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Thorax published online 11 Feb 2008;
doi:10.1136/thx.2007.085480

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Ambient Air Pollution Triggers Wheezing Symptoms in Infants

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Word count (excluding title page, references and tables): Abstract 237, Main text 3.665.

Key words: air pollution, asthma, infants, traffic, wheeze.

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ABSTRACT

Rationale: There is limited evidence for the role of air pollution in the development and triggering of wheezing symptoms in young children.

Objectives: To study the impact of exposures to air pollution on wheezing symptoms in children under the age of three and with genetic susceptibility to asthma.

Methods and Measurements: Daily symptoms recordings were obtained for a panel of 205 children participating in the birth cohort study Copenhagen Prospective Study on Asthma in Children and living in Copenhagen in the first three years of life. Daily air pollution levels for particulate matter less than 10 μm in diameter (PM_{10}), number concentration of ultrafine particles, nitrogen dioxide (NO_2), nitrogen oxide (NO_x), and carbon monoxide (CO) were available from a central background monitoring station in Copenhagen. The association between incident wheezing symptoms and air pollution on the concurrent and previous 4 days were estimated by a logistic regression model (generalized estimating equation) controlling for temperature, season, gender, age, exposure to smoking, and paternal history of asthma.

Main Results: We found significant positive associations between concentrations of PM_{10} , NO_2 , NO_x , CO and wheezing symptoms in infants (age 0-1) with 3 to 4 days delay. Only the traffic related gasses, NO_2 , NO_x showed significant effects throughout the three years of life, albeit attenuating after the age of one.

Conclusions: Air pollution related to traffic is significantly associated with triggering of wheezing symptoms in the first three years of life.

INTRODUCTION

Recurrent wheeze represents a serious health burden in young children and is the cause of considerable hospitalization and other health care utilizations [1]. Evidence on the role of air pollution in causing such symptoms remains mixed [2, 3]. Particulate air pollution, in terms of PM₁₀ and PM_{2.5} (particulate matter less than 10 and 2.5 µm in diameter), has been linked to asthma exacerbation in school children [4-12], suggesting a role as a trigger of symptoms, although two large multi-centre efforts failed to confirm this [13-14]. Recently, ultrafine particles (UFPs; particles less than 0.1 µm in diameter) generated by traffic emissions were suggested to have particularly strong effects in the airways due to high pulmonary deposition and ability to induce inflammation and oxidative stress [15]. However, the only epidemiological study of UFPs effects in children to date found weak association with asthma symptoms in school children [8], whereas traffic-related air pollution assessed by NO₂ (nitrogen dioxide) exposure or proximity to traffic, has been more consistently associated with asthma exacerbation in school children, suggesting air pollution is an important trigger of symptoms [16-20].

Only a few studies of air pollution effects are available in young children and offer mixed evidence [21-27]. Exaggerated susceptibility of younger children as compared to school children may be suspected due to the development of the lung and the higher ventilation rates in early life [2]. A case-control study has found significant associations between traffic pollution and asthma before the age of three, which attenuated from age three to 14 [20]. Understanding the effects of early air pollution exposures on the development and triggering of wheeze may give important clues to the role in the development of asthma later in life [3]. So far, most studies in young children used geographical variation in air pollutant levels and focused on traffic [20-21, 23-26], only one studied PM₁₀ [22], one O₃ (ozone) [27], with no evidence to date on the effect of UFPs. Two studies in preschool children found an association between soot and incident wheeze, asthma, and ear/nose/throat infections in a birth cohort of children up to age four [21], and association between PM₁₀ and prevalence and incidence of respiratory symptoms in a survey of children from age one to five [22]. While proximity to traffic was recently associated to wheeze in infants [23], only two birth cohort studies examined susceptibility in small children by age, reporting associations between doctor diagnosed asthma [24] and respiratory symptoms (cough) [25] and traffic to be stronger in the first than in second year of life. Finally, the potential importance of genetic susceptibility has been suggested by a particularly strong association between triggering of wheezing bronchitis or wheeze and PM_{2.5} and O₃, respectively, among infants with a family history of asthma [26, 27].

In this study we utilized the unique data on daily symptom recording in the birth cohort of children of mothers with asthma from the Copenhagen Prospective Study on Asthma in Childhood (COPSAC), to determine the short-term impact of particulate (PM₁₀ and UFPs) and gaseous (NO_x (nitrogen oxide), NO₂, and CO (carbon monoxide)) air pollution on the triggering of wheezing symptoms during the first three years of life in this high-risk population. We examined the susceptibility by age (infants (0-1 year), 1-2 years, and 2-3 years), to study the hypothesis of increased susceptibility of infants to air pollution, and tested for effect modification by medication use, gender and paternal asthma history.

