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## **Effect of prenatal exposure to fine particles and postnatal indoor air quality on the occurrence of respiratory symptoms in the first two years of life**

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**Abstract:** The purpose of this study was to assess an impact of prenatal exposure to fine particles (PM<sub>2.5</sub>) on the risk of developing respiratory symptoms in early childhood. The study was carried out in a cohort of 465 newborns in Krakow (Poland) who have been followed over the first two years of life. The children exposed to medium level of PM<sub>2.5</sub> (>35.3–53.4 µg/m<sup>3</sup>) had 13% more wheezing days (IRR = 1.13; 95% CI: 1.03–1.23), and those exposed to higher PM<sub>2.5</sub> (>53.4 µg/m<sup>3</sup>) had on average 62% more wheezing days (IRR = 1.62; 95% CI: 1.42–1.86) compared with the low exposure group (≤35.3 µg/m<sup>3</sup>). The presence of moulds in the household (IRR = 1.13; 95% CI: 1.03–1.24), parity (IRR = 1.18; 95% CI: 1.10–1.28), and maternal atopy (IRR = 1.45; 95% CI: 1.28–1.63) were also significantly associated with the number of wheezing days. Children only exposed to higher PM<sub>2.5</sub> (>53.4 µg/m<sup>3</sup>) had significantly more days with difficult breathing (IRR = 1.54; 95% CI: 1.34–1.78). Since that prenatal exposure to fine particles increases burden of respiratory symptoms among infants and young children the current PM<sub>2.5</sub> health air quality guidelines may be too high to protect the sensitive subgroups of population.

**Keywords:** respiratory symptoms; early childhood; prenatal and perinatal exposure; fine particles; indoor air quality; cohort study.

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Umberto Maugeri is currently the President of the Institute for Curative Medicine and Rehabilitation (Salvatore Maugeri Foundation) located in Pavia, Italy. In 1975, he was appointed as the Lecturer of Applied Hygiene at the Engineering Faculty in the Pavia University and since 1983, he is the Acting Professor of this faculty. He received the Medical Doctor's diploma from the Florence University in 1964. Subsequently, he developed his research activities at the Chair of Occupational Medicine of the Florence and Pavia Universities. Professor Maugeri is the author or co-author of about 130 publications in the field of environmental and occupational medicine.

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Susan Edwards is the Coordinator of the Krakow longitudinal cohort study in the USA. She has completed her Masters in Public Health (MPH) at Columbia University's Mailman School of Public Health in the department of Environmental Health Sciences. Her areas of interest include molecular toxicology and epidemiology.

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## **1 Introduction**

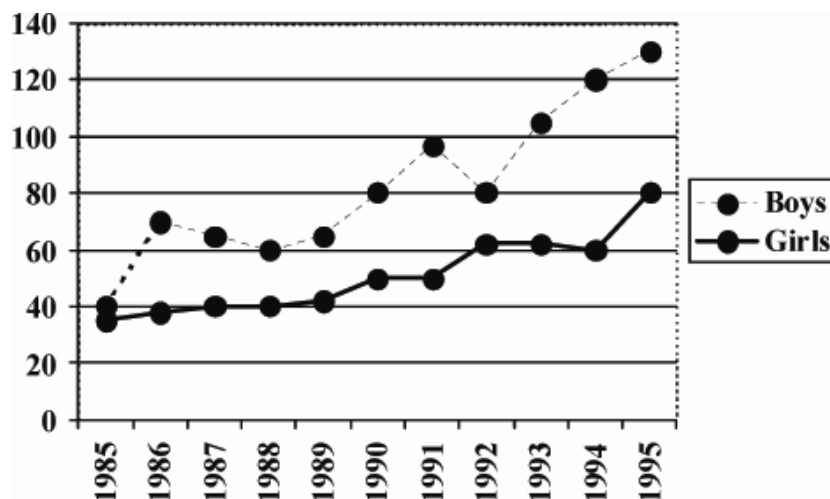
Over the last two or three decades, an increase in the prevalence and severity of respiratory diseases, in particular, asthma in children in many countries has been observed (Burr et al., 1989; Yunginger et al., 1992; Woolcock and Peat, 1997; Holgate, 1999; D'Amato et al., 2001). For example, in Poland we have observed a most significant rise in hospitalisation rates from asthma in very young children (Figure 1).

