using Poisson regression models allowing for overdispersion. In a first step, a basic confounder model was set up a priori for all cities based on a review of the current literature. The basic model included long-term time-trend, dummy variables for day of the week (Monday to Sunday), a dummy variable for public holidays referring to single days (holidays vs. non-holidays), a dummy variable for the decrease of the populations present in the city during longer vacation periods with high travel activities for example during school vacation (Christmas, Easter, summer vacation), a dummy variable for influenza epidemics (where available), air temperature (average of lags 0–1 [lag 0: same-day; lag 1: one day before the event] to represent effects of high temperatures and average of lags 2–13 [lag 2: two days prior to the event; lag 13: 13 days prior to the event] to represent effects of low temperatures), and relative humidity (average of lags 0–1 and average of lags 2–13). Penalised regression splines with natural cubic regression splines as smoothing basis were used to allow for non-linear confounder adjustment. The spline for time-trend was fixed to have four degrees of freedom per year to sufficiently represent long-term trend and seasonality since previous studies in Germany reported a negative residual autocorrelation when using more degrees of freedom per year (Peters et al., 2009). Splines for meteorological variables were fixed to three degrees of freedom. We performed single-lag models from lag 0 (same day of the event) up to lag 5 (five days prior to the event) to visually examine the lag structure of the association between particle exposures and health outcomes. Cumulative effect models were used representing immediate (2-day average: lag 0–1), delayed (average of lag 2–5) and prolonged effects (6-day average: lag 0–5).

City-specific effect estimates were combined with random-effects models. For each meta-analytical estimate, a  $\chi^2$ -test for heterogeneity was performed and the corresponding p-value reported, together with the  $I^2$ -statistic, which represents the proportion of total variation in effect estimates that is due to between-cities heterogeneity. Cities were weighted according to the precision of the city-specific effect estimates. For pooling the city-specific estimates the maximum likelihood effects estimator after van Houwelingen was used (van Houwelingen et al., 2002). The analyses were conducted for all ages (increasing the statistical power of the analysis) as well as stratified for deaths among those below 75 years of age and above 75 years. Moreover, we conducted the analyses for females and males separately in order to test effect modification by sex. Effect modification by season (October–March vs. April–September) was analysed by including an interaction term in the model. We estimated two-pollutant models to assess interdependencies of UFP and PM<sub>2.5</sub> as well as UFP and NO<sub>2</sub> effects.

### 2.2. Sensitivity analyses

The sensitivity of the air pollution effects was assessed by re-running the above-described analyses with the following variations:

- (1) Different values of smoothness for time-trend (6 and 3 degrees of freedom per year) and meteorological variables (5 degrees of freedom) were specified.
- (2) Air temperature and relative humidity were replaced by apparent temperature, a combination of both. Apparent temperature was calculated using the following formula (Kalkstein and Valimont, 1986; Steadman, 1979):  $at = -2.653 + (0.994 \times temp) + (0.0153 \times dp \times dp)$  with  $at$  = apparent temperature,  $temp$  = air temperature and  $dp$  = dew point temperature (Supplemental Material Formula (1)).
- (3) Air pollution effects were adjusted for air temperature by using temperature above the median for heat effects and below the median for cold effects (Stafoggia et al., 2013).
- (4) We adjusted for air temperature and relative humidity including the average of lag 0–1 and average of lag 2–5.
- (5) Barometric pressure was additionally included in the models.
- (6) Effect estimates for Augsburg and Prague were recalculated using a dataset with imputed missing data.
- (7) We analysed air pollution effects using distributed lag non-linear models as described by Gasparrini (2011). We assessed up to 7 and 14 lags using a second- and third-degree polynomial. Results of polynomial distributed lag models were pooled according to Gasparrini et al. (2012).

Effects of UFP on mortality are presented as percent changes in mortality outcomes ((relative risk from Poisson regression models – 1) \* 100) per 2750 particles/cm<sup>3</sup> increase (average IQR across all five cities) in daily UFP. Effects of PM<sub>2.5</sub> on mortality are presented as percent changes in mortality outcomes per 12.4 µg/m<sup>3</sup> increase (average IQR across Augsburg, Dresden, Ljubljana and Prague) in daily PM<sub>2.5</sub>. As pollutants of secondary interest, we also analysed effects of PNC (20–800 nm [20–500 nm in Prague]), PM<sub>10</sub>, PM<sub>2.5–10</sub>, and NO<sub>2</sub> on (cause-specific) mortality. Results of secondary pollutants are presented as percent changes in (cause-specific) mortality per 3675 particles/cm<sup>3</sup>, 16 µg/m<sup>3</sup>, 4.7 µg/m<sup>3</sup>, and 15.4 µg/m<sup>3</sup> (average IQRs across all five cities) increase in PNC, PM<sub>10</sub>, PM<sub>2.5–10</sub>, and NO<sub>2</sub>, respectively.

Data management was conducted using SAS statistical package (version 9.3; SAS Institute Inc., Cary, NC). Statistical analyses were performed using R project for statistical computing (version 2.15.3, <http://www.r-project.org/>) using the “mgcv”, “splines”, “dlnm”, “metafor” and “mvmeta” packages.

**Table 1**  
Socio-demographical information of the five UFIREG cities.

City	Year	Population	City area (km <sup>2</sup> )	Density of population <sup>a</sup>	Newborns	Deceased persons
Augsburg	2011	266,647	146.9	1815.8	2253	2820
	2012	272,699	146.9	1857.0	2465	2950
Chernivtsi	2013	258,371	153.0	1688.7	2751	2447
	2011	517,765	328.3	1577.1	5907	4772
Dresden	2012	525,105	328.3	1599.4	6001	5040
	2012	280,607	275.0	1020.4	3084	2272
Ljubljana	2013	282,994	275.0	1029.1	2982	2242
	2012	1,246,780	496.2	2512.7	14,176	12,411
Prague	2013	1,243,201	496.2	2505.4	13,867	12,149

<sup>a</sup> Inhabitants/km<sup>2</sup>.