METHODS

Study Population

The COPSAC prospective longitudinal birth cohort study including 411 Danish children born to mothers with asthma was designed to study the gene-environment interaction in the development of asthma and other atopic diseases [28-30]. The COPSAC study was conducted in accordance with the Declaration of Helsinki and approved by the Copenhagen Ethics Committee and the Danish Data Protection Agency. In brief, pregnant women identified through the Danish National Birth Cohort Study and prenatal clinics, living in Copenhagen, fluent in Danish, with history of physician's diagnosed asthma after the age of 7, and a history of daily treatment with inhaled β_2 -agonists or glucocorticoids (minimum of 2 weeks during 2 seasons or continuously for 1 year), were invited to participate. Infants with a severe congenital abnormality, a gestational age younger than 36 weeks, a need for mechanical ventilation, or a lower respiratory tract infection were excluded. The eligible 411 children of 394 mothers (9 pair of twins and 8 siblings) were born between 02.08.1998 and 12.12.2001, and enrolled in the cohort at the Clinical Research Unit (CRU) visit at 1 month age, with follow-up clinical investigations every 6 months until age of 7 years, as well as at any symptomatic episode.

At the first CRU visit parents were given diary cards (http://ipaper.dk/copsac/Asthma_in_young) and instructed to record their child's wheezing symptoms daily as dichotomized scores (yes/no), as previously described [29]. The CRU doctor reviewed symptom definition and the diary entries with the parents at the 6-monthly clinical sessions and at acute episodes of wheeze. Diary recordings for the first three years of life were used in the current analyses. Information on exposure to smoking at home and paternal history of asthma were obtained by interviews. Medications followed the algorithm previously described in details [29].

Address information, including relocations, were available from the COPSAC database and the Danish National Person Registry, allowing for geocoding and calculation of the exact distance from the residence to the air pollution monitor in the centre of Copenhagen on a daily basis. Thus, 205 children living within a 15km radius of the central monitor during first three years of life were selected for this study.

Air Pollution Exposure Assessment

The pollutant and meteorological data were measured by the Danish National Environmental Research Institute at a fixed urban background monitor (20m height) in the centre of Copenhagen, with minimal contribution from local sources, in accordance with WHO guidelines. Main streets are located about 300m west and 50m east of the monitor, with 26000 and 56000 vehicles passing per working day, respectively. For the study period (12.12.1998 - 19.12.2004), daily (24 h, midnight-to-midnight) mean concentrations were available for PM₁₀, measured by a SM200 monitor (Opsis, Sweden), CO (M 300; API, San Diego, USA), NO₂ and NO_x (M 200A; API, San Diego, USA), O₃ (M400; API, San Diego USA), and temperature, with missing data on days with equipment malfunctions. Although the total number concentration measurements included particles 10-700nm in diameter, we define them as UFP_{NC} in this study, because particles smaller than 100nm (by definition UFP) comprised more than 95% of total number concentration. Monitoring of UFP_{NC} (Differential Mobility Particle Sizer, Roskilde, Denmark) [31] and PM_{2.5} (TEOM Ambient Particulate Monitor; Rupprecht & Patashnick, USA) began in Denmark on 15.05.2001 and 03.10.2003, respectively.

Statistical Analysis

The incidence of wheezing symptoms was defined as the first day of a registered symptom. We performed logistic regression analyses using generalized estimating equations [32], by GENMOD procedure with exchangeable correlation structure, in SAS (version 9.1; SAS Institute Inc., Cary, NC). Pollutant concentrations were log-transformed. Analyses were conducted for all three years and separately for infants, and age groups 1-2 and 2-3 years. We first fitted single pollutant models, adjusted for age (dummy for each year), gender, exposure to passive smoking, paternal history of asthma, 24-h mean temperature (linearly) and calendar season (dummy). We considered pollutant concentrations on the same day (lag 0), previous day (lag 1), and up to four days (lag 4) prior to a new symptom, and the 3-day mean (2-4 days). In case of missing monitor data from single days, 3-day means of available measurements were used. Two-pollutant models were fitted for 3-day mean pollutant concentrations to examine the robustness of one pollutant associations. Separate analyses were conducted for a subset of data where UFP_{NC} data were available (15.05.2001-19.12.2004) to ensure comparability between the effect of UFP_{NC} and other pollutant. Finally, we tested for effect modification by gender, medication use, and paternal history of asthma. Effects were reported as odds ratios (ORs) per inter-quartile range (IQR) increase in exposure.