The annual increase in hospitalisation rates was 12.2% in boys and 10.6% in girls (Jedrychowski et al., 2004) and the highest annual intensification occurred in children under 4 years of age (20.3% for boys and 18.9% for girls).

Since genetic traits probably do not contribute to this rising trend, prenatal and perinatal environmental factors might play a considerable role in the development of respiratory diseases and asthma in early childhood. Reproductive epidemiology provides evidence that the foetus and infants are likely to be significantly more sensitive to a

variety of environmental toxicants than adults. This results from the fact that many environmental toxicants absorbed by mother easily cross the placenta and accumulate in the foetus sometimes at higher concentrations than in mothers (Perera et al., 1998; Perera et al., 2004).

**Figure 1** Hospitalisation rates/100,000 due to asthma in children in the age group under 4 years in Poland



The rising trends in respiratory diseases in children are of great public health concern since respiratory infections in early life can lead to persistent lung damage and a long-term increased susceptibility to various lung diseases in adulthood. Over the last decades, many studies analysed respiratory health in young children in terms of exposure to various air pollutants such as fine particulate matter or Polycyclic Aromatic Hydrocarbons (PAHs). Major sources of these pollutants in indoor air include emissions from the combustion of fuel for residential heating (e.g. coal or wood stoves, fireplaces, kerosene heaters), unvented gas appliances, Environmental Tobacco Smoke (ETS) and fumes from cooking, grilling and frying (Zedeck, 1980; IARC, 1983; Knize et al., 1999; Spengler et al., 2001). Major outdoor sources of emissions, such as automobiles and power plants, which are mainly responsible for fine particles concentrations in outdoor air, affect levels indoors.

However, these studies were mostly concerned with respiratory morbidity in infants and young children in the context of ambient air pollution in the postnatal period. Although there is already some epidemiologic evidence linking prenatal exposure to tobacco smoke with respiratory health of children (Tager et al., 1993; Stick et al., 1996; DiFranza et al., 2004; Miller et al., 2004), there is a scarcity of research on the effects of prenatal and perinatal determinants of respiratory health in early childhood resulting from prenatal exposure to air pollutants measured on an individual basis.

The main purpose of the study was to measure the effect of prenatal exposure to fine particles, assessed in pregnant women with personal dosimeters, on the occurrence of respiratory symptoms in offsprings monitored over the first two years of life.

## **2 Materials and methods**

This study uses data from an earlier established Krakow birth cohort of children, being part of the collaborative study with Columbia University in New York. The design of this cohort prospective study and the detailed selection of the population have been described previously (Jedrychowski et al., 2003). The Ethical Committee of the Jagiellonian University approved the study.

The analysis was carried out in 465 women who gave birth between 29 and 43 weeks of gestation from January 2001 to February 2004. The women attending ambulatory prenatal clinics in the first and second trimesters of pregnancy were eligible for the study. The enrolment included only non-smoking women with singleton pregnancies aged 18–35 years and who were free from chronic diseases such as diabetes and hypertension. Upon enrolment, a detailed questionnaire was administered to each subject at the entry of the study to solicit information on demographic data, house characteristics, medical and reproductive history, occupational hazards and smoking practices of other household components.

After delivery, newborns were followed-up over 2 years and, during this period, trained interviewers visited children's house every 3 months to carry out standardised interviews on infant's health, breast feeding and home environment. All interviews were performed with the mothers of infants and the following respiratory health variables were analysed: (1) cough, irrespective of cold; (2) wheezing or whistling in the chest, irrespective of respiratory infection and (3) difficult breathing. For each of the symptoms, the duration in days over a given period was recorded in the questionnaire.

Maternal education (years of schooling) was treated as a proxy for the socioeconomic status. Maternal atopy was assumed when mother reported allergic skin disorders or allergy-related respiratory diseases. Postnatal ETS was defined as reported ETS at home from any household members and the definition of moulds in the household was based on questions regarding noticeable moisture stains and visible mould growth on the walls within the household reported at the interviews. In the present analysis, ETS and mould variables were ranked as: 0 = no exposure over the follow-up, 1 = exposure present for 6 months, 2 = exposure for 12 months, 3 = exposure for 18 months and 4 = exposure confirmed over the whole postnatal period.

