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PEAK EXPIRATORY FLOW RATES IN GERMAN CHILDREN EXPOSED TO AMBIENT OUTDOOR AIR POLLUTION, PARTICULATE MATTER AND TRACE ELEMENTS

presented by

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By

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ABSTRACT

PEAK EXPIRATORY FLOW RATES IN GERMAN CHILDREN EXPOSED TO AMBIENT OUTDOOR AIR POLLUTION, PARTICULATE MATTER AND TRACE ELEMENTS

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In light of inconsistencies among existing reports and the continued public health impact, the objective of this report is to investigate associations between childhood exposures to ambient particulate matter, gaseous, and trace metal pollution and observable decrements in peak expiratory flow.

We longitudinally followed 70 German children. Linear regression analyses of moving averages of pollution variables and changes in peak flow values were performed utilizing the mixed procedure of SAS.

Analysis of changes in afternoon peak flow yielded a statistically significant negative association for NO_3^-, strong acid aerosol, and antimony concentrations. No associations were seen for changes in morning peak flow.

In conclusion, this study failed to demonstrate consistent associations between pollutants and peak expiratory flow rates. The data does provide additional evidence for reductions in lung function associated with exposures to low level NO_3^- and strong acid aerosol concentrations. The significant association observed for antimony suggests further research into health effects of various transition metal species is warranted. This inability to demonstrate consistent relationships supports previous assertions that future research endeavors investigating associations regarding particulate matter and its speciation should target susceptible children with chronic respiratory complaints.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ETS.....Environmental Tobacco Smoke

SAS.....Statistical Analysis Software

$PM_{2.5}$.....Particulate Matter smaller than 2.5 micrometers

PM_{10}.....Particulate Matter smaller than 10 micrometers

SO_2.....Sulfur Dioxide

SO_4^{2-}.....Sulfate Ion

NO_2.....Nitrogen Dioxide

NO_3^-.....Nitrate Ion

Co.....Cobalt

Cd.....Cadmium

As.....Arsenic

Pb.....Lead

Va.....Vanadium

Sb.....Antimony

Pt.....Platinum

PEFR.....Peak Expiratory Flow Rate

FEV_1.....Forced Expiratory Ventilation in 1 second

EPA.....Environmental Protection Agency

NAAQS.....National Ambient Air Quality Standards

CI.....Confidence Intervals

BHR.....Bronchial Hyperreactivity

INTRODUCTION

Public health concern regarding possible associations between adverse health outcomes and exposure to air pollutants has been mounting over the last several decades. The effect increasing levels of air pollution may have on childhood health outcomes has been of particular concern. This concern has been prompted in part by observed increases in the prevalence of asthma and asthma-like symptoms in children who live in developed countries. (Peat, van den Berg et al. 1994; Mannino, Homa et al. 1998; Sullivan 2003) Due to the substantial social and economic cost that is associated with asthma, researchers have attempted to identify prospective risk factors amenable to intervention. (Rusznak, Devalia et al. 1994; Zmirou, Deloraine et al. 1999; Zanobetti, Schwartz et al. 2000; Piecoro, Potoski et al. 2001; Park, Lee et al. 2002; Mo, Robinson et al. 2003)

Childhood exposure to air pollution warrants special consideration based on a variety of factors. Chief among these is the knowledge that a child's immune system and lung function are not fully developed. (Dockery, Speizer et al. 1989; Wang, Dockery et al. 1993; Roth-Kleiner and Post 2003) This physiological immaturity raises the suspicion that children's sequelae from environmental exposures that occur during a sensitive target window may be different from those of adults. (Chew, Goh et al. 1999; Braga, Zanobetti et al. 2001; Fusco, Forastiere et al. 2001) Furthermore, it is reasonable to assume that since children spend more time outdoors during warmer months and evening hours that have a greater exposure to peak air pollution levels. This temporal trend of childhood outdoor activity and higher ambient levels of air pollution is made more ominous by the

tendency for childhood outdoor activities to increase ventilatory rate. (Mercier, Varray et al. 1991; Rowland and Cunningham 1997)

There are a variety of postulated mechanisms mitigating the association between ambient air pollution and respiratory compromise. The two most prominent include inflammatory processes (Ferin, Oberdorster et al. 1992; Utell and Frampton 2000; Goto, Ishii et al. 2004) and oxidative stress. (Dick, Brown et al. 2003; Hirano, Furuyama et al. 2003) Both of these mechanisms involve chronic exposure to industrial ambient air pollutants including ozone, nitrogen dioxide, sulfur dioxide, various heavy metals, and particulate matter. Each of these pollutants will be examined briefly.

Chapter 1

BACKGROUND

Overview of current research regarding air pollution and childhood lung function

Ozone (O_3)

Unlike the other listed pollutants, ozone is not directly emitted from an identifiable polluting source. It is readily produced through photochemical thermal reactions between volatile organic compounds and NO_2 species, and is the principal component of smog. The basic requirements of sunlight and heat explain the observed seasonal and diurnal variations in ozone concentration. (Porter, Rao et al. 2001; Wolff, Dunker et al. 2001) The organic compound precursors of ozone are emitted from a variety of sources including motor vehicles, chemical plants, refineries and factories. The NO_2 species are mainly released from motor vehicle exhaust, power plant emissions and as by-products of various combustion reactions. The Environmental Protection Agency (EPA) 1997 National Ambient Air Quality Standards (NAAQS) have established a one hour O_3 exposure level of 0.12 ppm. This standard is the threshold exposure level that individuals should not exceed more than once per year. In addition, the NAAQS has established an 8-hour O_3 exposure limit of 0.08 ppm. This level is the 8-hour O3 exposure averaged over a three year time span.

A variety of studies have established an association between increases in ozone levels and increases in hospital emergency room admissions for respiratory illness. (Hajat, Haines et al. 1999; Neas, Schwartz et al. 1999; Sheppard, Levy et al. 1999; Fauroux, Sampil et al. 2000; Gouveia and Fletcher 2000; Schwela 2000; Atkinson,

Anderson et al. 2001; Braga, Zanobetti et al. 2001; Friedman, Powell et al. 2001; Fusco, Forastiere et al. 2001; Delfino, Zeiger et al. 2002; Martins, Latorre Mdo et al. 2002; Park, Lee et al. 2002; Peel, Tolbert et al. 2005) In addition, researchers have demonstrated that exposure to ambient ozone concentrations can lead to respiratory compromise (Gent, Triche et al. 2003) and reductions in lung function. (Gold, Damokosh et al. 1999; Jalaludin, Chey et al. 2000; Mortimer, Neas et al. 2002)

Particulate Matter (PM)

Particulate matter is a mixture of solid particles and liquid droplets found in the air. The particles emitted by an identifiable source are commonly referred to as direct emissions, while those that are formed through atmospheric processes are known as secondary particles. There are a variety of factors that determine the role particulate matter has on health outcomes. (Harrison and Yin 2000)

One key determinate is the size of particulate matter. (Harkema, Keeler et al. 2004) PM can be classified as either large diameter, coarse, fine, or ultrafine. Large diameter particles are those that are greater than 10 micrometers in diameter. These particles rarely penetrate beyond the upper airways. (Ferin, Oberdorster et al. 1992) They are readily removed by the body's main defenses including coughing and transport via the mucociliary ladder. Particles of this size are generally regarded as having little affect on the development of respiratory disease and will not be discussed further.