A number of sensitivity analyses were performed. The results remained unchanged when fitting GEE model with alternative correlation structures (independent, autoregressive). Various adjustments for meteorology and seasonal/time trend effects were tested. A model with temperature defined by a dummy per 25th percentile showed similar results and indicated a strong negative trend on symptom development by increasing quartiles of temperature, validating linear modeling of temperature. Season was modeled by dummy variables for calendar seasons, with a significant trend seen for increase of symptoms in colder seasons. After adjusting for season and temperature, additional time trends by including calendar time (linearly and by smoothing spline), and dummy for each month, were not significant and not included in the model. Finally, we performed analyses for an alternative choice of study population with 110 COPSAC children living within a 5km radius from the central monitor.

RESULTS

The 205 COPSAC children (99 male) lived within a 15km radius of the central monitor, with average and minimum distance of 6.1km (standard deviation 4.0km) and 0.3km respectively. Of the 205 children, 35 had a paternal asthma history, whereas 94, 64 and 47 were exposed to passive smoking for less than 10, between 10 and 100, and more than 100 days/year, respectively. The 205 children seemed representative of the whole COPSAC cohort [29], with dropout rate of 3 (1.5%), 6 (3%), and 2 (1%) children in the first, second and third year of life respectively, and 194 (95%) active children at their third birthday. The average observation period per child was 850 (min-max: 23-1097) with a total of 174,259 person-days and 15.4% diary entries missing. Thirty three children (16%) experienced no wheezing symptoms in the first three years of life. The prevalence of 6.2 per 100 person-days (total of 10779 symptom-days) and an incidence of 1 per 100 person-days (1591 new symptom-days), was observed.

Table 1 and 2 describe the pollution and meteorological conditions in Copenhagen during the

Table 1. Air Pollutant Levels and Meteorological Conditions in Copenhagen from 12.12.1998 until 19.12.2004 (2199 days).

	N	Mean (SD)	25 th percentile	75 th percentile	IQR [*]
Pollutant (units)					
PM ₁₀ (µg/m ³)	1749	25.1 (16.7)	15.7	30.2	14.5
PM _{2.5} [†] (µg/m ³)	453	9.8 (4.5)	6.8	11.7	4.9
UFP _{NC} [‡] (particles/cm ³)	602	8092 (3470)	5706	9825	4119
NO ₂ (ppb)	2045	11.8 (5.1)	8.1	14.6	6.5
NO _x (ppb)	2045	15.2 (8.7)	9.5	18.4	8.9
CO (ppm)	2068	0.29 (0.10)	0.22	0.34	0.12
O ₃ (ppb)	680	25.0 (9.9)	18.2	31.8	13.6
Meteorology (units)					
Temperature (C)	2143	9.3 (6.6)	3.9	14.7	10.8
Relative Humidity (%)	2142	75.2 (11.4)	67.3	83.7	16.4
Wind Speed (m/s)	2092	4.2 (1.5)	3.1	5.0	1.9
Global Radiation (W/m ²)	2128	114.1 (95.5)	25.5	189.3	163.8

^{*}IQR (interquartilerange) = 75th-25th percentile; [†] Measurements of PM_{2.5} were carried out by a TEOM instrument operated at 50°C in order to dry the aerosol, resulting in loss of volatile compounds (ammonium nitrate and semivolatile organic compounds). Thus TEOM measurements give in general smaller PM_{2.5} values compared to the gravimetric or beta-attenuation method, with difference of about 8-10 µg/m³; [‡] Total number concentration of ultrafine particles 10-700 nm in diameter (95% of total number concentration comes from particles < 100nm).

study period. Frequent break-down or other utilization of the UFP_{NC} measuring equipment caused missing data gaps (54%). PM_{2.5} and O₃ data were not sufficient for analyses of all three age groups, due to the late start of PM_{2.5} monitoring (03.10.2003) and a monitoring gap for O₃ in the middle of the study period (13.07.1999-01.07.2003). Strong correlations were observed between PM₁₀ and PM_{2.5}, CO and NO₂/NO_x and UFP_{NC} and NO₂/NO_x (Table 2), and weak between PM₁₀ and UFP_{NC} and PM₁₀ and NO₂/NO_x.