### *2.1 Dosimetry of prenatal personal exposure to fine particles*

During the second trimester, a member of the air monitoring staff instructed the woman for the use of the personal monitor, which is lightweight, quiet and worn in a backpack. The woman was asked to wear the monitor during the daytime for two consecutive days and to place the monitor near the bed at night. During the morning of the second day, the air monitoring staff person and interviewer visited the woman's home to change the battery pack and administer the full questionnaire. They also checked the continuous running of the monitor to avoid technical or operating failures. A staff member returned to the woman's home in the morning of the third day to pick up the equipment.

Personal Environmental Monitoring Sampler (PEMS) was used to measure particle mass, gravimetrically. The PEMS is designed to achieve the target particle size of  $\leq 2.5 \mu\text{m}$  at a flow rate of 2.0 litres per minute over the 48-hour period. Flow rates are calibrated (with filters in place) using a bubble meter prior to the monitoring, and are checked again with a change of the battery pack on the second day and at the conclusion

of the monitoring. Particles were collected on Teflon membrane filter (37 mm Teflo™, Gelman Sciences). The combination of low pressure drop (permitting use of a low power sampling pump), low hygroscopicity (minimising bound water interference in mass measurements) and low trace element background (improving analytical sensitivity) of these filters makes them highly appropriate for personal particle sampling.

To evaluate the relationship between the level of PM<sub>2.5</sub> measured over 48 hours in the second trimester of pregnancy with those in the first and third trimesters, a series of repeated measurements in each trimester was carried out in a subsample of 85 pregnant women who were recruited in the first trimester (Table 1). The data show that the levels of prenatal exposure to PM<sub>2.5</sub> (tertiles of exposure) in the second trimester are well correlated with the measurements taken in the first and third trimesters.

**Table 1** Rank correlation between levels of personal exposure to PM<sub>2.5</sub> (categorised by tertiles of distribution) taken in the subsample of subjects ( $N = 85$ ) with repeated measurements in each trimester of pregnancy

<i>1st trimester</i>	<i>2nd trimester</i>			<i>Total</i>
	<i>Low</i>	<i>Medium</i>	<i>High</i>	
Low	18	3	0	21
Medium	0	38	4	42
High	0	0	22	22
Total	18	41	26	85
Kendall's rank correlation coefficient (tau $b$ ) = 0.910; 95% CI: 0.846–0.974, $p = 0.000$				
<i>3rd trimester</i>	<i>2nd trimester</i>			<i>Total</i>
	<i>Low</i>	<i>Medium</i>	<i>High</i>	
Low	10	0	0	10
Medium	8	39	0	47
High	0	2	26	26
Total	18	41	26	85
Kendall's rank correlation coefficient (tau $b$ ) = 0.881; 95% CI: 0.815–0.946, $p = 0.000$				

## 2.2 Statistical analysis

The purpose of the statistical analysis was to correlate the prenatal exposure to fine particles with respiratory symptoms monitored in children over the first two years of life. To identify potential confounders, associations between population characteristics and outcome variables were investigated. Differences between subgroups, with lower and higher particulate matter exposure, were tested by chi-square statistics (categorical variables) or by  $t$ -test (numerical variables). Dependent variables were counts of observed total number of days a given symptom was present in subsequent periods of the follow-up. Initially, we wanted to assess the effect of prenatal exposure on respiratory events in children at 3, 6, 9, 12, 15, 18, 21 and 24 months of age using the Poisson distribution, but the observed over-dispersion index (variance/mean) showed that the Poisson distribution would be an inappropriate model. Therefore in the further course of the statistical analysis, we applied the negative binomial regression models, which accommodate over-dispersion and are now widely used to model over-dispersed count data in many disciplines (Liang and Zeger, 1986; Kohler and Kreuter, 2005; Rabe-Hesketh and Skrondal, 2005). The effect of prenatal exposure on respiratory events

recorded over the follow-up was assessed by the Generalised Estimating Equations (GEE) model. GEE model estimated regression coefficients, taking into account the correlation between symptoms at various ages of children and computed regression coefficients of dependent variable (individual respiratory symptoms) and the main PM<sub>2.5</sub> exposure variable, which was divided into three exposure levels: (1) low level, i.e. below median ( $\leq 35.3 \mu\text{g}/\text{m}^3$ ); (2) medium level, in the range between median and 75th percentile value ( $>35.3\text{--}53.4 \mu\text{g}/\text{m}^3$ ) and (3) high level, above 75th percentile ( $>53.4 \mu\text{g}/\text{m}^3$ ). In the GEE models, a set of potential confounders or modifiers (maternal education, maternal atopy, parity, gender of child, postnatal ETS and moulds in the household) was taken into consideration. Association between dependent and independent variables was expressed as IRR and standard errors were scaled using square root of deviance-based dispersion to allow for possible over-dispersion. Statistical analyses were performed with Stata version 10 (data analysis and statistical software) for Windows (2007).