Coarse particles are those that are between 2.5 and 10 micrometers in diameter. These particles are generally made up of silica, aluminum, and iron, and have been demonstrated to be deposited high in the endobronchial tree. Various research studies have linked ambient concentrations of coarse particulate matter, commonly denoted as

PM_{10}, to a variety of respiratory and other health outcomes. (Pope, Dockery et al. 1991; Pope and Dockery 1992; Neas, Dockery et al. 1995; Abbey, Nishino et al. 1999; Gold, Damokosh et al. 1999; Neas, Schwartz et al. 1999; Sheppard, Levy et al. 1999; Gouveia and Fletcher 2000; Zanobetti, Schwartz et al. 2000; Anderson, Bremner et al. 2001; Atkinson, Anderson et al. 2001; Braga, Zanobetti et al. 2001; Lacasana, Esplugues et al. 2005) The studies include large scale epidemiologic studies in both Europe (Pekkanen, Timonen et al. 1997; Roemer, Clench-Aas et al. 1999; Tiittanen, Timonen et al. 1999; Samoli, Analitis et al. 2005) and the United States. (Keeler, Dvonch et al. 2002; Mortimer, Neas et al. 2002; Peel, Tolbert et al. 2005)

Similarly, fine particles are the components of PM that are less than 2.5 micrometers in size. These particles are deposited in the terminal bronchioles and alveoli, and are regarded as the most closely associated with adverse health outcomes (Osornio-Vargas, Bonner et al. 2003; Choi, Kim et al. 2004) such as emergency rooms admissions (Sheppard, Levy et al. 1999; Anderson, Bremner et al. 2001; Barnett, Williams et al. 2005), respiratory compromise (Romieu, Meneses et al. 1996; Ostro, Lipsett et al. 2001; Pino, Walter et al. 2004; Ranzi, Gambini et al. 2004), and decrements in lung function. (Koenig, Larson et al. 1993; Gold, Damokosh et al. 1999; Neas, Schwartz et al. 1999; Tiittanen, Timonen et al. 1999; Schwartz and Neas 2000; Delfino, Quintana et al. 2004) They are composed of a mixture of particles formed in the air from chemical reactions involving gaseous pollutants including sulfates, nitrates and other organic compounds.

Finally, the subset of $PM_{2.5}$ that ranges from 0.1 to 1 micrometers is sometimes referred to as ultrafine particles. This range of PM has not been extensively studied.

Particles less than 0.1 nm remain in the air stream and are not believed to settle within the respiratory tract.

The EPA NAAQS for PM_{10} sets an annual mean ambient air concentration < 50 $\mu g/m^3$ averaged over three years. In addition, the NAAQS specifies a standard concentration of 150 $\mu g/m^3$ that should not be exceeded more than once per year over a three year time period. For $PM_{2.5}$ the standards are 15 $\mu g/m^3$ and 65 $\mu g/m^3$ respectively.

Trace Elements

Another recently hypothesized explanation of the relationship between PM and adverse health outcomes is exposure to trace amounts of heavy metals in the atmosphere. One of the leading mechanistic theories explaining these adverse outcomes is based on the oxidation-reduction capacity of various metals which leads to the production of free radicals via the Fenton reaction. (Dick, Brown et al. 2003; Jimenez Del Rio and Velez-Pardo 2004) The generation of these free radicals results in widespread tissue inflammation. Metals which have been demonstrated to undergo Fenton-type oxidation-reduction reactions include iron, cobalt, lead, cadmium and mercury. Recent studies have begun investigating the potential role air particulate metal exposure may have on human health. (Burnett, Brook et al. 2000; Claiborn, Larson et al. 2002)

Sulfur Dioxide (SO_2)

Sulfur dioxide is formed primarily from either the burning of fossil fuels that contain sulfur, including emissions from coal burning electric utilities or as a by-product during industrial processes such as metal smelting. Reports have linked elevated levels of SO_2 with increased mortality (Wichmann and Heinrich 1995; Vigotti 1999; Wichmann, Spix et al. 2000), asthma exacerbations in children (Chew, Goh et al. 1999;

de Diego Damia, Leon Fabregas et al. 1999; Vigotti 1999; Herbarth, Fritz et al. 2001), decrements in lung function (Roemer, Hoek et al. 1993; Timonen and Pekkanen 1997; Roemer, Clench-Aas et al. 1999; van der Zee, Hoek et al. 1999), and aggravation of cardiovascular disease. (Vigotti 1999; Venners, Wang et al. 2003; Fung, Luginaah et al. 2005) The established NAAQS for SO_2 include a maximum concentration of 0.14 ppm over 24 hours that should not be exceeded more than once per year. Additionally, a specified annual mean of 0.030 ppm should not be surpassed more than once in any given year.

Nitrogen Dioxide (NO_2)

NO_2 is a highly reactive gas that is formed via oxidation of nitric oxide. Anthropogenic sources of NO_2 include high-temperature combustion processes primarily via automobile combustion engines, power plants and electric utilities. There are a variety of natural sources of NO_2 include lightning, biological processes in the soil and stratospheric intrusion. Home heaters (Moseler, Hendel-Kramer et al. 1994; Garcia Algar, Pichini et al. 2004) and gas stoves (Goldstein, Melia et al. 1979; Brugge, Vallarino et al. 2003) have been shown to produce significant concentrations of indoor NO_2 pollution. Various European and American studies have shown childhood health effects including airway hyperresponsiveness (Boezen, van der Zee et al. 1999; Hirsch, Weiland et al. 1999), reductions in pulmonary function (Timonen and Pekkanen 1997), and relative increases in asthmatic symptoms and other respiratory illnesses. (Baldacci, Carrozzi et al. 1997; Just, Segala et al. 2002; van Strien, Gent et al. 2004) In addition, a recent study has suggested a positive association between increased respiratory symptoms

in childhood and combined exposure to NO_2 and ETS. (Emenius, Pershagen et al. 2003) The NAAQS index for maximum annual mean NO2 concentration is 0.053 ppm.

Current issues in air pollution and health impact studies

Although there is a growing body of evidence implicating various components of ambient air pollution to adverse health outcomes, there continue to be reports published that yield inconsistent findings. (Roemer, Hoek et al. 1998; Roemer, Clench-Aas et al. 1999) In light of these inconsistencies as well as the potential impact on public health, the objective of this report is to further investigate the potential associations between childhood exposure to ambient fine particulate matter, gaseous pollutants, and trace metal pollution and observable reductions in peak expiratory flow (PEF).