Air pollution showed delayed adverse effects, strongest and significant in infants with four days delay for PM₁₀ and three days delay for NO₂, NO_x, and CO, where one IQR increase in exposure lead to 23% (95% confidence interval 2%-48%; lag 4; IQR 14 µg/m³), 42% (15-77%; lag 3; 6.5 ppb), 30% (9-53%; lag 3; 8.9 ppb), and 47% (10-96%; lag 3; 0.12 ppm) increase in wheezing symptoms respectively (Table 3). Associations for NO₂ and NO_x were also significant with 4 day lag and 3-day means. Similar lag structures were observed across pollutants in all three

Table 2. Spearman Correlation Coefficients between Air Pollutants and Temperature in Copenhagen from 12.12.1998 until 19.12.2004 (2199 days).

	PM _{2.5}	UFP _{NC} *	NO ₂	NO _x	CO	O ₃	Temperature
PM ₁₀	0.79	0.37	0.43	0.40	0.45	-0.32	0.25
PM _{2.5}		0.40	0.41	0.39	0.45	-0.20	-0.01
UFP _{NC}			0.67	0.65	0.52	-0.12	-0.06
NO ₂				0.98	0.75	-0.58	-0.21
NO _x					0.74	-0.62	-0.21
CO						-0.63	-0.52
O ₃							0.43

* Total number concentration of ultrafine particles 10-700 nm in diameter (95% of total number concentration comes from particles < 100nm).

years, but effects attenuated after age one. Associations remained positive and significant in all three years only for NO₂ and NO_x for which one IQR increase in 3-day mean of exposure was associated with 19% (1-30%; 6.5 ppb) and 14% (0-30%; 8.9 ppb) increase in wheezing symptoms, respectively. The effects of NO₂ and NO_x were stronger than those of PM₁₀ throughout the study period (Table 3), as confirmed in two-pollutant models for infants (Table 4).

UFP_{NC} showed a relatively strong adverse effect with two to four days delays in infants, but without reaching statistical significance and this changed after age one to apparently protective effect. Analyses for 3-day mean concentrations of other pollutants (PM₁₀, NO₂, NO_x, and CO) were repeated for the subset of data where UFP_{NC} measurements were available (15.05.2001-19.12.2004) for infants, to enable direct comparisons (Table 5). Here, associations for all pollutants were enhanced, where estimates for UFP_{NC} were comparable to those of PM₁₀ and gasses in one pollutant models, but exceeded others in two pollutant models. For COPSAC children living within a 5km radius of the monitor effects of UFP_{NC} were enhanced reaching statistical significance for 3-day means (Table 6) as well as for lags 2-4 (data not shown).

No effect modification was detected between air pollution and gender, paternal asthma history or medication use.

Table 3. Associations between Incident Wheezing Symptoms for single-day and 3-day Means (lag2-4) Concentrations of Air Pollutants with Maximum Data Available for Each Pollutant (12.12.1998-19.12.2004).