### 3. Results

A description of the UFIREG cities is shown in Table 1 and Supplemental Fig. 1 shows the location of the five cities in Central and Eastern Europe. Prague was the largest of the five cities with about 1.2 million inhabitants and an area of almost 500 km<sup>2</sup>. Dresden was the second largest city in the UFIREG project with about 500,000 inhabitants within an area of more than 300 km<sup>2</sup>. The number of inhabitants in Augsburg, Ljubljana, and Chernivtsi was comparable and ranged from about 260,000 to 280,000 inhabitants. Ljubljana, however, was larger than Augsburg and Chernivtsi with an area of 275 km<sup>2</sup>. In all cities, except for Augsburg, the number of newborns was higher than the number of deceased persons during the respective study periods. The percentages of women and men were similar in all cities with about 52% women and 48% men. In Chernivtsi, 11% of the population were 65 years or older, whereas in the other cities the number of people aged 65 years or older ranged from 18% in Prague and Ljubljana to 20% and 22% in Augsburg and Dresden, respectively.

According to the WHO Report on the Global Tobacco Epidemic 2013, the Czech Republic showed the highest prevalence of tobacco smoking of countries within the study with 36.9% followed by the Ukraine with 28.8% in 2012 (WHO, 2013b). In the same year, the prevalence of tobacco smoking in Germany was 25.7% and in Slovenia 25.4%.

Table 2 shows a description of mortality outcomes by city for each year. In Augsburg, Dresden, Ljubljana, and Prague 40%–50% of natural death cases were due to cardiovascular diseases. In Chernivtsi, almost 70% of natural deaths were due to cardiovascular diseases in 2013. Chernivtsi was excluded from the analysis on respiratory mortality due to an insufficient number of respiratory death cases in the study period. Supplemental Table 1 presents a description of (cause-specific) mortality outcomes per 100,000 inhabitants.

A description of 24-hour averages of air pollution and meteorological variables by city is shown in Table 3. In Prague and Ljubljana UFP measurements started in mid-April 2012; hence, missing values of UFP were higher compared to the other air pollutants in these two cities. UFP were moderately correlated with PM<sub>10</sub>, PM<sub>2.5</sub> and PM<sub>2.5–10</sub> (Spearman's rank correlation coefficient  $0.3 \leq r_s \leq 0.5$ ) in all cities (Supplemental Table 2). Moreover, the correlation between air pollution and meteorological parameters was low to moderate ( $r_s < 0.6$ ) in all cities. High correlations were observed between PM<sub>10</sub> and PM<sub>2.5</sub> with  $r_s = 0.9$  in Augsburg, Dresden, Ljubljana and Prague. The seasonal variation of UFP and PM<sub>2.5</sub> for each city separately is shown in Supplemental Fig. 2. Prague and Dresden showed higher UFP concentrations during summer compared to the other cities, whereas, Chernivtsi showed highest concentrations during autumn (Supplemental Fig. 2A). In all cities PM<sub>2.5</sub> concentrations were higher during winter compared to the

**Table 3**

Description of 24-hour averages of air pollution and meteorological variables by city.

City (study period)	N	Min	Median	Mean (SD)	Max	IQR <sup>a</sup>
Augsburg (2011–2012)						
Air temperature (°C)	720	−13.4	9.9	10.0 (8.0)	26.8	12.4
Relative humidity (%)	720	39.6	78.3	77.1 (13.0)	100	20.3
PM <sub>10</sub> <sup>b</sup> (μg/m <sup>3</sup> )	725	2.7	17.2	20.0 (12.5)	91.5	14.5
PM <sub>2.5</sub> <sup>c</sup> (μg/m <sup>3</sup> )	720	1.7	12.4	14.9 (9.8)	86.3	10.8
PM <sub>2.5–10</sub> <sup>d</sup> (μg/m <sup>3</sup> )	714	0.1	5.3	6.0 (4.2)	36.0	5.3
UFP <sup>e</sup> (n/cm <sup>3</sup> )	712	1161	5172	5880 (3016)	28,800	3332
PNC <sup>f</sup> (n/cm <sup>3</sup> )	712	1369	6409	7239 (3643)	29,470	4124
NO <sub>2</sub> <sup>h</sup> (μg/m <sup>3</sup> )	718	4.2	26.9	28.0 (11.8)	74.0	16.1
Chernivtsi (2013)						
Air temperature (°C)	291	−7.4	13.9	11.9 (8.2)	27.4	13.8
Relative humidity (%)	291	31.7	74	74.0 (15.6)	100	22.6
PM <sub>10</sub> <sup>b</sup> (μg/m <sup>3</sup> )	.	.	.	.	.	.
PM <sub>2.5</sub> <sup>c</sup> (μg/m <sup>3</sup> )	.	.	.	.	.	.
PM <sub>2.5–10</sub> <sup>d</sup> (μg/m <sup>3</sup> )	.	.	.	.	.	.
UFP <sup>e</sup> (n/cm <sup>3</sup> )	340	1769	5018	5511 (2614)	19,160	3324
PNC <sup>f</sup> (n/cm <sup>3</sup> )	340	2212	6908	7775 (3782)	29,030	4325
NO <sub>2</sub> <sup>h</sup> (μg/m <sup>3</sup> )	.	.	.	.	.	.
Dresden (2011–2012)						
Air temperature (°C)	731	−13.4	11.7	11.7 (8.2)	29.6	12.8
Relative humidity (%)	731	36	69.6	69.5 (11.1)	94.3	16.7
PM <sub>10</sub> <sup>b</sup> (μg/m <sup>3</sup> )	726	2.2	16.5	20.9 (15.2)	103.5	14.3
PM <sub>2.5</sub> <sup>c</sup> (μg/m <sup>3</sup> )	720	1.5	11.6	16.2 (13.8)	95.7	13.1
PM <sub>2.5–10</sub> <sup>d</sup> (μg/m <sup>3</sup> )	717	0.0	4.3	4.7 (2.7)	21.6	3.0
UFP <sup>e</sup> (n/cm <sup>3</sup> )	639	677	3752	4286 (2338)	14,440	2882
PNC <sup>f</sup> (n/cm <sup>3</sup> )	639	855	5446	5851 (2902)	16,710	4068
NO <sub>2</sub> <sup>h</sup> (μg/m <sup>3</sup> )	719	3.9	20.4	22.3 (10.0)	67.3	12.9
Ljubljana (2012–2013)						
Air temperature (°C)	730	−8.8	12.2	11.7 (8.7)	29.4	14.0
Relative humidity (%)	731	37.8	74.3	73.8 (13.7)	97.5	23.6
PM <sub>10</sub> <sup>b</sup> (μg/m <sup>3</sup> )	682	3.0	20.0	24.9 (16.8)	130.0	18.0
PM <sub>2.5</sub> <sup>c</sup> (μg/m <sup>3</sup> )	694	3.4	16.5	20.7 (14.3)	114.8	14.4
PM <sub>2.5–10</sub> <sup>d</sup> (μg/m <sup>3</sup> )	646	0.0	3.9	5.0 (5.1)	29.8	5.8
UFP <sup>e</sup> (n/cm <sup>3</sup> )	435	855	4400	4693 (1896)	13,920	1935
PNC <sup>f</sup> (n/cm <sup>3</sup> )	435	1685	6071	6750 (3121)	24,360	2689
NO <sub>2</sub> <sup>h</sup> (μg/m <sup>3</sup> )	683	1.8	22.2	25.1 (14.8)	119.4	16.4
Prague (2012–2013)						
Air temperature (°C)	723	−13.7	9	9.2 (8.4)	27.2	13.1
Relative humidity (%)	704	40.8	78.2	77.3 (13.2)	98.9	20.4
PM <sub>10</sub> <sup>b</sup> (μg/m <sup>3</sup> )	681	5.1	22.2	26.2 (15.7)	100.9	17.2
PM <sub>2.5</sub> <sup>c</sup> (μg/m <sup>3</sup> )	612	1.6	13.1	16.2 (11.6)	78.8	11.4
PM <sub>2.5–10</sub> <sup>d</sup> (μg/m <sup>3</sup> )	579	1.7	9.2	9.8 (4.0)	44.6	4.6
UFP <sup>e</sup> (n/cm <sup>3</sup> )	464	960	3797	4197 (2010)	14,960	2278
PNC <sup>f</sup> (n/cm <sup>3</sup> )	464	1217	5417	5799 (2537)	16,950	3168
NO <sub>2</sub> <sup>h</sup> (μg/m <sup>3</sup> )	707	4.5	19.5	21.9 (11.7)	74.2	16.2