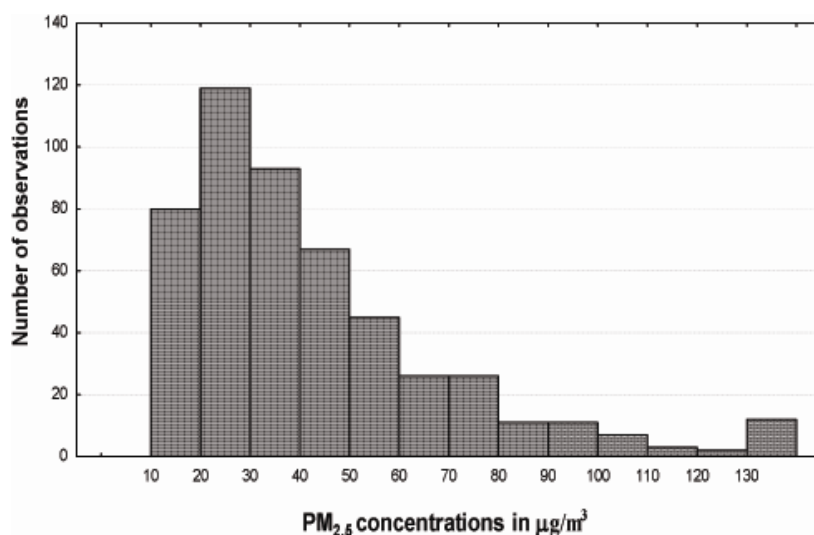
### 3 Results

Personal measurements of prenatal exposure to PM<sub>2.5</sub> particles in the study population were within a wide range of  $10.3 \mu\text{g}/\text{m}^3\text{--}294.9 \mu\text{g}/\text{m}^3$  (Figure 2), with the median of  $35.3 \mu\text{g}/\text{m}^3$  (95% Confidence Interval (CI): 33.3–37.5).

Only self-reported prenatal and postnatal ETS significantly differentiated the groups with higher and lower particulate matter exposure (Table 2).

Cough irrespective of cold was most frequently reported symptom over the follow-up; difficult breathing and wheezing was less frequently reported. Mean duration of various respiratory symptoms in 1-year olds was about the same (17–22 days), however in the 2-year olds, coughing persisted twice as long as difficult breathing or wheezing. Each of the respiratory symptoms lasted longer in children whose mothers were exposed to higher levels of PM<sub>2.5</sub> during pregnancy (Tables 3 and 4).

**Figure 2** Personal exposure to fine particles measured in the second trimester of pregnancy



**Table 2** Characteristics of the study sample by the prenatal PM<sub>2.5</sub> level

		Total (N = 465)	PM <sub>2.5</sub> level		p-value
			≤35.28 µg/m <sup>3</sup> (N = 235)	>35.28 µg/m <sup>3</sup> (N = 230)	
Maternal age	Mean	27.63	27.60	27.66	0.8521
	SD	3.519	3.337	3.702	
Maternal education (years)	Mean	15.57	15.75	15.39	0.1466
	SD	2.687	2.493	2.866	
Maternal atopy	(-) n (%)	351 (75.5)	173 (73.6)	178 (77.4)	0.4020
	(+) n (%)	114 (24.5)	62 (26.4)	52 (22.6)	
Gender	Boys n (%)	233 (50.1)	121 (51.5)	112 (48.7)	0.6103
	Girls n (%)	232 (49.9)	114 (48.5)	118 (51.3)	
Parity	1 n (%)	289 (62.2)	152 (64.7)	137 (59.6)	0.2976
	≥2 n (%)	176 (37.8)	83 (35.3)	93 (40.4)	
Prenatal ETS	(-) n (%)	342 (73.5)	186 (79.1)	156 (67.8)	0.0078
	(+) n (%)	123 (26.5)	49 (20.9)	74 (32.2)	
Moulds at home (-)	n (%)	405 (87.1)	203 (86.4)	202 (87.8)	0.9416
Present only 6 months	n (%)	36 (7.7)	20 (8.5)	16 (7.0)	
Present up to 12 months	n (%)	14 (3.0)	7 (3.0)	7 (3.0)	
Present 13–24 months	n (%)	10 (2.2)	5 (2.1)	5 (2.2)	
Postnatal ETS (-)	n (%)	386 (83.0)	211 (89.8)	175 (76.1)	0.0000
Present only 6 months	n (%)	16 (3.4)	10 (4.3)	6 (2.6)	
Present up to 12 months	n (%)	13 (2.8)	5 (2.1)	8 (3.5)	
Present 13–24 months	n (%)	50 (10.8)	9 (3.8)	41(17.8)	