The likely role of inflammation in regard to the adverse respiratory outcomes of air pollution has led many researchers to evaluate alternate means of assessing airway inflammation. One commonly used test believed to be a reasonable marker of inflammation is bronchial hyperreactivity to a pharmacologic challenge test. (Parker, Abu-Hijleh et al. 2003) The associated cost and relative invasiveness of these tests limit their utility as epidemiologic tools. These limitations have led researchers to evaluate alternative proxies for these pharmacologic challenge tests.

One such commonly proposed surrogate is the peak expiratory flow rate (PEFR). The diurnal variability in PEF has been shown to be correspond well to the degree of bronchial hyperresponsiveness following pharmacologic challenge tests, (Ryan, Latimer et al. 1982; Gern, Eggleston et al. 1994) although some researchers have disputed the clinical and diagnostic value of this association. (den Otter, Reijnen et al. 1997; Goldstein, Veza et al. 2001) The physiologic pattern of diurnal variation in PEF has been

well demonstrated. (Hetzel 1981) Subsequently, several studies have identified a variety of factors which contribute to the observed variation. These factors include age (Le Souef 1997; Lebowitz, Sherrill et al. 1997), gender (Lebowitz, Sherrill et al. 1997; Holcroft, Eisen et al. 2003; Ones, Somer et al. 2004), race (Le Souef 1997; Ones, Somer et al. 2004), height (Ones, Somer et al. 2004), weight (Ones, Somer et al. 2004), and smoking exposure. (Holcroft, Eisen et al. 2003).

Peak expiratory flow is obtained from the maximum flow achieved during a maximal expiratory effort following maximum inspiration. Since PEF is generally attained within the first 0.1 seconds of a forced expiratory maneuver, it has potential advantages over other pulmonary function determinants for long-term epidemiologic monitoring in a childhood population. The most likely advantage in measuring PEF is that it makes the prolonged and challenging expiration maneuvers necessary to calculate forced expiratory ventilation in 1 second (FEV_1) and forced vital capacity unnecessary.

A variety of portable hand-held peak flow monitors have been manufactured to reliably record PEF. One of the most widespread meters, the mini-Wright flow meter, has been shown to be reliable after 5 years of continued use. (Douma, van der Mark et al. 1997) In addition, the reproducibility of PEF obtained by the mini-Wright peak flow meter is also reported to be good. (Enright, Sherrill et al. 1995; Paggiaro, Moscato et al. 1997; Holcroft, Eisen et al. 2003)

Several studies have confirmed that the mini-Wright flow meter over estimates PEF values in the mid-flow ranges and under records values at the lower and higher flow ranges. (Miller, Dickinson et al. 1992) Subsequent authors have determined a correction formula for the observed non-linearity of the mini-Wright peak flow meter (Miles,

Tunnicliffe et al. 1996), although recent studies have confirmed that correction for this factor does not increase reproducibility. (Douma, Kerstjens et al. 2000) Taking into consideration the widespread availability, ease of use, good reproducibility, good reliability and the relative inexpensiveness of hand-held spirometers, it is reasonable to conclude that they serve as a valuable epidemiologic monitoring tool.

Chapter 2

METHODS

Study population

Data was collected in a longitudinal study designed to assess the potential impact of ambient air pollution on childhood lung function in a cohort of children residing in seven largely urban Southwestern German communities. Children eligible to participate were those who had previously been enrolled in a large scale environmental cohort (Kuehr, Frischer et al. 1992; Kuehr, Frischer et al. 1998) determined to live within one km of the seven primary schools selected as sites for air pollution monitoring stations. The communities from which children were recruited include: Crumstadt, Eschollbrüken-Pfungstandt, Biebesheim, Gernsheim, Bickenbach, Auf Esch-Groß-Gerau, and Michelstadt (See Figure 1).

School site selection

As the children spent a large proportion of their days at school and all participating children lived within close proximity of the school, we elected to collect air pollution measurements on the rooftops of the schools. Due to limited funds, we were forced to select only seven locations to measure air pollution data. Of the 18 schools from which children participated, we selected those buildings that provided the best measurement conditions (flat roof, not more than one story, and readily accessible electrical power). The selected schools maintained representation of the three regions from which children were recruited for the larger study.

Determination and specification of PM2.5

Air pollution monitoring stations were established on the rooftops of the seven primary schools as discussed above. All air samples were conducted with a filter unit (VDI guideline No. 2463). Each filter sample was collected for 24 hr through a Harvard-Marple impactor (Air Diagnostics, Harrison, ME, USA) with a 50% aerodynamic diameter cutpoint of 2.5 μm. The selection of PM was based on impactor principle and required constant flow and volumes during the sampling period. The sampling units were equipped with volume regulators to ensure a constant flow of 2 m^3 per hour.

For the deposition of particulate matter, adhesive-free silica fiber filters (Munktell, Type MK 360, 47 mm) were utilized. Throughout the study, three filters were used per week. The rotation of filter replacement was coordinated to ensure an equal distribution of each week day. Therefore, the sampling duration was 24 hours responding to a volume of 48 m^3. In order to maintain adequate quality control measures, following three sample data collection visits, two unused filters which had previously been transported to the sampling unit site were analyzed.

To maximize consistency, we equilibrated each sample filters at 21 ± 2°C and 35 ± 5% relative humidity for 24 hr before weighing. We used an analytical balance designed for weighing filters with a resolution of 0.1 μg (M5P-000V001; Sartorius, Goettingen, Germany). We calculated ambient $PM_{2.5}$ concentrations based on sample weight and air volume.

Determination of metals and anions required two different preparations. Filters were divided into equal halves (1% weight difference). For the analyses of metals, samples were prepared by chemical decomposition with nitric acid and hydrogen fluoride in encased pressure containers using microwave treatment (VDI guideline No. 2268).

Metals were determined with ICP-MS (inductively coupled plasma - mass spectrometry, PerkinElmer). For the analyses of ion species, the remaining half of each filter was used for aqueous extraction in a microwave bath. Ions were then determined using ion chromatographic methods. Detection limits based on a sampling volume of 48 m^3 were: 0.5 ng/m^3 for cobalt, cadmium, antimony and vanadium; 3.3 ng/m^3 for lead; 1.0 ng/m^3 for platinum; 0.33 $\mu g/m^3$ for sulfate; 0.21 $\mu g/m^3$ for nitrate and 0.06 $\mu g/m^3$ 'strong acid' (chloride anions).

The mini-Wright peak flow meters (Clement Clarke International Ltd., London UK) were distributed to each child. The study was approved by the Data Protection Agency of the State of Hamburg, Germany, by the Ministry of Cultural Affairs of Hessen, Germany, and by the local school committees. According to the requirements of the Ethical Committee of the Board of Physicians and the Data Protection Agency of the State of Hamburg, informed consent was obtained from the parents or legal guardians of all participating children.