<sup>a</sup> Interquartile range.

<sup>b</sup> Particulate matter with a size range of <10 μm in aerodynamic diameter.

<sup>c</sup> Particulate matter with a size range of <2.5 μm in aerodynamic diameter.

<sup>d</sup> Coarse particles with a size range of 2.5–10 μm in aerodynamic diameter.

<sup>e</sup> Ultrafine particles with a size range of 0.02 to 0.1 μm in aerodynamic diameter (20–100 nm).

<sup>f</sup> Particle number concentration with a size range of 0.02 to 0.8 μm in aerodynamic diameter (20–800 nm).

<sup>g</sup> Particle number concentration with a size range of 0.02 to 0.5 μm in aerodynamic diameter (20–500 nm).

<sup>h</sup> Nitrogen dioxide.

**Table 2**

Description of (cause-specific) mortality outcomes by city.

City	Year	Population	Mean daily natural death counts (SD)	Mean daily cardiovascular death counts (SD)	Mean daily respiratory death counts (SD)
Augsburg <sup>a</sup>	2011	266,647	6.9 (2.5)	3.1 (1.7)	0.5 (0.8)
	2012	272,699	7.2 (2.8)	3.1 (1.7)	0.4 (0.6)
Chernivtsi	2013	258,371	6.3 (2.7)	4.3 (2.1)	0.1 (0.4)
	2011	517,765	11.5 (3.4)	5.3 (2.3)	0.6 (0.9)
Dresden <sup>a</sup>	2012	525,105	12.2 (3.7)	5.4 (2.4)	0.6 (0.8)
	2012	280,607	5.8 (2.5)	2.3 (1.5)	0.4 (0.6)
Ljubljana	2013	282,994	5.7 (2.4)	2.3 (1.5)	0.3 (0.5)
	2012	1,246,780	27.1 (5.7)	13.7 (4.1)	1.5 (1.3)
Prague	2013	1,243,201	26.5 (5.9)	12.8 (3.8)	1.7 (1.4)

Outcome definitions: natural causes ICD-10 A00–R99, cardiovascular diseases ICD-10 I00–I99, respiratory diseases ICD-10 J00–J99.

<sup>a</sup> Reference: Research Data Centres of the Federal Statistical Office and the Statistical Offices of the Länder, Death Statistics, 2011–2012, own calculations.

other seasons (Supplemental Fig. 2B). Ljubljana showed the highest PM<sub>2.5</sub> concentrations in winter compared to the other cities.

#### 3.1. Associations between air pollutants and (cause-specific) mortality

Most of the associations reported in this section were not statistically significant. However, we still interpret these findings as potentially important as results indicated delayed and prolonged associations between UFP and PM and cause-specific mortality. The strongest associations between air pollutants and mortality outcomes were observed for the cumulative lag periods. Table 4 shows percent changes in natural and cause-specific mortality in association with an average IQR increase in air pollutants for the 2-day average, the average of lag 2 to lag 5 and the 6-day average. Results on single time lags are presented in Supplemental Table 3. We observed no associations between UFP and natural

**Table 4**

Percent changes (95%–CI) in (cause-specific) mortality with each average IQR increase in air pollutants.