**Table 3** Occurrence of respiratory symptoms among children during their first year and second year of life

	Total number of children with a given symptom	Total sum of days	Mean duration per child with the symptom mean (SD)
<i>First year (N = 465)</i>			
Cough	325 (69.9%)	6302	19.39 (21.99)
Difficult breathing	141 (30.3%)	1845	13.08 (17.10)
Wheezing in the chest	83 (17.8%)	1205	14.52 (20.96)
<i>Second year (N = 465)</i>			
Cough	381 (81.9%)	8595	22.56 (21.85)
Difficult breathing	99 (21.3%)	1048	10.59 (14.09)
Wheezing in the chest	71 (15.3%)	844	11.89 (12.06)
<i>Total (N = 465)</i>			
Cough	420 (90.3%)	14,897	35.47 (33.79)
Difficult breathing	201 (43.2%)	2893	14.39 (18.40)
Wheezing in the chest	125 (26.9%)	2049	16.39 (21.99)

**Table 4** Occurrence of respiratory symptoms among children during their first year and second year of life by prenatal PM<sub>2.5</sub> level

Symptoms	Total number of children with a given symptom		Duration in days mean (SD)	
	Low PM <sub>2.5</sub> (≤35.28 µg/m <sup>3</sup> )	High PM <sub>2.5</sub> (>35.28 µg/m <sup>3</sup> )	Low PM <sub>2.5</sub> (≤35.28 µg/m <sup>3</sup> )	High PM <sub>2.5</sub> (>35.28 µg/m <sup>3</sup> )
<i>First year (N = 465)</i>				
Cough	154 (65.5%)	171 (74.3%)	19.32 (23.21)	19.45 (19.86)
Difficult breathing	67 (28.5%)	74 (32.2%)	11.75 (16.26)	14.30 (17.84)
Wheezing in the chest	41 (17.4%)	42 (18.3%)	12.19 (21.42)	16.79 (20.50)
<i>Second year (N = 465)</i>				
Cough	187 (79.6%)	194 (84.3%)	21.44 (18.63)	23.64 (24.56)
Difficult breathing	47 (20.0%)	52 (22.6%)	9.64 (9.54)	11.44 (17.26)
Wheezing in the chest	33 (14.0%)	38 (16.5%)	9.15 (4.93)	14.26 (15.54)
<i>Total (N = 465)</i>				
Cough	208 (88.5%)	212 (92.2%)	33.58 (32.52)	37.32 (34.96)
Difficult breathing	98 (41.7%)	103 (44.8%)	12.65 (16.31)	16.05 (20.12)
Wheezing in the chest	60 (25.5%)	65 (28.3%)	13.37 (20.31)	19.18 (23.24)

The adjusted IRR for individual respiratory symptoms related to PM<sub>2.5</sub> exposure level estimated from the GEE models are presented in Tables 5–7.

No relationship was found between the adjusted risk of coughing and medium or higher PM<sub>2.5</sub> exposure level (Table 5), but only with parity (IRR = 5.04; 95% CI: 2.69–9.43).