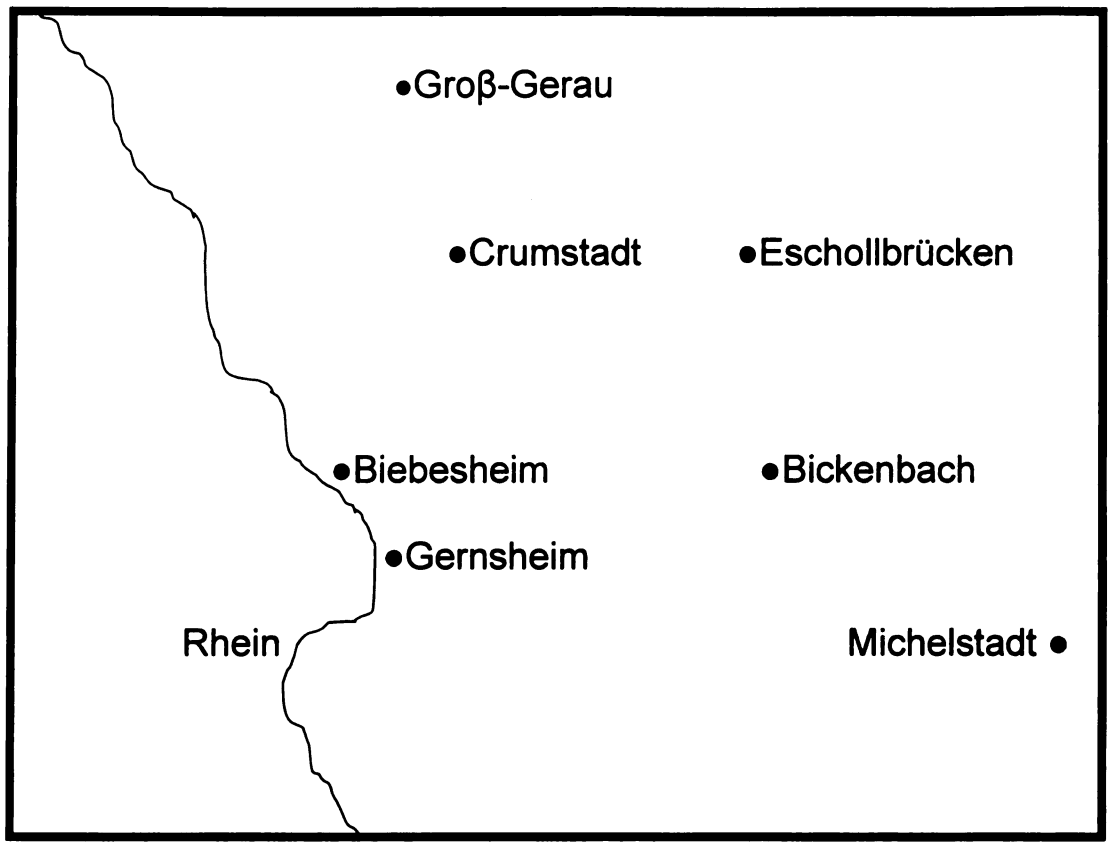

Figure 1: Map of the seven Southwestern German communities with children participating in peak flow measurements

Questionnaires

A questionnaire designed to investigate a variety of environmentally related health outcomes had previously completed for each child in 1995 as part of the larger cohort investigation. In addition, a follow-up questionnaire was distributed and completed by the parents in 1996. A history of physician diagnosed asthma or current wheezy bronchitis, of asthmatic complaints in the preceding 12 months, and of parental atopy (ascertained as maternal or parental history of asthma, hay fever, or eczema) were determined based on the 1995 and 1996 questionnaires. Additional information collected on the children included gender, birth-date, environmental tobacco smoke exposure (defined as never, 1 day a week, a couple of times a week, or daily), height (centimeters), weight (kilograms), as well as any respiratory symptoms experienced during the study period. Height and weight were recorded directly on the data sheet (see Appendix A) based upon the child's height and weight on the first day of participation in both February and March. For February and March three and six children respectively were missing either height or weight measurements. For these children height and weight information was obtained either from questionnaire data (7 children) or the preceding month's measurement (2 children) depending on which date was closer to the child's first PEFR trial. All measurements taken from questionnaire data were determined within one week of the first trial. Similarly, the presence of any of the following symptoms were recorded directly onto the data sheet for each day of participation: coughing or shortness of breath at night or in the morning, coughing during exertion or upon exposure to cold air, or any wheezing or whistling sounds. In addition to the respiratory symptoms, parents were

instructed to record any use of prescription medication and to specify which medications had been used each day.

Peak Expiratory Flow Rate

Children and parents were instructed in the correct use of the mini-Wright peak flow meter, and were instructed to perform all tests in a standing position. Parents were also instructed to record the best of three readings (Enright, Sherrill et al. 1995; Holcroft, Eisen et al. 2003) achieved by their child twice each day. The morning period, between 7am-9am, was prior to the use of any medications. The evening measurement was to occur between 4pm-7pm. Measurements were recorded on a sheet that reinforced the instructions (see Appendix A). The importance of the correct time for measurements was emphasized and parents were encouraged to insert blanks rather than inaccurate data. PEFR measurements were recorded over a maximum of 10 consecutive days in February 1996 and again in March 1996.

Statistical analysis

For each child, the ΔPEF was defined as the deviation of each PEFR measurement from the child's mean PEFR. This value was calculated for ΔPEF_{am} and ΔPEF_{pm} separately by subtracting the child's mean PEFR over the entire study from the recorded value for that day and subsequently dividing by the child's mean PEFR. These child-specific deviations were then standardized by multiplying each observation by 199.1, the mean PEFR for the entire population. (Neas, Dockery et al. 1995) Values determined to be outlier, greater than three standard deviations from the mean, were removed from analysis. Linear regression analyses of 5 day moving averages of air pollution variables and the ΔPEF values controlling for gender, age, linear time-trend,

BMI, and ETS exposure were performed for each pollutant separately utilizing the mixed procedure of SAS. For all repeated measurements, an unstructured covariance matrix was assumed. All analyses were performed using SAS statistical software version 9.1. (SAS Institute Inc., Cary, NC, USA)

Hypertonic saline challenge test

A pharmacologic hypertonic saline challenge tests was completed to assess bronchial hyperreactivity (BHR) at the time of the third survey in 1997. Subjects were excluded from the tests if their baseline FEV1 was less than 65% of predicted value. We obtained a history of respiratory infection that occurred during the week preceding bronchial hyperreactivity testing. The BHR test was done at a later visit if the child had been suffering from a respiratory infection in the last 7 days. The bronchial challenge test was done following standard procedures. The 4.5% hypertonic solution was prepared by adding 45g of dialysis-grade sodium chloride to 1,000 ml of sterile pyrogen-free water. The aerosolized solution was delivered using an ultrasonic nebulizer (particle-size distribution, 0.5-5 mm; mean, 2.8 mm; De Vilbiss Ultraneb 2000, De Vilbiss, Langen, Germany), connected to 60 cm of corrugated aerosol tubing (inner diameter, 2.2 cm) and two non-rebreathing valves (Hans Rudolph 1410, Hans Ruldolph, Inc., Kansas City, MO). The mean output of the nebulizer was 2.14 ml/min. During the test, the dose of hypertonic saline was increased successively by doubling the aerosol inhalation time, starting with 30 sec up to 8 min. One minute after each inhalation, a flow-volume curve was recorded. The challenge test ended when FEV1 had fallen >=15%, or after a cumulative inhalation time of greater than 15.5 min or a cumulative dose of 23 ml of hypertonic saline had been used. BHR was defined as a fall in FEV1 >=15%.