	Age 0-1	Age 1-2	Age 2-3	Age 0-3
	OR* (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
n [†]	189	171	155	195
PM₁₀ (µg/m³)				
Lag0	1.05 (0.88-1.25)	1.00 (0.86-1.15)	0.87 (0.72-1.06)	0.97 (0.87-1.08)
Lag1	1.00 (0.82-1.22)	1.02 (0.87-1.19)	0.95 (0.78-1.15)	0.99 (0.89-1.10)
Lag2	1.01 (0.83-1.23)	1.05 (0.93-1.19)	0.99 (0.82-1.17)	1.01 (0.92-1.12)
Lag3	1.20 (0.98-1.46)	0.96 (0.84-1.09)	1.03 (0.84-1.25)	1.03 (0.93-1.14)
Lag4	1.23 (1.02-1.48) [‡]	1.04 (0.90-1.21)	0.89 (0.74-1.09)	1.04 (0.94-1.15)
3-day Mean [§]	1.21 (0.99-1.48)	1.03 (0.88-1.22)	0.94 (0.74-1.19)	1.04 (0.92-1.17)
n	144	157	151	179
UFP_{NC}[§] (part./m³)				
Lag0	0.71 (0.44-1.16)	0.82 (0.62-1.09)	1.00 (0.67-1.49)	0.85 (0.68-1.05)
Lag1	0.88 (0.56-1.38)	0.92 (0.70-1.21)	0.93 (0.68-1.26)	0.91 (0.75-1.10)
Lag2	1.60 (0.92-2.67)	0.88 (0.67-1.16)	1.03 (0.73-1.44)	1.00 (0.81-1.24)
Lag3	1.07 (0.67-1.73)	0.79 (0.59-1.06)	0.89 (0.63-1.27)	0.84 (0.70-1.02)
Lag4	1.50 (0.89-2.54)	0.99 (0.76-1.29)	0.62 (0.44-0.89)	0.88 (0.73-1.05)
3-day Mean	1.92 (0.98-3.76)	0.83 (0.58-1.17)	0.72 (0.49-1.04)	0.85 (0.68-1.07)
n	190	171	155	196
NO₂ (ppb)				
Lag0	0.78 (0.61-1.00)	0.99 (0.85-1.17)	1.00 (0.82-1.22)	0.93 (0.82-1.05)
Lag1	0.82 (0.67-1.01)	1.03 (0.86-1.24)	0.94 (0.78-1.13)	0.95 (0.84-1.06)
Lag2	1.12 (0.88-1.42)	1.07 (0.90-1.26)	1.12 (0.94-1.36)	1.09 (0.97-1.21)
Lag3	1.42 (1.15-1.77) [‡]	0.99 (0.80-1.22)	1.20 (0.98-1.46)	1.13 (0.99-1.30)
Lag4	1.33 (1.06-1.68) [‡]	1.06 (0.89-1.26)	1.00 (0.82-1.21)	1.09 (0.96-1.23)
3-day Mean	1.45 (1.08-1.95) [‡]	1.09 (0.85-1.40)	1.19 (0.98-1.45)	1.19 (1.01-1.30) [‡]
n	190	171	155	196
NO_x (ppb)				
Lag0	0.84 (0.68-1.02)	0.98 (0.85-1.12)	1.00 (0.84-1.19)	0.94 (0.85-1.04)
Lag1	0.82 (0.69-0.98)	1.02 (0.87-1.19)	0.90 (0.77-1.05)	0.92 (0.84-1.02)
Lag2	1.05 (0.87-1.28)	1.06 (0.92-1.22)	1.11 (0.95-1.30)	1.07 (0.97-1.17)
Lag3	1.30 (1.09-1.53) [‡]	0.99 (0.83-1.18)	1.12 (0.95-1.33)	1.09 (0.98-1.22)
Lag4	1.26 (1.03-1.54) [‡]	1.03 (0.89-1.19)	1.01 (0.85-1.19)	1.07 (0.96-1.19)
3-day Mean	1.30 (1.03-1.65) [‡]	1.09 (0.89-1.32)	1.14 (0.97-1.35)	1.14 (1.00-1.30) [‡]
n	190	172	154	196
CO (ppm)				
Lag0	0.82 (0.61-1.10)	1.04 (0.80-1.36)	1.04 (0.75-1.44)	0.96 (0.80-1.15)
Lag1	0.77 (0.57-1.04)	1.05 (0.79-1.40)	0.94 (0.68-1.29)	0.92 (0.77-1.10)
Lag2	1.05 (0.77-1.42)	1.10 (0.86-1.42)	1.16 (0.90-1.50)	1.08 (0.92-1.28)
Lag3	1.47 (1.10-1.96) [‡]	0.89 (0.67-1.18)	1.08 (0.80-1.46)	1.07 (0.90-1.26)
Lag4	1.19 (0.86-1.64)	0.94 (0.72-1.22)	1.04 (0.76-1.43)	1.02 (0.84-1.23)
3-day Mean	1.33 (0.94-1.90)	0.97 (0.70-1.34)	1.13 (0.81-1.55)	1.07 (0.87-1.32)

* Odds ratio is estimated per IQR of a pollutant, in a single pollutant model, adjusted for age, gender, smoking, paternal asthma history, temperature, and season; [†] Number of COPSAC children in the model; [‡] p < 0.05; [§] Total number concentration of ultrafine particles 10-700 nm in diameter (95% of total number concentration comes from particles < 100nm).

Table 4. Associations between Incident Wheezing Symptoms for 3-day Mean (lag2-4) Concentrations of Air Pollutants in Infants (age 0-1 years) in Period with Available PM₁₀ Measurements (12.12.1998-19.12.2004).

	One Pollutant Model	Two Pollutant Model	Two Pollutant Model	Two Pollutant Model
	OR* (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
<i>n</i> [†]	189	189	189	189
PM ₁₀ (µg/m ³)	1.21 (0.99-1.48)	1.13 (0.88-1.45)	1.16 (0.90-1.48)	1.23 (0.96-1.57)
NO ₂ (ppb)	1.46 (1.05-2.02) [‡]	1.34 (0.90-1.99)		
NO _x (ppb)	1.31 (1.01-1.68) [‡]		1.21 (0.89-1.64)	
CO (ppm)	1.27 (0.86-1.89)			1.03 (0.64-1.6)

* Odds ratio is estimated per IQR of a pollutant and 3DayMean = (lag2-4), adjusted for age, gender, exposure to smoking, paternal asthma history, temperature, and season; [†] Number of COPSAC children in the model; [‡] p < 0.05.