**Table 5** Occurrence of cough (number of days cough symptom occurred) reported for children over the two years related to main exposure variables and confounders (the GEE population-averaged negative binomial model)

Predictor variables	IRR	Std. err.	z	P > z	95% conf. interval
Maternal education*	0.837	0.101	-1.48	0.139	0.661–1.060
Parity**	5.036	1.612	5.05	0.000	2.670–9.430
Gender of child***	0.735	0.219	-1.03	0.301	0.410–1.317
Medium PM <sub>2.5</sub> level (35.3–53.4 µg/m <sup>3</sup> )	1.973	0.767	1.75	0.080	0.92–4.226
Higher PM <sub>2.5</sub> level (>53.4 µg/m <sup>3</sup> )	1.434	0.550	0.94	0.348	0.676–3.043
Postnatal ETS****	1.324	0.238	1.56	0.118	0.931–1.882
House moulds****	1.811	0.621	1.73	0.084	0.924–3.548
Maternal atopy*****	2.054	0.782	1.89	0.059	0.974–4.330

N = 3720 measurements, number of groups = 465.

\*Maternal: education years of schooling.

\*\*Parity: 0 = first child, 1 = other.

\*\*\*Boys = 0, girls = 1.

\*\*\*\*0 = no exposure over the follow-up, 1 = exposure present for 6 months, 2 = exposure for 12 months, 3 = exposure for 18 months and 4 = exposure confirmed over the whole postnatal period.

\*\*\*\*\*Maternal atopy absent = 0, maternal atopy present = 1.

The risk of wheezing symptoms was significantly associated with the prenatal exposure to PM<sub>2.5</sub>. Children exposed to medium level of PM<sub>2.5</sub> (>35.3–53.4 µg/m<sup>3</sup>) had 13% more wheezing days (IRR = 1.13; 95% CI: 1.03–1.23), those exposed to higher PM<sub>2.5</sub> (>53.4 µg/m<sup>3</sup>) had on average 62% more wheezing days (IRR = 1.62; 95% CI: 1.42–1.86) compared to the low exposure group (≤35.3 µg/m<sup>3</sup>). Presence of moulds in the household (IRR = 1.13; 95% CI: 1.03–1.24), parity (IRR = 1.18; 95% CI: 1.10–1.28) and maternal atopy (IRR = 1.45; 95% CI: 1.28–1.63) were also significantly associated with the number of wheezing (Table 6). The occurrence of wheezing was lower in girls (IRR = 0.83; 95% CI: 0.77–0.89).

The risk of difficult breathing was associated with the higher PM<sub>2.5</sub> level (IRR = 1.54; 95% CI: 1.34–1.78) compared to the low exposure group (≤35.3 µg/m<sup>3</sup>). The risk of difficult breathing was also associated with parity (IRR = 1.41; 95% CI: 1.28–1.55), with the presence of moulds in the household (IRR = 1.35; 95% CI: 1.19–1.54) and maternal atopy (IRR = 1.39; 95% CI: 1.22–1.60). Like with other respiratory symptoms, the difficult breathing occurred less frequently in girls (Table 7).

**Table 6** Occurrence of wheezing in chest (duration in days) in children over the 2-year follow-up related to exposure variables and confounders (the GEE population-averaged, negative binomial model)

<i>Predictor variables</i>	<i>IRR</i>	<i>Std. err.</i>	<i>z</i>	<i>P &gt; z</i>	<i>95% conf. interval</i>
Maternal education	0.979	0.014	1.54	0.124	0.952–1.006
Parity	1.184	0.045	4.49	0.000	1.099–1.275
Gender of child	0.829	0.032	−4.88	0.000	0.768–0.894
Medium PM <sub>2.5</sub> level (35.3–53.4 µg/m <sup>3</sup> )	1.127	0.050	2.67	0.008	1.032–1.230
Higher PM <sub>2.5</sub> level (>53.4 µg/m <sup>3</sup> )	1.622	0.112	7.03	0.000	1.418–1.857
Postnatal ETS	0.989	0.019	−0.57	0.569	0.954–1.026
House moulds	1.133	0.054	2.63	0.008	1.032–1.244
Maternal atopy	1.446	0.090	5.94	0.000	1.280–1.633

*N* = 3720 measurements, number of groups = 465.

\*Predictors variables were defined as in Table 5.