Association under investigation	IQR <sup>a</sup>	2-day average (lag 0–1)	Average of lag 2–5	6-day average (lag 0–5)
<b>Natural mortality</b>				
UFP <sup>b</sup> (n/cm <sup>3</sup> )	2750	0.1 (–2.0; 2.4)	–1.2 (–4.0; 1.8)	–0.3 (–3.8; 3.2)
PNC <sup>c</sup> (n/cm <sup>3</sup> )	3675	–0.2 (–2.4; 2.1)	–1.2 (–4.1; 1.8)	–0.6 (–4.0; 2.9)
PM <sub>2.5</sub> (μg/m <sup>3</sup> )	12.4	–0.5 (–2.2; 1.2)	0.9 (–3.4; 5.4)*	0.3 (–3.7; 4.5)*
PM <sub>10</sub> (μg/m <sup>3</sup> )	16.0	–0.2 (–1.9; 1.5)	0.8 (–3.3; 5.1)*	0.8 (–3.6; 5.3)*
PM <sub>2.5–10</sub> (μg/m <sup>3</sup> )	4.7	1.1 (–0.8; 3.0)	1.2 (–1.6; 4.0)	1.7 (–1.9; 5.4)*
NO <sub>2</sub> (μg/m <sup>3</sup> )	15.4	0.5 (–1.6; 2.8)	0.1 (–3.4; 3.7)	0.4 (–3.1; 4.1)
<b>Cardiovascular mortality</b>				
UFP <sup>b</sup> (n/cm <sup>3</sup> )	2750	–0.5 (–3.6; 2.8)	–0.5 (–5.3; 4.5)	–0.2 (–5.5; 5.4)
PNC <sup>c</sup> (n/cm <sup>3</sup> )	3675	–0.7 (–3.9; 2.5)	–0.1 (–5.5; 5.6)	–0.1 (–5.8; 5.9)
PM <sub>2.5</sub> (μg/m <sup>3</sup> )	12.4	–0.4 (–2.9; 2.2)	3.0 (–2.7; 9.1)*	1.6 (–2.8; 6.2)
PM <sub>10</sub> (μg/m <sup>3</sup> )	16.0	–0.3 (–2.7; 2.1)	3.2 (–2.4; 9.2)*	2.3 (–2.9; 7.8)*
PM <sub>2.5–10</sub> (μg/m <sup>3</sup> )	4.7	0.9 (–2.3; 4.3)	4.1 (0.4; 8.0)	4.2 (–0.6; 9.1)
NO <sub>2</sub> (μg/m <sup>3</sup> )	15.4	–1.1 (–4.3; 2.2)	2.2 (–3.4; 8.0)	0.8 (–3.7; 5.5)
<b>Respiratory mortality</b>				
UFP <sup>b</sup> (n/cm <sup>3</sup> )	2750	3.7 (–5.8; 14.2)	8.5 (–4.8; 23.7)	9.9 (–6.3; 28.8)
PNC <sup>c</sup> (n/cm <sup>3</sup> )	3675	1.5 (–8.0; 12.1)	5.8 (–6.4; 19.7)	5.6 (–8.3; 21.7)
PM <sub>2.5</sub> (μg/m <sup>3</sup> )	12.4	–3.4 (–9.9; 3.6)	–0.9 (–8.1; 6.9)	–2.4 (–10.5; 6.4)
PM <sub>10</sub> (μg/m <sup>3</sup> )	16.0	–3.6 (–9.8; 3.0)	–3.6 (–10.4; 3.6)	–5.1 (–12.6; 3.1)
PM <sub>2.5–10</sub> (μg/m <sup>3</sup> )	4.7	–2.2 (–9.3; 5.4)	–2.1 (–10.3; 6.7)	–4.1 (–13.1; 5.9)
NO <sub>2</sub> (μg/m <sup>3</sup> )	15.4	2.7 (–6.1; 12.4)	–1.3 (–13.7; 12.9)	–1.2 (–12.8; 11.9)

<sup>a</sup> Average interquartile range across all cities.<sup>b</sup> Ultrafine particles with a size range 20–100 nm.<sup>c</sup> Particle number concentration with a size range 20–800 nm (for Prague 20–500 nm).\* Heterogeneity p-value < 0.1 and I<sup>2</sup> > 50%.

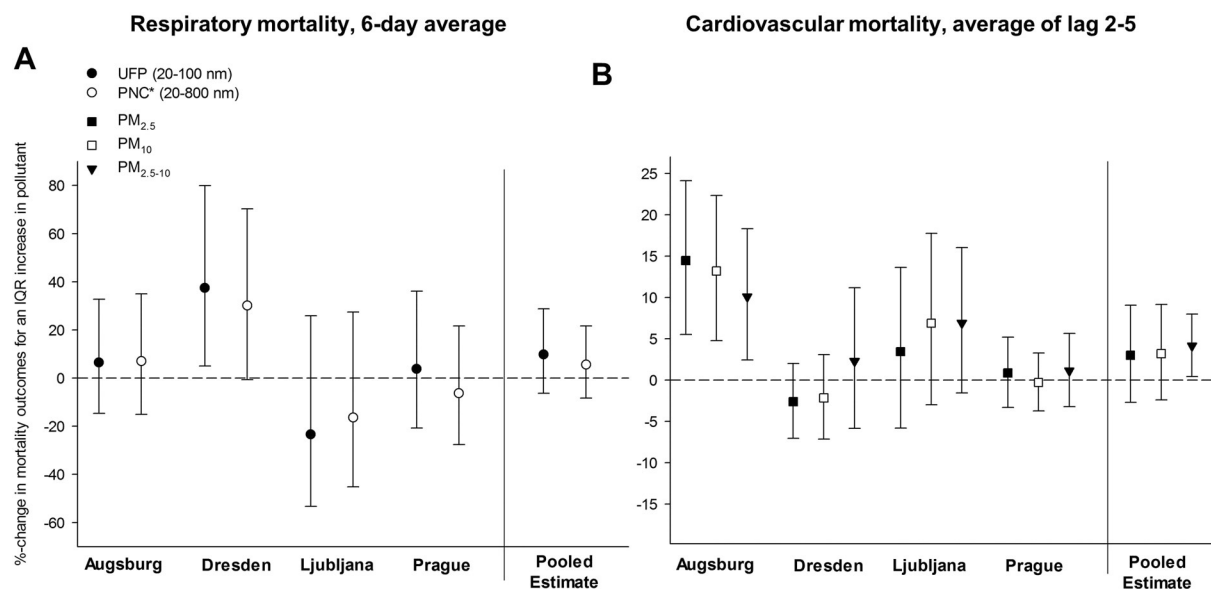
or cardiovascular mortality for all cities combined. However, results indicated delayed and prolonged effects of UFP on respiratory mortality. For example, the pooled effect estimate of respiratory mortality increased by 9.9% [–6.3%; 28.8%] in association with a 2750 particles/cm<sup>3</sup> increase in the 6-day average of UFP. Results of PNC were similar, showing delayed and prolonged effects on respiratory mortality.