Chapter 3

RESULTS

Selection and description of the study population

Of the 84 children eligible to participate in the study 72 (85.7%) agreed to participate. Of the 72 children who were enrolled in the study, 70 (97%) participated in both the February and March trial periods. The average morning PEF in February was 294.0 ± 62.4 L/min, whereas the average evening PEF was 298.1 ± 62.3 L/min. The values were similar for March with 303.4 ± 53.6 L/min and 306.6 ± 53.3 L/min respectively. The average variation between morning and afternoon PEF was 4.4 ± 29.4 L/min and 4.3 ± 25.2 L/min for February and March respectively.

The demographics of the children studied are summarized in Table 1. A slightly higher percentage of girls than boys participated in the study, 54.3% versus 45.7%. The average age of the children at their first PEF measurement was 9.23 ± 0.37 years old. All ages were calculated directly from the child's birth date. Sixty-one children (87%) had complete height and weight information for both months. A majority of children (62.9%) had no reported ETS exposure within the previous week. Fifty-nine (84.3%) of the children had complete ETS exposure information for February and March. For those children (7) who were missing either February or March data, ETS exposure was determined based on the value reported in the preceding or subsequent month. For the 3 children who were missing ETS for both February and March, ETS exposure was taken from a 1997 questionnaire completed by the child's parents ascertaining the average ETS exposure of the child per week.

Outdoor air pollution

During the study period, the mean air pollution concentration for $PM_{2.5}$ ranged from 33.35 $\mu g/m^3$ in Bickenbach to 41.0 $\mu g/m^3$ in Gernsheim (Table 2). The mean 24 hour concentrations on $PM_{2.5}$ exceeded the World Health Organization and the United States NAAQS recommendation on 11 out of 55 days between February 4, 1996 and March 31, 1996. The mean concentration for SO_4^{2-} ranged from 4.55 $\mu g/m^3$ in Bickenbach to 5.11 $\mu g/m^3$ in Michelstadt. The mean concentration for NO_3^- ranged from 3.29 $\mu g/m^3$ in Bickenbach to 4.02 $\mu g/m^3$ in Gernsheim. With respect to trace element speciation, there was a noticeably higher mean cobalt concentration for Michelstadt compared with the other communities. Similarly, there was a higher mean platinum concentration in Crumstadt compared to the other sites.

There was a high degree of inter-correlation between the air pollution variables (Table3). Twenty-four hour $PM_{2.5}$ concentrations were found to be statistically correlated with levels of SO_4^{2-} ($r_{Spearman}$: 0.69, $p<0.001$), arsenic ($r_{Spearman}$: 0.84, $p<0.001$), lead ($r_{Spearman}$: 0.0.76, $p<0.001$) and antimony ($r_{Spearman}$: 0.85, $p<0.001$).

Table 1: Demographics of 70 German Children Participating in the Human Health Bio-monitoring Program

Age (years)		9.23 ± 0.37	
Height (cm)		137.5 ± 6.8	
Weight (kg)		31.6 ± 6.2	
		Number	Percent
Gender	Boys	64	45.7
	Girls	76	54.3
ETS: number of days within previous week where child was exposed	Never	44	62.86
	One	23	32.86
	3.5	3	4.29
History of physician diagnosed asthma	Yes	3	4.3
	No	67	95.7
Parental atopy	Yes	24	34.3
	No	46	65.7

Table 2a: Daily daytime mean and maximum concentrations of $PM_{2.5}$, SO_4^{2-}, NO_3^- and Strong Acid across seven Southwestern German communities for 26 days between February and March 1996

	Location	Mean	Std Deviation	Minimum	Maximum	#	# missing
$PM_{2.5}$ ($\mu g/m^3$)	CR	37.88	22.66	10.40	98.00	19	7
	MI	39.28	17.36	9.30	70.20	23	3
	GG	37.80	16.89	10.70	69.40	26	0
	PF	35.08	16.20	10.10	62.80	24	2
	BI	37.32	16.73	11.10	69.00	25	1
	GE	41.00	19.38	13.30	71.10	24	2
	BB	33.35	15.90	10.40	69.40	25	1
SO_4^{2-} ($\mu g/m^3$)	CR	4.94	3.612	0.84	13.22	19	7
	MI	5.11	3.48	0.79	12.65	24	2
	GG	4.93	3.32	0.88	12.74	25	1
	PF	4.70	3.26	0.75	12.72	24	2
	BI	4.81	3.28	0.89	13.22	25	1
	GE	5.03	3.49	0.91	13.58	25	1
	BB	4.55	3.13	0.73	11.77	25	1
NO_3^- ($\mu g/m^3$)	CR	3.34	1.82	0.55	7.06	19	7
	MI	3.29	2.04	0.30	9.25	24	2
	GG	3.34	2.05	0.17	10.15	26	0
	PF	3.35	1.75	0.16	6.98	24	2
	BI	3.81	1.79	1.27	9.00	25	1
	GE	4.02	2.05	1.16	9.19	25	1
	BB	3.20	1.89	0.14	6.67	25	1
Strong Acid: Cl^- ($\mu g/m^3$)	CR	0.10	0.14	0.02	0.54	17	9
	MI	0.09	0.11	0.02	0.41	19	7
	GG	0.08	0.10	0.02	0.40	20	6
	PF	0.13	0.18	0.02	0.66	19	7
	BI	0.13	0.16	0.02	0.63	18	8
	GE	0.10	0.15	0.02	0.58	19	7
	BB	0.09	0.12	0.02	0.46	20	6

CR = Crumstadt, MI = Michelstadt, GG = Auf Esch-Groß-Gerau, PF = Eschollbrüken-Pfungstandt, BI = Biebesheim, GE = Gernsheim, BB = Bickenbach

Table 2b: Daily daytime mean and maximum concentrations of 7 select trace elements across seven Southwestern German communities for 26 days between February and March 1996