**Table 7** Occurrence of difficult (puffy) breathing (duration in days) in children over the 2-year follow-up related to exposure variables and confounders (in the GEE population-averaged negative binomial model)

<i>Predictor variables</i>	<i>IRR</i>	<i>Std. err.</i>	<i>Z</i>	<i>P &gt; z</i>	<i>95% conf. interval</i>
Maternal education	0.997	0.017	−0.17	0.864	0.965–1.030
Parity	1.408	0.069	6.97	0.000	1.279–1.551
Gender of child	0.822	0.038	−4.28	0.000	0.751–0.899
Medium PM <sub>2.5</sub> level (35.3–53.4 µg/m <sup>3</sup> )	1.090	0.058	1.63	0.103	0.983–1.209
Higher PM <sub>2.5</sub> level (>53.4 µg/m <sup>3</sup> )	1.543	0.114	5.89	0.000	1.335–1.782
Postnatal ETS	1.011	0.024	0.46	0.646	0.964–1.060
House moulds	1.353	0.091	4.49	0.000	1.186–1.544
Maternal atopy	1.393	0.094	4.91	0.000	1.220–1.590

*N* = 3720 measurements, number of groups = 465.

\*Predictors variables were defined as in Table 5.

#### 4 Discussion

Analysis of personal air samples collected from pregnant female residents in Krakow showed that PM<sub>2.5</sub> median exposure averaged 35.3 µg/m<sup>3</sup> with a wide range of concentrations between 10.3 and 249.9 µg/m<sup>3</sup>. Up to 17% of study subjects was exposed to fine particle levels above the EPA guidelines (65.0 µg/m<sup>3</sup>) for daily exposure (EPA, 2004) and 28.2% was exposed to levels above the WHO standards (50 µg/m<sup>3</sup>) (WHO, 2005). The study has demonstrated that the prenatal exposure to fine particles was significantly associated with the high incidence of wheezing symptoms recorded systematically over eight 3-month periods in the first two years of infants' life and the relationship showed a clear dose–effect pattern. The impact of the exposure on the occurrence of difficult breathing was only seen in the group exposed to higher PM<sub>2.5</sub> level (above 53.4 µg/m<sup>3</sup>). It is important to mention that the analysis repeated in the strata of children without both ETS and house mould exposure (data not presented) produced the same estimates of PM<sub>2.5</sub> exposure on wheezing and difficult breathing. The effect of prenatal PM<sub>2.5</sub> exposure on cough was insignificant. Although the magnitude of the prenatal exposure on respiratory symptoms was relatively small, it may have a biological relevance since persistent wheezing symptoms in early childhood are associated with poorer lung function and respiratory health in early childhood and, possibly, in adulthood as well (Dezateux and Stocks, 1997; Stick, 2000; Love et al., 2005).

Apart from the prenatal PM<sub>2.5</sub> exposure, the risk of wheezing and puffy breathing increased with parity, the presence of moulds in house and maternal atopy. Neither the effects of the prenatal or postnatal ETS were significantly associated with symptom incidence nor was this due to the fact that both correlated with the prenatal PM<sub>2.5</sub>. Although Spearman correlation coefficient between prenatal ETS and PM<sub>2.5</sub> was lower ( $r = 0.21$ ) than that between postnatal ETS and PM<sub>2.5</sub> ( $r = 0.26$ ), the difference was statistically insignificant ( $z = 0.805$ ,  $p = 0.421$ ).

The data argue for the hypothesis that higher risk of respiratory symptoms in young children may result from biological effects of prenatal PM<sub>2.5</sub> exposure on developing foetus or in early infancy. The biological mechanisms whereby prenatal PM<sub>2.5</sub> might lead to excessive respiratory outcomes are yet unclear. Studies in animals and human beings have revealed strong proinflammatory effects of particulate matter involving lung epithelial cells and alveolar macrophages through oxidant pathways and subsequent stimulation of cytokine and mediator release (Holgate, 2005). It is well established that inhalation of particles with a Mass Median Aerodynamic Diameter (MMAD) of 10 µm or less is associated with bronchiolar irritation and lower tract infections, while exposure to particles of 2.5 µm and smaller exhibit a stronger epidemiological link with respiratory inflammatory effects. Particles even smaller, 0.1 µm or less, are thought to move beyond the respiratory system and may reach the bloodstream and cross placenta.