We found no changes in natural and respiratory mortality in association with increases in PM<sub>2.5</sub>, PM<sub>10</sub> and PM<sub>2.5–10</sub> for all cities combined. However, our findings pointed to delayed increases in cardiovascular mortality in association with IQR increases in the averages of lag 2 to lag 5 of PM<sub>2.5</sub>, PM<sub>10</sub> and PM<sub>2.5–10</sub>, respectively. A 12.4 μg/m<sup>3</sup> increase in the PM<sub>2.5</sub>-average of lag 2 to lag 5 was associated with a 3.0% [–2.7%; 9.1%] increase in cardiovascular mortality. Results of PM<sub>10</sub> were similar. However, we also found heterogeneity in the pooled effect estimates of PM<sub>2.5</sub> as well as PM<sub>10</sub> and cardiovascular mortality. A

4.7 μg/m<sup>3</sup> increase in the PM<sub>2.5–10</sub> average of lag 2 to lag 5 led to a significant increase in cardiovascular mortality by 4.1% [0.4%; 8.0%]. There was no association between increases in NO<sub>2</sub> and mortality outcomes.

City-specific and pooled effect estimates of respiratory mortality with increases in the 6-day average of UFP and PNC for Augsburg, Dresden, Ljubljana and Prague are presented in Fig. 1A. All cities except Ljubljana showed (slight) increases in the relative risk of respiratory mortality in association with UFP; Dresden showed the strongest effect. Results of PNC and respiratory mortality were similar.

Fig. 1B shows city-specific and pooled effect estimates for the PM<sub>2.5</sub>, PM<sub>10</sub>- and PM<sub>2.5–10</sub>-averages of lag 2 to lag 5 and cardiovascular mortality. Chernivtsi was excluded here since PM data were not available. Augsburg showed a statistically significant increase in deaths due to cardiovascular diseases in association with increases in PM<sub>2.5</sub>, PM<sub>10</sub> and PM<sub>2.5–10</sub>; whereas Dresden showed decreases in the relative risk of



**Fig. 1.** A) Percent change in the city-specific and pooled effect estimates of respiratory mortality with each IQR increase in UFP and PNC, 6-day average. B) Percent change in the city-specific and pooled effect estimates of cardiovascular mortality with each IQR increase in PM<sub>2.5</sub>, PM<sub>10</sub> and PM<sub>2.5–10</sub>, average of lag 2–5. \*Prague: PNC 20–500 nm.

cardiovascular mortality with increases in PM<sub>2.5</sub> and PM<sub>10</sub>. Non-significant but positive effect estimates were found for Ljubljana. Prague showed no association between PM<sub>2.5</sub>, PM<sub>2.5–10</sub> or PM<sub>10</sub> and cardiovascular mortality.

Moreover, city-specific effect estimates for Augsburg showed a significant five-days delayed increase in cardiovascular mortality by 6.0% [1.0%; 11.4%] in association with an IQR increase in UFP (Supplemental Fig. 3).

Effect modification by age, sex and season were only investigated for respiratory mortality and the 6-day average of UFP and for cardiovascular mortality and the PM<sub>2.5</sub>-average of lag 2 to lag 5 since UFP and PM<sub>2.5</sub> were of primary interest and strongest effects were observed for these cumulative lags. Effects of UFP and PM<sub>2.5</sub> were not significantly modified by age, sex and season (Supplemental Table 4). However, effects of UFP as well as PM<sub>2.5</sub> seemed to be driven by the older age group and females. But, due to limited power the results of effect modification analyses should be interpreted with caution.

### 3.2. Sensitivity analyses and two-pollutant models

Table 5 shows the results of the sensitivity analyses for respiratory mortality and the 6-day average of UFP and cardiovascular mortality and the PM<sub>2.5</sub>-average of lag 2 to lag 5.

- (1) Increasing the degrees of freedom for the smooth function of trend decreased the pooled effect estimate for UFP and respiratory mortality and to a lower extent the pooled effect estimate for PM<sub>2.5</sub> and cardiovascular mortality. Using fewer degrees of freedom for the trend did not change the effect estimates.
- (2) Increasing the degrees of freedom for smooth functions of air temperature and relative humidity slightly increased the pooled effect estimate for UFP and respiratory mortality, whereas weakened the association between PM<sub>2.5</sub> and cardiovascular mortality.
- (3) Replacing air temperature and relative humidity by apparent temperature in the model slightly decreased the pooled effects of UFP on respiratory mortality and marginally increased the pooled effects of PM<sub>2.5</sub> on cardiovascular mortality.

**Table 5**

Sensitivity analyses, percent change (95%-CI) in respiratory mortality per IQR increase in UFP and percent change in cardiovascular mortality per IQR increase in PM<sub>2.5</sub>.

Sensitivity analysis	UFP <sup>a</sup> and respiratory mortality (6-day average)	PM <sub>2.5</sub> <sup>b</sup> and cardiovascular mortality (average of lag 2–5)
Original model	9.9 (–6.3; 28.8)	3.0 (–2.7; 9.1)
More DF <sup>c</sup> (DF = 6 df/year) for smooth function of trend	5.6 (–9.0; 22.4)	2.6 (–3.9; 9.7)
Fewer DF <sup>c</sup> (DF = 3 df/year) for smooth function of trend	9.9 (–9.3; 33.1)	2.9 (–2.7; 8.9)
More DF <sup>c</sup> (DF = 5) for smooth functions of meteorological variables	10.9 (–5.9; 30.6)	1.9 (–2.5; 6.5)
Use of apparent temperature	7.5 (–10.7; 29.4)	3.4 (–2.6; 9.7)
Adjusting for air temperature by using temperature above the median for heat effects and below the median for cold effects	14.1 (–2.1; 32.9)	1.3 (–2.1; 4.8)
Adjusting for air temperature and relative humidity average of lag 0–1 and average of lag 2–5	11.8 (–6.7; 34.0)	2.1 (–3.9; 8.4)
Inclusion of barometric pressure	4.1 (–14.7; 27.0)	3.2 (–3.4; 10.2)

Average interquartile range for UFP across all cities: 2750 particles/cm<sup>3</sup>.