Table 5. Associations between Incident Wheezing Symptoms for 3-day Mean (lag2-4) Concentrations of Air Pollutants in Infants (age 0-1 years) in Period with Available UFP_{NC} Measurements (15.05.2001-19.12.2004).

	One Pollutant Model	Two Pollutant Model	Two Pollutant Model	Two Pollutant Model	Two Pollutant Model
	OR* (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
<i>n</i> [†]	144	144	144	144	144
UFP _{NC} [§] (part./m ³)	1.92 (0.98-3.76)	1.86 (0.88-4.14)	1.82 (0.62-5.34)	2.04 (0.68-6.16)	1.67 (0.69-4.02)
PM ₁₀ (µg/m ³)	2.05 (1.26-3.32) [‡]	1.76 (1.12-2.76) [‡]			
NO ₂ (ppb)	2.15 (1.02-4.57) [‡]		1.47 (0.53-4.06)		
NO _x (ppb)	1.73 (0.94-3.22)			1.18 (0.50-2.78)	
CO (ppm)	2.17 (1.01-4.66) [‡]				1.44 (0.51-4.06)

* Odds ratio is estimated per IQR of a pollutant and 3DayMean = (lag2-4), adjusted for age, gender, exposure to smoking, paternal asthma history, temperature, and season; [†] Number of COPSAC children in the model; [‡] p < 0.05; [§] Total number concentration of ultrafine particles 10-700 nm in diameter (95% of total number concentration comes from particles < 100nm).

Table 6. Associations between Incident Wheezing Symptoms for 3-day Means (lag2-4) of Air Pollutants in 110 Children Living within a 5 km radius from the Central Monitor.

	Age 0-1	Age 1-2	Age 2-3	Age 0-3
	OR* (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
<i>n</i> [†]	102	81	70	103
PM ₁₀ (µg/m ³)	1.32 (0.95-1.82)	1.20 (0.87-1.67)	0.78 (0.52-1.16)	1.11 (0.88-1.39)
<i>n</i>	76	78	70	95
UFP _{NC} [§] (part./m ³)	2.46 (1.04-5.84) [‡]	1.09 (0.61-1.94)	0.40 (0.21-0.76) [‡]	0.92 (0.63-1.34)
<i>n</i>	103	81	70	
NO ₂ (ppb)	1.84 (1.07-3.17) [‡]	1.36 (0.90-2.05)	0.92 (0.62-1.35)	1.28 (0.99-1.65)
<i>n</i>	103	81	70	104
NO _x (ppb)	1.46 (0.97-2.21)	1.37 (0.98-1.91)	0.93 (0.67-1.28)	1.23 (1.00-1.51) [‡]
<i>n</i>	103	83	70	104
CO (ppm)	1.40 (0.83-2.36)	1.32 (0.75-2.34)	0.81 (0.46-1.40)	1.18 (0.85-1.63)

* Odds ratio is estimated in a single pollutant model per log IQR of a pollutant and 3DayMean = (lag2-4), adjusted for age, gender, exposure to smoking, paternal asthma history, temperature, and season. [†] Number of COPSAC children in the model. [‡] p < 0.05. [§] Total number concentration of ultrafine particles 10-700 nm in diameter (95% of total number concentration comes from particles < 100nm).

DISCUSSION

We found strong adverse effects of air pollution in terms of PM₁₀, NO₂, NO_x and CO on the triggering of wheezing symptoms in infants, attenuating after the age of one. Independent effects of PM₁₀ and gases were observed, with consistently stronger estimates for gases (NO₂ and NO_x), proxies of traffic-related pollution. Furthermore, adverse effects of UFP_{NC} in infants, which were enhanced for infants living within a 5km radius from monitor, support the relevance of traffic, although an apparently protective effect of UFP_{NC} was observed in the third year of life.

The finding of traffic relevance in the triggering of wheezing in infants is in agreement with recent evidence associating infant wheezing to proximity to traffic [23] and infant wheezing bronchitis to PM_{2.5} [26]. Furthermore, two studies associated doctor diagnosed asthma and respiratory symptoms (cough) in children under the age of 2 to modeled levels of PM_{2.5} and NO₂ at the children's residence [24, 25]. These associations were strongest in the first years of life, as observed in our study. Lung anatomy and physiology, ventilation rates, and organ maturity change with age which may explain a greater vulnerability to air pollution in infants, and suggest biological plausibility to these findings [2]. Consistently with our findings, a study of asthma symptoms in preschool children [21] and several studies of asthma exacerbation in school children have also shown adverse effects of various proxies of traffic [16-19].