It was recently documented in one of our studies that personal prenatal PM<sub>2.5</sub> exposure of pregnant women in the second trimester of pregnancy was associated with significant foetal growth retardation in terms of lower weight, length and head circumference at birth (Jedrychowski et al., 2005), which might be related to impaired lung growth and increased risk of lower respiratory tract infections in early childhood. Newborn infants with evidence of intrauterine growth retardation have reduced lung function in childhood and more spells of pneumonia or bronchiolitis (Rona et al., 1993; Shaheen et al., 1994). Although it is difficult in epidemiological studies to establish the association between ambient air pollution, impaired lung growth in utero and adult

lung function, it was shown that the schoolchildren born in the urban areas with higher ambient air pollution had lower adjusted lung function and slower rate of body growth in preadolescence than those born in lower polluted areas (Jedrychowski et al., 1999; Jedrychowski et al., 2002).

PM<sub>2.5</sub> may be treated as a proxy measure of a whole complex of toxic agents present in the environment because it contains constituents of soots including PAHs, tobacco, wood smoke, organic compounds, sulphates and metals (Spengler et al., 2001). Absorbed toxic air pollutants may affect DNA as evidenced by the observations that placental DNA adducts are more common in areas with higher levels of pollution and that altered foetal growth has been associated with PAH-DNA adducts (Perera et al., 2004). It is believed that an inhibition of the production of immunocompetent cells is one of the important mechanisms contributing to the immunosuppression due to air pollutants. For example, exposure to PAHs inhibits the differentiation of human monocytes, but mature differentiated immune cells also constitute targets for PAHs. Prenatal exposure to xenobiotics may enhance allergic reactions within the bronchial tract and may subsequently be responsible for an increased susceptibility of newborns and young infants to respiratory infections (Ward et al., 1984; Wojdani and Alfred, 1984; Lyte and Bick, 1986; Davila et al., 1996; Nel et al., 1998; Saxon and Diaz-Sanchez, 2000; Devouassoux et al., 2002; Laupeze et al., 2002; Van Grevenynghe et al., 2003).

The weakness of our study results from the fact that we could not unmistakably distinguish the effect of prenatal PM<sub>2.5</sub> exposure from that of the postnatal exposure since the PM<sub>2.5</sub> postnatal measurements were not repeated. Therefore, we are not certain whether our findings represent delayed effects of prenatal PM<sub>2.5</sub> exposure on infants or more immediate effects of postnatal PM<sub>2.5</sub> exposure over the first two years of life. Moreover, postnatal air indoor quality was only based on questionnaire data regarding passive smoking and the presence of moulds in the households.

To underline strength of our study, we have to mention very careful design which enabled us to limit measurement error in estimating realistic prenatal exposure to fine particles by assigning personal exposure level to each child. The personal monitoring of ambient PM<sub>2.5</sub> exposure is a highly relevant measure of individual exposure incorporating outdoor and indoor exposure. Since there was very good agreement between the PM<sub>2.5</sub> measurements in the second and the first and third trimesters of pregnancy, we think the measured fine particles in the second pregnancy trimester reflected the true gradient of prenatal exposure between individuals under study. Previous studies have attempted to quantify the concentration of air pollutants measured in the residence area and assign these exposure values to the study subjects. Estimating individual average exposures during specific gestational months by relying on the ambient air monitoring stations even close to the maternal residence may result in exposure misclassification.

Furthermore, in our study important potential confounders of the relationship between prenatal ambient risk factors and the respiratory outcomes of infants such as chronic diseases or active tobacco smoking by mothers have been removed through entry criteria. Other risk factors that are thought to affect the probability of respiratory diseases in infants such as parity, maternal atopy, postnatal indoor air quality have been taken into consideration and the estimates of main effects were adjusted in the multivariate GEE statistical models. The GEE regression model is a new statistical tool for the analysis of longitudinal studies. A great advantage of the GEE model is the fact that regression coefficients are calculated taking into account the correlation between observed number of days a given symptom occurred in subsequent periods of the follow-up.

Summing up, the results of our study demonstrated that prenatal exposure to PM<sub>2.5</sub> produced a biological effect in terms of higher occurrence of respiratory inflammatory symptoms in early childhood and that this effect was independent from that associated with confounders. The study provided additional evidence to suggest that the current PM<sub>2.5</sub> health limits may be too high to protect the sensitive subgroups of population. Planned extension of the study over next years should hopefully provide new arguments to the debate on the eventual need of the revision of air pollutant guidelines.

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