Average interquartile range for PM<sub>2.5</sub> across all cities: 12.4 µg/m<sup>3</sup>.

<sup>a</sup> Ultrafine particles with a size range of 0.02 to 0.1 µm in aerodynamic diameter (20–100 nm).

<sup>b</sup> Particulate matter with a size range of <2.5 µm in aerodynamic diameter.

<sup>c</sup> Degrees of freedom.

- (4) Adjusting for air temperature by using temperature above the median for heat effects and below the median for cold effects strengthened the association between UFP and respiratory mortality. Pooled effect estimates for PM<sub>2.5</sub> and cardiovascular mortality decreased when using this method.
- (5) Adjusting for air temperature and relative humidity average of lag 0–1 and average of lag 2–5 strengthened the association between UFP and respiratory mortality; whereas, weakened the association between PM<sub>2.5</sub> and cardiovascular mortality.
- (6) Additionally adjusting for barometric pressure decreased the pooled effect estimate of respiratory mortality in association with UFP increases. The association between PM<sub>2.5</sub> and cardiovascular mortality slightly increased when barometric pressure was included in the model.
- (7) Effect estimates for Augsburg and Prague did not change when the data set with imputed missing data was used (data not shown).
- (8) Results of second- and third-degree polynomial distributed lag models supported delayed and prolonged associations between UFP and PM<sub>2.5</sub> and cardio-respiratory mortality (Supplemental Figs. 4 and 5).

We analysed two-pollutant models for PM<sub>2.5</sub> and UFP as well as for UFP and NO<sub>2</sub> in order to test interdependencies of the pollutants. UFP and PM<sub>2.5</sub> were moderately correlated in all cities with  $r_s \leq 0.4$  (Supplemental Table 2). The correlation between UFP and NO<sub>2</sub> was also moderate in all cities with  $r_s \leq 0.5$ . Since we did not find an association between PM<sub>2.5</sub> or UFP and natural mortality, we only calculated two-pollutant models for cause-specific mortality. The association between UFP and cause-specific mortality was similar in single- and two-pollutant models with PM<sub>2.5</sub> (Fig. 2). Including NO<sub>2</sub> strengthened the decrease in cardiovascular and the increase in respiratory mortality in association with UFP. The association between PM<sub>2.5</sub> and cause-specific mortality was similar in single- and two-pollutant models with UFP.

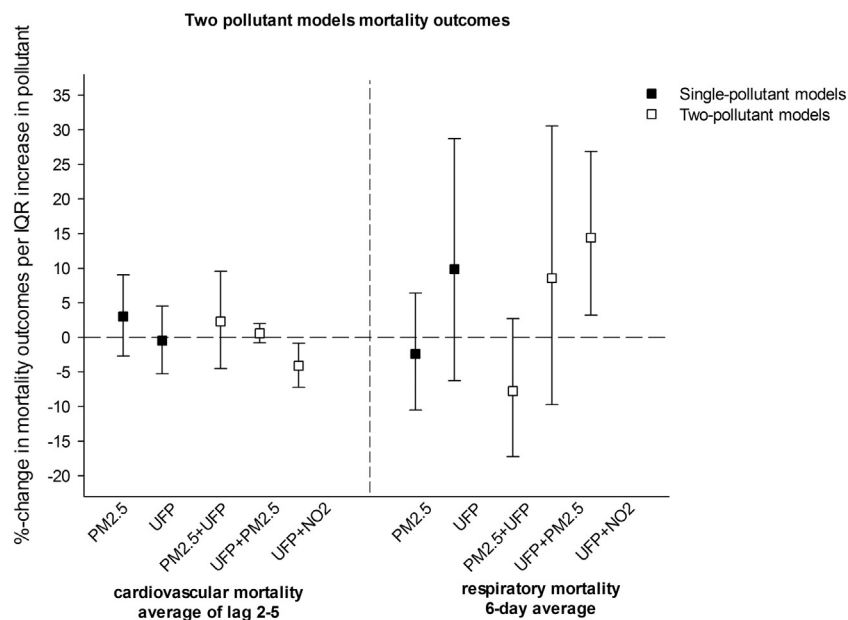
## 4. Discussion

### 4.1. Summary

We observed positive but not statistically significant associations between exposures to UFP and respiratory mortality. Our results indicated a delayed and prolonged association between UFP and respiratory mortality (9.9% [–6.3%; 28.8%] increase in association with a 2750 particles/cm<sup>3</sup> increase in the 6-day average of UFP). Effect estimates for PNC and respiratory mortality were weaker, but consistent. Moreover, findings pointed to a delayed increase in cardiovascular mortality (3.0% [–2.7%; 9.1%]) per 12.4 µg/m<sup>3</sup> increment in the PM<sub>2.5</sub>-average of lag 2 to lag 5. Results of PM<sub>10</sub> were similar, but we observed heterogeneity in the pooled effect estimates of PM<sub>2.5</sub> as well as PM<sub>10</sub> and cardiovascular mortality. A 4.7 µg/m<sup>3</sup> increase in the PM<sub>2.5–10</sub>-average of lag 2 to lag 5 led to a significant increase by 4.1% [0.4%; 8.0%] in cardiovascular mortality.