Our study is first on the effects of UFPs on triggering of wheezing symptoms in young children, and we found a positive association in the first year of life, which was significant among children living within 5km from our monitor. The only other study on UFPs effects in children found significant effect of PM₁₀ and comparable but not significant effect of UFPs on peak expiratory flow in 39 asthmatic school children over 57 days [8]. Three studies of pollution effects in adult asthma exacerbation found greater importance of UFPs and PM_{2.5} over larger particles [34-36], using 2km [34] and 5km [35] radius from pollution monitor. However, our results should be taken with caution and need confirmation due to the surprising change of effects of UFPs toward protective after the age of one.

To date, PM₁₀ has been considered in only one survey study of preschool children [22], and never in infants. In Copenhagen, the main source of PM₁₀ is long-range transport from secondary, biomass and oil combustion sources, with minor contribution from traffic [33], implying that PM₁₀ is a poor proxy of traffic and allowing for separation and comparison of its effects with traffic proxies, NO₂, NO_x, and UFP_{NC}. We found adverse effects of PM₁₀ significant only in infants (lag 3), and generally smaller than those of gasses (Table 4) and UFP_{NC} (Table 5). This suggests independent effects of particle mass and gasses or particle numbers mainly related to traffic.

The increased susceptibility to air pollution in children genetically susceptible was earlier suggested by increased association of incident wheezing bronchitis with PM_{2.5} in children with a family history of asthma [26], and stronger effects of O₃ on triggering wheeze in children of mothers with asthma [27]. In our cohort of high-risk infants, associations with traffic related air pollution seem higher than those reported in healthy infants elsewhere [22, 24, 25], but direct comparisons are difficult due to differences in outcome definition, and the use of geographical variation in exposure assessment in those studies. Furthermore, associations of asthma prevalence with traffic were surprisingly stronger for school children without familial asthma history [17]. This may indicate that early symptoms in infants and later diagnosed asthma, although strongly related, may represent a mixture of children with different susceptibilities

The incidence of wheezing symptoms was chosen as the health outcome in this study rather than diagnoses of asthma because of the inaccurate asthma diagnostic criteria in early life [29-30]. As the term “wheeze” carries little specific meaning in lay-terms and even between specialists [37], we have explained the term to the parents in the generic sense of lung symptoms severely affecting the child’s wellbeing including wheeze or whistling sounds, breathlessness, shortness of breath and persistent troublesome cough. This approach was supported by diary reviews at the 6-monthly visits together with a dedicated book describing the relevant lung symptoms. The diagnosis and day-to-day management of respiratory conditions were conducted solely by the doctors employed for this purpose at the CRU in accordance with predefined algorithms, minimizing the risk of symptom misclassification from influence of the prevailing and variable diagnostic criteria and treatment traditions in the medical community. The inaccuracy of symptom recognition and reporting bias was reduced by all mothers having a personal asthma history and familiarity with the disease. Furthermore, the state-of-the art information on the incidence of wheezing symptoms in COPSAC cohort provided the opportunity of studying the short-term effects of air pollution on triggering of the symptoms, which was reported earlier only in two studies in infants [26-27]. Analyses of the cohort at later ages, when diagnoses of health outcomes are more specific, will help determine the long-term impact of early air pollution exposures on the development of specific respiratory diseases, including asthma.

The strengths of our study include the prospective daily recording of symptoms allowing for the study of symptom incidence, as well as long study period of five years (12.12.1998-19.12.2004) giving sufficient exposure contrasts over time and power to detect adverse effects. This is an advantage over earlier studies with a short follow-up of a few months [4, 6-10, 27], which may limit the study power to detect pollutant effects [38]. An additional strength is the well-defined birth-cohort of children genetically predisposed to asthma, allowing for a unique possibility of studying early exposures of air pollution effects in a high-risk group. Furthermore, the continuously updated residential information allowed inclusion criteria to be based on address (vicinity to the monitor) at the time of symptoms and not at the time of birth, as done previously [21, 24]. Finally, the availability of both gaseous and particulate pollutants of different size cuts, allowed for the first to date comprehensive comparisons of effects of traffic (gasses and UFPs) and non-traffic (PM₁₀) related air pollution in development of respiratory disease in young children.