	Location	Mean	Std Deviation	Minimum	Maximum	#	# missing
Cobalt (ng/m^3)	CR	0.55	0.02	0.51	0.57	16	10
	MI	0.71	0.62	0.55	3.40	21	5
	GG	0.56	0.01	0.55	0.58	23	3
	PF	0.57	0.01	0.56	0.58	21	5
	BI	0.56	0.01	0.55	0.57	22	4
	GE	0.56	0.01	0.55	0.57	22	4
	BB	0.56	0.01	0.55	0.58	22	4
Cadmium (ng/m^3)	CR	2.14	2.34	1.13	9.31	19	7
	MI	1.96	2.33	1.23	10.43	24	2
	GG	1.83	2.10	1.23	10.39	26	0
	PF	1.73	1.89	1.24	10.37	24	2
	BI	1.63	1.85	1.23	10.53	25	1
	GE	1.66	1.23	1.22	6.42	25	1
	BB	1.58	0.96	1.22	5.04	25	1
Arsenic (ng/m^3)	CR	2.71	2.88	0.80	10.10	17	9
	MI	3.30	3.04	0.88	9.82	23	3
	GG	3.14	3.09	0.88	10.92	25	1
	PF	2.98	2.95	0.89	9.57	24	2
	BI	2.77	2.90	0.88	10.19	24	2
	GE	3.12	3.14	0.88	10.01	25	1
	BB	2.95	2.91	0.87	10.09	25	1
Lead (ng/m^3)	CR	30.58	17.33	8.07	63.37	19	7
	MI	33.63	19.07	8.97	72.34	24	2
	GG	30.30	18.41	7.56	67.70	26	0
	PF	32.58	17.20	11.38	71.32	24	2
	BI	31.79	18.03	8.52	68.75	25	1
	GE	33.19	22.09	6.55	97.27	25	1
	BB	27.72	16.01	6.81	63.07	25	1

CR = Crumstadt, MI = Michelstadt, GG = Auf Esch-Groß-Gerau, PF = Eschollbrüken-Pfungstandt, BI = Biebesheim, GE = Gernsheim, BB = Bickenbach

Table 2b (continued): Daily daytime mean and maximum concentrations of 7 select trace elements across seven Southwestern German communities for 26 days between February and March 1996

	Location	Mean	Std Deviation	Minimum	Maximum	#	# missing
Antimony (ng/m^3)	CR	1.30	0.97	0.12	2.97	16	10
	MI	1.36	0.93	0.13	3.23	21	5
	GG	1.36	1.03	0.13	4.23	23	3
	PF	1.44	0.90	0.13	3.16	21	5
	BI	1.38	0.95	0.12	3.34	22	4
	GE	1.36	0.93	0.12	3.05	22	4
	BB	1.23	0.79	0.13	2.67	22	4
Vanadium (ng/m^3)	CR	2.79	1.82	0.48	6.28	14	12
	MI	3.13	2.21	0.47	6.80	20	6
	GG	2.95	2.01	0.48	6.67	22	4
	PF	2.76	1.86	0.49	6.17	21	5
	BI	2.78	1.81	0.48	6.44	21	5
	GE	3.20	2.35	0.47	8.97	22	4
	BB	2.94	2.02	0.47	6.24	22	4
Platinum (ng/m^3)	CR	0.25	0.50	0.11	2.11	16	10
	MI	0.13	0.00	0.12	0.13	21	5
	GG	0.13	0.00	0.12	0.13	23	3
	PF	0.13	0.00	0.13	0.13	21	5
	BI	0.13	0.00	0.12	0.13	22	4
	GE	0.13	0.00	0.12	0.13	22	4
	BB	0.17	0.22	0.12	1.18	22	4

CR = Crumstadt, MI = Michelstadt, GG = Auf Esch-Groß-Gerau, PF = Eschollbrüken-Pfungstandt, BI = Biebesheim, GE = Gernsheim, BB = Bickenbach

Table 3: Spearman correlation coefficients for measured mean ambient outdoor air pollutants

	$PM_{2.5}$	SO_4^{2-}	NO_3^-	Strong Acid	Co	Cd	As	Pb	Sb	Va	Pt
$PM_{2.5}$ $\mu g/m^3$	1 n=135	0.688 n=135 p<0.001	0.235 n=125 p=0.006	-0.118 n=135 p=0.174	0.054 n=130 p=0.541	-0.229 n=135 p=0.008	0.836 n=130 p<0.001	0.763 n=135 p<0.001	0.848 n=130 p<0.001	0.555 n=130 p<0.001	-0.023 n=130 p=0.794
SO_4^{2-} $\mu g/m^3$		1.000 n=135	-0.214 n=135 p=0.013	-0.160 n=135 p=0.063	0.045 n=130 p=0.611	-0.479 n=135 p<0.001	0.793 n=135 p<0.001	0.804 n=135 p<0.001	0.762 n=130 p<0.001	0.754 n=130 p<0.001	-0.060 n=130 p=0.500
NO_3^- $\mu g/m^3$			1.000 n=135	0.343 n=135 p<0.001	-0.148 n=130 p=0.092	0.066 n=135 p=0.445	0.101 n=135 p=0.245	-0.039 n=135 p=0.655	0.198 n=130 p=0.024	-0.437 n=130 p<0.001	-0.163 n=130 p=0.064
Strong Acid $\mu g/m^3$				1.000 n=135	-0.102 n=130 p=0.249	0.082 n=135 p=0.082	-3.030 n=135 p<0.001	-0.252 n=135 p=0.003	-0.070 n=130 p=0.431	-0.428 n=130 p<0.001	-0.142 n=130 p=0.108
Co ng/m^3					1.000 n=130	0.426 n=130 p<0.001	0.005 n=130 p=0.959	0.034 n=130 p=0.703	-0.072 n=130 p=0.413	0.022 n=130 p=0.808	0.751 n=130 p<0.001
Cd ng/m^3						1.000 n=135	-0.397 n=135 p<0.001	-0.336 n=135 p<0.001	-0.326 n=130 p<0.001	-0.507 n=130 p<0.001	0.322 n=130 p<0.001
As ng/m^3							1.000 n=135	0.829 n=135 p<0.001	0.866 n=130 p<0.001	0.667 n=130 p<0.001	-0.067 n=130 p=0.447
Pb ng/m^3								1.000 n=135	0.872 n=130 p<0.001	0.678 n=130 p<0.001	-0.011 n=130 p=0.905
Sb ng/m^3									1.000 n=130	0.444 n=130 p<0.001	-0.135 n=130 p=0.125
Va ng/m^3										1.000 n=130	0.053 n=130 p=0.546
Pt ng/m^3											1.000 n=130

Description of outcome and external validity

The relative proportion of children completing both morning and afternoon peak expiratory flow rate maneuvers are presented in Table 4. The participation proportions ranged from 4.3-87% for February and between 15.7%-100% for March. If the final two days of the study are excluded, the values are between 71.4%-87% and 97.1%-100% respectively.