### 4.2. Associations between air pollutants and (cause-specific) mortality

Short-term exposure to fine particles has been shown to be associated with natural all-cause and cause-specific mortality (Rückerl et al., 2011; WHO, 2013a). For example, studies conducted in 112 U.S. cities (Zanobetti and Schwartz, 2009) or in 10 areas of the European Mediterranean Region (Samoli et al., 2013) found increases in natural mortality of 1.0% and 0.6% per 10 µg/m<sup>3</sup> increment in PM<sub>2.5</sub>, respectively. A recent meta-analysis by Atkinson et al. (2014) based on estimates from single-city and multi-city studies worldwide confirmed previous findings. They reported increases of 1.0% in all-cause and 1.5% in respiratory



**Fig. 2.** Percent change in cause-specific mortality per IQR increase in air pollutants using single- and two-pollutant models. PM<sub>2.5</sub>: main effects of PM<sub>2.5</sub>, UFP: main effects of UFP, PM<sub>2.5</sub> + UFP: effects of PM<sub>2.5</sub> adjusted for UFP, UFP + PM<sub>2.5</sub>: effects of UFP adjusted for PM<sub>2.5</sub>, UFP + NO<sub>2</sub>: effects of UFP adjusted for NO<sub>2</sub>.

mortality in association with a 10 µg/m<sup>3</sup> increase in PM<sub>2.5</sub>. In contrast to these studies we did not observe an association between PM<sub>2.5</sub> and natural or respiratory mortality as other single-city studies conducted in Prague or Erfurt, Germany, reported (Branis et al., 2010; Peters et al., 2009). However, it is important to point out that the confidence intervals are wide and do include the estimates obtained in meta-analyses.

Moreover, Atkinson et al. (2014) reported a summary increase in cardiovascular mortality by 2.3% [1.2%; 3.3%] per 10 µg/m<sup>3</sup> increment in PM<sub>2.5</sub> for the European Region based on estimates from studies conducted in Austria, the Czech Republic, France, Spain and the UK. We observed a similar increase in cardiovascular mortality (pooled effect estimate of 2.4% [−2.3%; 7.3%]) in association with the same increment in PM<sub>2.5</sub>. However, studies conducted in Prague or London, UK, reported no evidence of associations between PM<sub>2.5</sub> and cardiovascular mortality (Atkinson et al., 2010; Branis et al., 2010). Comparing our results to a national U.S. analysis, our findings for PM<sub>2.5</sub> and cardiovascular mortality are comparable to U.S. regions with a dry climate as New Mexico, Arizona and Nevada (lag 0–1: 3.1% [−0.02%; 6.3%]) and to U.S. regions with a dry together with continental climate as Montana, Idaho, Wyoming, Utah and Colorado (lag 0–1: 1.7% [−0.8%; 4.2%]) (Zanobetti and Schwartz, 2009). However, we have found strongest associations for the PM<sub>2.5</sub>-average exposure of lag 2–5, whereas no association for the 2-day average of lag 0–1. Other studies have found an association for the 2-day average (Zanobetti and Schwartz, 2009), but also for the average of lag 2–5 and the 6-day average exposure (Samoli et al., 2013). For example Samoli et al. (2013) observed an increase in the association between PM<sub>2.5</sub> and cardiovascular mortality with longer cumulative lag periods. Furthermore, we observed an increase in cardiovascular mortality with an increase in the PM<sub>2.5–10</sub>-average of lag 2–5. Zanobetti and Schwartz (2009) also reported an association between PM<sub>2.5–10</sub> and cardiovascular mortality in 47 U.S. cities and Samoli et al. (2013) observed a non-significant but positive association between PM<sub>2.5–10</sub> and cardiovascular mortality in the European Mediterranean Region. Moreover, in general there is growing evidence on the association between PM<sub>2.5–10</sub> and natural and cause-specific mortality (Adar et al., 2014).

Evidence from epidemiological studies on UFP and mortality is still limited. A small number of studies on UFP and cause-specific mortality also reported increases in the risk of respiratory mortality (Atkinson et al., 2010; Stolzel et al., 2007; Wichmann et al., 2000). Wichmann

et al. (2000) observed a significant increment of 15.5% [5.5%; 26.4%] in respiratory mortality per IQR increase in UFP of 12,690 particles/cm<sup>3</sup> with a delay of one day in Erfurt. Associations between UFP and respiratory mortality in Erfurt were also shown by Stolzel et al. (2007) for an extended period of the previous study by Wichmann et al. (2000). An IQR increase of 9748 particles/cm<sup>3</sup> in UFP led to an immediate increase of 5.0% [−1.9%; 12.3%] and to a one-day delayed increase of 5.3% [−1.4%; 12.4%] in the relative risk of respiratory mortality. However, in our study the association between UFP and respiratory mortality was more delayed compared to previous studies. We observed a 9.9% [−6.3%; 28.8%] increase in respiratory mortality in association with a 2750 particles/cm<sup>3</sup> increase in the 6-day average of UFP. Studies also reported increases in all-cause or natural as well as cardiovascular deaths in association with increases in UFP or different size ranges of PNC (Atkinson et al., 2010; Breitner et al., 2009; Breitner et al., 2011; Forastiere et al., 2005; Stolzel et al., 2007; Wichmann et al., 2000). In contrast to previous studies, our pooled effect estimates did not show any associations. However, city-specific effect estimates for Augsburg showed five-days delayed effects of UFP on cardiovascular mortality (6.0% [1.0%; 11.4%]). We assume that our non-significant results for the other cities might be at least partly due to missing data and insufficient statistical power. However, Branis et al. (2010) also found no associations between PNC and total, cardiovascular and respiratory mortality in Prague. Moreover, a Finish study conducted in Helsinki reported only weak associations between PNC in the size range 30–100 nm and cause-specific mortality (Halonen et al., 2009).

We found heterogeneous effects of PM<sub>2.5</sub> and PM<sub>10</sub> on cardiovascular mortality between the cities, in particular between Augsburg and Dresden. While Augsburg showed a significant increase in the relative risk of cardiovascular mortality with increases in PM<sub>2.5</sub> and PM<sub>10</sub>, Dresden showed negative effect estimates. The negative effects in Dresden were more pronounced during April–September but were also there during the colder period from October–March. There are no plausible biological mechanisms explaining a protective effect of PM<sub>2.5</sub> and PM<sub>10</sub> on the cardiovascular system, the heterogeneous findings might be due to different sources and compositions of PM<sub>2.5</sub> and PM<sub>10</sub>. PM<sub>2.5</sub> and PM<sub>10</sub> might be influenced by a local source that could be more pronounced in Dresden compared to the other cities. Additional analyses on the source apportionment are necessary to support this assumption. Moreover, the air mass origin might also play a role in the heterogeneity



of the results. Nevertheless, we cannot exclude uncontrolled residual confounding or chance as possible explanations especially since we observed no heterogeneous effects of UFP.