The limitations of the study include exposure assessment from a single monitor implying exposure misclassification. A 15km radius from the central monitor was chosen as it represents the municipality limits of Copenhagen City with similar population and traffic density, and assumed representative of air pollution levels measured at the central monitor. We have previously shown that the UFP_{NC} levels at this site correlated well with levels at a monitoring site at the kerbside (2m height) of a busy street (spearman correlation coefficient $R_s=0.62$) 3000m away, and a rural monitoring site ($R_s=0.80$) located in the residential area 30km southwest [40]. This was despite that UFP_{NC} levels were 4.6 times higher at the kerbside and 0.7 times lower at the rural site on average as compared to urban background site. This indicates that the daily oscillations in traffic-related air pollutants at busy streets due to variation in traffic intensity, weather conditions and other factors are also reflected by urban background monitoring, and that 15km radius from the background monitor is reasonable for the assessment of daily variation in population exposure. Among children living closer to the central monitor (5km), we found slightly stronger associations between UFP_{NC} and triggering of wheeze, reaching statistical significance and exceeding those of other pollutants (Table 6). However, if this apparent difference between estimates for 5km and 15km radius is real, we cannot determine whether it is due to larger exposure misclassification with the large radius or due to real higher

effects of traffic closer to the monitor, where traffic density and emissions and traffic pollutant levels are higher. The limitation of the study also include the counterintuitive and biologically implausible change of the effect of UFP_{NC} from adverse in infants to protective in children above age 2, statistically significant in children living within 5km from air pollution monitor (Table 6). However, the effect of all other pollutants (PM₁₀, NO₂, NO₂ and CO) also attenuated after age of 2 to apparently protective, although not reaching statistical significance (Table 6). This shows that decrease in susceptibility to air pollution after age of 2 is not confined only to UFPs and thus not likely explained by measurement error or change in exposure, but rather by underlying disease process and/or the treatment of wheezing symptoms in this group of children. As the medication protocol in COPSAC cohort presumes history of recurring wheezing episodes, children typically don't receive medication in the first, but during second year of life or later. Thus, this attenuation of adverse air pollution effects after age of 1 and furthermore after age of 2, is most likely explained by the drop in children's susceptibility to air pollution achieved by medication of wheezing symptoms. Likewise, the statistical significance with respect to UFP_{NC} is most likely explained by a type I error. Nevertheless, it may question the findings for UFP_{NC} in the first year of life as no other supportive data are available. Further limitations of the study include the large number (54%) of missing data for UFP_{NC}. However, missing data gaps were examined and assessed to be at random and not associated with pollution levels or weather conditions. Still, our series with UFP_{NC} measurements for 602 days are the longest available to date with respect to study of UFP effects on respiratory symptoms [8, 34-36].

To summarize, we found independent effects of PM₁₀ and traffic related pollution, measured by exposure to NO₂/NO_x, CO, and UFP_{NC} (5km radius) for the triggering of wheezing symptoms in young children genetically predisposed to asthma. Infants were found to be particularly vulnerable. Follow-up of the cohort at later ages will determine the effect of these associations on the development of asthma.

ACKNOWLEDGEMENTS

We thank the children and parents participating in this cohort.

FUNDING

The statistical analyses were supported by the Danish Research Council, grant number 2052-03-16, AIRPOLIFE (Air Pollution in a Life Time Health Perspective) and the Danish Environmental Protection Agency. The COPSAC cohort study is funded by research funds: the Pharmacy Foundation of 1991; the Lundbeck Foundation; The Augustinus Foundation; Ronald McDonald House Charities; the Danish Medical Research Council; The Danish Pediatric Asthma Center; Direktør, cand.pharm. K. Gad Andersen og Hustrus Familiefond; Aage Bangs Fond; Danish Lung Association; Kai Lange og Gunhild Kai Langes Fond; Direktør Ib Henriksens Fond; Gerda og Aage Hensch's Fond; Rosalie Petersens Fond; Hans og Nora Buchards Fond; Dagmar Marshalls Fond; Foundation of Queen Louise's Children Hospital; the Danish Hospital Foundation for Medical Research, Region of Copenhagen, the Faroe Island, and Greenland; Gangsted Fond; Højmosegård-Legatet; Fonden til Lægevidenskabens Fremme; A.P. Møller og Hustru Chastine Mc-Kinney Møllers Fond til almene Formaal; The Danish Ministry of the Interior and Health's Research Centre for Environmental Health. The study received support from the following pharmaceutical companies: AstraZenaca; LEOpharma; Pharmacia-Pfizer and Yamanouchi Pharma.

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