The relationship between the children's mean morning PEFR, mean afternoon PEFR, ΔPEFam and ΔPEF_{pm} with various external measures of bronchial hyperreactivity are presented in Table 5. For morning and afternoon PEFR, the mean values coincide favorably with all three external measures of lung function. Similar results are seen with ΔPEF_{am} and ΔPEF_{pm}.

Table 4: Number of children completing both morning and afternoon PEFR measurements by day				
	Feb-96		Mar-96	
Day	Number of Children	Percent	Number of Children	Percent
1	54	77.1	70	100.0
2	61	87.0	69	98.6
3	52	74.3	68	97.1
4	56	80.0	69	98.6
5	57	81.4	69	98.6
6	55	78.6	69	98.6
7	53	75.7	69	98.6
8	50	71.4	70	100.0
9	4	5.7	11	15.7
10	3	4.3	11	15.7

Table 5: Relationship between study mean peak expiratory flow rate and external measures of bronchial hyperreactivity							
Measures of hyperreactivity Hyperreactivity score	Outcome	# of children	# of observations	Mean	Std	05% CL	95% CL
0	Morning PEF (L/min)	48	742	303.7	61.0	299.3	30.8
1		15	232	295.7	49.5	289.2	302.1
≥2		7	102	268.6	47.3	259.3	277.9
0	Afternoon PEF (L/min)	48	732	306.8	60.8	30.2	311.2
1		15	231	300.1	51.5	293.4	306.7
≥2		7	102	274.9	44.5	266.1	283.6
0	ΔPEF_{AM} (%)	48	732	0.91	2.89	0.70	1.12
1		15	227	0.37	3.15	-0.04	0.78
≥2		7	104	0.31	3.63	-0.39	1.02
0	ΔPEF_{PM} (%)	48	732	0.87	2.64	0.68	1.06
1		15	231	0.62	3.45	0.17	1.07
≥2		7	102	0.37	3.77	-0.37	1.11
Hypertonic saline test							
Response missing	Morning PEF (L/min)	7	106	304.7	38.2	297.3	312.0
<15% FEV1 reduction		53	809	300.2	61.4	295.9	304.4
≥15% FEV1 reduction		10	161	286.9	52.1	278.8	295.0
Response missing	Afternoon PEF (L/min)	7	106	308.9	40.1	301.2	316.6
<15% FEV1 reduction		53	801	302.4	59.8	298.3	306.6
≥15% FEV1 reduction		10	158	297.0	60.2	287.6	306.5
Response missing	ΔPEF_{AM} (%)	7	109	0.42	2.96	-0.14	0.98
<15% FEV1 reduction		53	793	0.81	2.94	0.60	1.01
≥15% FEV1 reduction		10	161	0.60	3.51	0.06	1.15
Response missing	ΔPEF_{FM} (%)	7	106	0.65	2.49	0.17	1.13
<15% FEV1 reduction		53	801	0.81	2.78	0.62	1.00
≥15% FEV1 reduction		10	158	0.62	3.98	-0.01	1.24

Table 5: Relationship between study mean peak expiratory flow rate and external measures of bronchial hyperreactivity							
Measures of hyperreactivity Diagnosed Asthma	Outcome	# of children	# of observations	Mean	Std	05% CL	95% CL
No	Morning PEF (L/min)	67	1034	300.3	58.0	296.8	303.9
Yes		3	42	256.9	51.9	240.7	273.1
No	Afternoon PEF (L/min)	67	1024	303.6	58.1	300.1	307.2
Yes		3	41	269.0	51.6	252.7	285.3
No	ΔPEF_{AM} (%)	67	1018	0.75	3.02	0.57	0.94
Yes		3	45	0.41	3.36	-0.60	1.42
No	ΔPEF_{FM} (%)	67	1024	0.77	2.90	0.60	0.95
Yes		3	41	0.59	4.16	-0.72	1.91

Estimate coefficients for the ΔPEF_{am} and ΔPEF_{pm} are summarized in tables 6 and 7 respectively. Regarding ΔPEF_{am}, no pollutants attained statistical significance. Analysis of ΔPEF_{pm} yielded a statistically significant adverse association between increasing levels of NO_3^- and afternoon PEF rates. The estimate corresponds to a 0.11 L/min reduction in mean afternoon PEFR per 1 $\mu g/m^3$ increase in NO_3^- concentration. A similar adverse association was seen with strong acid concentration. A 1 $\mu g/m^3$ increase in mean chloride concentration resulted in a 12.7 L/min reduction in afternoon PEFR. A borderline significant adverse association was also seen for antimony. A 1 ng/m3 increase in mean antimony concentration resulted in a 3 L/min reduction in afternoon PEFR. After specification of a two-pollutant model with PM2.5 and antimony the effect estimates no longer showed a clear association. Nitrate ion was the only pollutant to have an adverse impact on both morning and afternoon PEFR. Increasing levels of cobalt, SO_4^{2-}, arsenic, lead and vanadium all had an apparent protective affect with respect to both morning and afternoon PEFR.

Table 6: ΔPEF_{am} effect-estimates with standard error of air pollution on peak expiratory flow			
Controlling for site, gender, age, time trend, ETS and BMI			
	Estimate	std error	p-value
$PM_{2.5\ (\mu g/m^3)}$	0.01835	0.05990	0.7594
$SO_4^{2-}{}_{(\mu g/m^3)}$	0.1223	0.2773	0.6594
$NO_3^-{}_{(\mu g/m^3)}$	-0.1079	0.4033	0.7892
Strong Acid: Cl^- ($\mu g/m^3$)	4.8594	5.1664	0.3472
Cobalt (ng/m^3)	37.6544	64.6885	0.5607
Cadmium (ng/m^3)	0.2454	0.4134	0.5529
Arsenic (ng/m^3)	0.07751	0.3216	0.8096
Lead (ng/m^3)	0.02947	0.05614	0.5998
Vanadium (ng/m^3)	0.8732	0.4710	0.0641
Antimony (ng/m^3)	0.5774	1.469	0.6944
Platinum (ng/m^3)	-0.9654	10.4256	0.9262

Table 7: ΔPEF_{pm} effect-estimates with standard error of air pollution on peak expiratory flow			
Controlling for site, gender, age, time trend, ETS and BMI			
	β Coefficient	std error	p-value
$PM_{2.5\ (\mu g/m^3)}$	-0.03234	0.06110	0.5968
$SO_4^{2-}{}_{(\mu g/m^3)}$	0.4837	0.2823	0.0870
$NO_3^-{}_{(\mu g/m^3)}$	-1.1174	0.4103	0.0066
Strong Acid: Cl^- ($\mu g/m^3$)	-12.7643	5.2326	0.0149
Cobalt (ng/m^3)	123.11	69.6449	0.0775
Cadmium (ng/m^3)	-0.1404	0.4224	0.7396
Arsenic (ng/m^3)	0.1201	0.3253	0.7120
Lead (ng/m^3)	0.003911	0.05633	0.9447
Vanadium (ng/m^3)	0.8339	0.4752	0.0797
Antimony (ng/m^3)	-2.9691	1.4838	0.0458
Platinum (ng/m^3)	20.8023	10.9793	0.0585