However, the seasonal variation of UFP differed between the cities. New particle formation events during summer were observed particularly in Dresden and Prague which might be an explanation for the high UFP concentrations during summer in these two cities. Moreover, leaf burning might lead to the high UFP levels in autumn in Chernivtsi. The high PM<sub>2.5</sub> concentration in Ljubljana during winter might be explained by temperature inversion.

#### 4.3. Plausible biological mechanisms

Fine and ultrafine particles can cause oxidative stress in the lungs which can further lead to lung inflammation (Brook et al., 2010; Newby et al., 2015; Rückerl et al., 2011). Oxidative stress has also been suggested to play a role in the development of certain lung diseases as asthma (Mazzoli-Rocha et al., 2010). In general, the extra-pulmonary effects of PM and UFP are explained by three pathways. First, systemic oxidative stress and inflammation may be caused by the release of pro-inflammatory mediators or vasoactive molecules from lung cells upon fine and UFP exposure. This may lead to a change in vascular tone (endothelial dysfunction), adverse cardiac outcomes, and a pro-coagulation state with thrombus formation and ischemic response as well as promotion of atherosclerotic lesions as suggested by Utell et al. (2002). Second, an imbalance of the autonomic nervous system or heart rhythm may occur due to fine and UFP deposited in the pulmonary tree. These effects can be either triggered directly, by stimulating pulmonary neural reflexes (Widdicombe and Lee, 2001), or indirectly, by provoking oxidative stress and inflammation in the lung. Third, translocation of UFP and PM constituents into the blood may cause endothelial dysfunction and vasoconstriction, increased blood pressure and platelet aggregation (Brook et al., 2010; Rückerl et al., 2011). Once in the circulation, UFP might also have direct effects on the heart and other organs.

It is assumed that the three pathways do not act independently. Moreover, it is very likely that UFP are also linked to different biological pathways than fine particles because of the different deposition pattern and the fact that UFP are not well recognized and cleared by the immune system and can escape natural defence mechanisms. In contrast to larger particles, UFP have a higher biological reactivity and surface area and by reaching the bloodstream UFP can be transported to other organs (Brook et al., 2004; HEI, 2013; Rückerl et al., 2011; WHO, 2013a). However, we observed UFP effects rather on the respiratory than cardiovascular system, whereas other studies found an association between UFP and cardiovascular health as well as UFP and respiratory health outcomes (Breitner et al., 2009; Breitner et al., 2011; Stolzel et al., 2007; Wichmann et al., 2000).

#### 4.4. Strengths and limitations

The study presented here was a prospective planned multi-centre effort with the aim to provide two years of continuous time-series data. Within the framework of the UFIREG project the association between UFP and (cause-specific) mortality was investigated at multiple locations using the same UFP measurement device.

Compared to earlier studies in European cities we observed slightly lower PNC values, especially for Prague (Borsos et al., 2012; von Bismarck-Osten et al., 2013). However, it has to be mentioned that we excluded the size range 10–20 nm due to measurement uncertainties. Furthermore, a decreasing trend in PNC in European regions was observed during the last decades (Asmi et al., 2013; Birmili et al., 2015). In contrast to the other cities, the monitoring station in Prague was located at a suburban background site. In all the five cities, exposure was measured at one fixed monitoring site with the same instrumentation following joint standard operating procedures and quality

measures. Exposure misclassification might be possible especially for UFP as it was shown that the spatial variability of UFP was higher than for fine particles. However, PNC showed high temporal correlations across different sites in the city area of Augsburg despite differing magnitudes in space (Cyrys et al., 2008). Birmili et al. (2013) reported low spatial variability in PNC among urban background stations in Dresden. Therefore, it is suggested that UFP exposure of the average population might be adequately characterized by one monitoring site in short-term effect studies like UFIREG if the fixed urban background station is chosen carefully (Cyrys et al., 2008). However, the real location of the stations is always a compromise between the requirements on the type of station and real possibilities as requirements for operation and connection to electricity. Moreover, UFP does not penetrate indoors well and hence indoor exposures may not be well presented (HEI, 2013).

There might be differences in coding of the primary causes of death that might explain the differences in death counts between the countries. For example, it might be possible that in Chernivtsi respiratory diseases were often coded as cardiovascular diseases explaining the low number of deaths due to respiratory diseases during the study period in Chernivtsi. With regard to health effects, different lifestyles might also play an important role. The prevalence of tobacco smoking was higher in Czech Republic and Ukraine compared to Germany and Slovenia (WHO, 2013b). It might be possible that air pollution plays a smaller role with regard to health effects in countries with higher smoking prevalence. Air pollution is responsible for 3.7 million premature deaths worldwide, whereas, tobacco causes 6 million deaths per year (WHO, 2013b).

Due to different starting dates of UFP measurements and because of a delayed availability of health data in Germany it was not possible to use the same analysing periods for all five cities. Moreover, for Chernivtsi only one full year could be investigated due to limited data availability. Due to the short study period we observed effect estimates with relatively large confidence intervals. Especially for investigating rare death cases in smaller cities such as Augsburg, Chernivtsi and Ljubljana it is important for future studies to use longer time periods to produce more precise effect estimates. In our study the small power is partly compensated by the multi-cities and meta-analysis design. Nevertheless, UFIREG was one of the very few multi-centre studies investigating the associations between UFP and fine particles and (cause-specific) mortality including cities from Central and Eastern European countries since most research activities so far were concentrated on Western European countries (HEI, 2013).

## 5. Conclusions

We observed positive but not statistically significant associations between prolonged exposures to UFP and respiratory mortality, which were independent of particle mass exposures. Effects of PM<sub>2.5</sub> on cardiovascular mortality were comparable to results from other European regions and U.S. regions with a dry climate and a dry together with continental climate (Atkinson et al., 2014; Zanobetti and Schwartz, 2009).

The study adds to the growing scientific literature on health effects of UFP and indicates that dedicated efforts are needed allowing time-series data collections over extended periods. Therefore, it is important to integrate UFP into routine measurement networks in order to provide data for future epidemiological studies especially those which will consider spatio-temporal variation in UFP.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.envint.2015.12.006>.

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