Chapter 3

DISCUSSION

This study found evidence supporting a relationship between ambient NO_3^- and strong acid aerosols air pollution concentration and a reduction in afternoon peak expiratory flow rate. We found no clear associations between ambient $PM_{2.5}$, SO_4^{2-}, or trace metals with respect to reductions in PEFR in a cohort of primary school children. These results support other recent European reports that failed to show clear associations between these air pollutants and PEF. (Roemer, Hoek et al. 1998; Peacock, Symonds et al. 2003)

Previous studies have documented reductions in various lung function determinants in children exposed to increasing levels of NO_2. (Timonen and Pekkanen 1997; Peters, Avol et al. 1999; Peacock, Symonds et al. 2003) In their study, Peters et al. reported a negative association between NO_2 and forced vital capacity, FEV_1, and maximal midexpiratory flow rates in girls. No similar effect was seen with respect to boys. Timonen and Pekkanen demonstrated a significant association between NO_2 levels and reductions in morning PEFR in a cohort of Finnish school children. They reported no effect on afternoon PEFR. In another report, Peakcock et al. 2003 reported statistically significant reductions when stratifying for children who experienced large reduction in PEFR measurements. The results of the current study, showing a significant reduction in afternoon rather than morning PEF rates among school age children, suggest the possibility that may be differences between this report and previous studies. It is possible that monitoring NO_3^- rather than NO_2 concentrations, contributed to the

differences. In addition, since we are unable to control for ozone levels, it is possible that the affects attributed to NO_3^- levels in the current study may in fact be due to exposure to ambient ozone concentrations. (Just, Segala et al. 2002)

The association with strong acid vapor is similar to that reported by Neas et al 1995. (Neas, Dockery et al. 1995) They reported a 2.5 L/min reduction in PEF with strong acid aerosols measured as H^+ ion concentration. The magnitude of the effect estimate in the current study is surprising given the low levels of acid aerosols detected. Given that the standard error was large it is likely that the true effect estimate is much lower.

The observed effect between antimony and afternoon peak expiratory flow rate was unexpected. Although previous studies have implicated various transition metals, no study has indicated antimony specifically. It is possible that this was a chance association. It is also possible that the affects attributed to antimony may be confounded by another metal species such as iron. (Roemer, Hoek et al. 2000) This hypothesis is supported by the high correlation between various metal species in this study.

The lack of effect for other pollutants in this study cannot be adequately explained by low levels of exposures. The average $PM_{2.5}$ and SO_4^{2-} concentrations for the study period were similar to or higher than other reported European studies. (Roemer, Hoek et al. 2000) It is noteworthy that the average NO_3^- concentrations during this study period were actually lower than NO_2 concentrations reported in previous studies. It is acknowledged that the lack of any additional associations could also be explained by low power. The study population consisted of only 70 children and a maximum of 1,145 child-day observations. It is reasonable to conclude that the current study would not have

sufficient power to detect the small decrements reported by previous studies. (Neas, Dockery et al. 1995; Hoek, Dockery et al. 1998)

One proposed difference between those studies which have reported a positive association and those studies which no association was observed concerns the make-up of the various study populations. In many studies reporting an inconsistent relationship between air pollutants and various outcomes involving PEF, researchers have operated under the population average model. This model assumes that all children in the population will have a similar sensitivity to the effects of air pollutants.

A competing theory which was first proposed by Hoek et al. 1998 (Hoek, Dockery et al. 1998) operates on the assumption that there is a heterogeneous response to air pollution among the childhood population. In this model, the authors only suggest that only a small proportion of children will be sensitive to the effects of respiratory insults. The conclusion based upon the population susceptible model is that overall effects observed in outcomes based upon continuous variable analyses will be diluted by the majority of children who are not susceptible. Evidence for this theoretical model is provided by several studies verifying a positive association between levels of air pollution and childhood lung function deficits in children with chronic respiratory complaints. (Neas, Dockery et al. 1995; Yu, Sheppard et al. 2000) Further support has been provided researchers having demonstrated a more consistent association between ambient air pollution concentration and large PEFR decrements. (Hoek, Dockery et al. 1998; Peacock, Symonds et al. 2003) As only three of the children in this study were confirmed to be asthmatic and only four used bronchodilators during the course of the study, subgroup analysis in the current study was not feasible.

In addition, the lack of daily determinations of air pollutants is a potential limitation of the study. It is possible that the lack of complete measurements obscured possible associations due to misclassification of the actual 5 day average pollutant exposure. This hypothesis is supported by significant fluctuations in pollutant levels for several of the communities. However, this is unlikely to systematically bias the data since each center collected data on a similar time schedule and any missing values would have been unknown to the children or parents.

Similarly, the lack of site-specific temperature readings could have obscured any associations. This, however, is also unlikely as all of the communities except Michelstadt are in the Rhine Valley and therefore, regional climate trends would not be expected to vary dramatically between sites.

Although the multiple locations of air pollution collection data, the establishment of schools as the sites of collection, and the restriction of the study population to those children within one kilometer of the schools, reduces the possibility of exposure misclassification, the possibility remains. The inability to account for time spent outdoors may have accounted for the observed lack of association. Neas et al. have reported that accounting for individual time spent outdoors increases the magnitude of effect estimates and reduced variation in outcomes. (Neas, Dockery et al. 1995)

Furthermore, since the PEFR determinations were made during colder months, it may be reasonable to assume that children would spend less time outdoors and have had less exposure to ambient air pollution. With the exception of exposure to NO_3^-, less time spent outdoors would be expected to bias the results toward a null association. It is possible that during colder months, the use of home heating oil would increase thereby

resulting in increasing indoor concentrations of NO_3^-. The combination of higher indoor NO_3^- concentrations and children spending more time indoors could drastically alter the reported association between ambient outdoor NO_3^- concentrations and peak flow rate measurements.

Another potential limitation in the current study is the inability to account for respiratory infections and other infectious outbreaks during the study period. It is possible that infectious agents may have influenced the children's lung function and biased the effect estimates on PEF. As infectious processes would have varied in time, severity and location, any potential association between air pollution and PEF measurements based upon communities may have been biased. Due to the nature of these infections, it is reasonable to conclude that these biases could have occurred in either direction. In one scenario, an influenza outbreak in a community with low average daily pollution could bias the results toward the null whereas an infectious outbreak in a community with high daily averages of pollutants could bias the results in a positive direction.

Similarly, the inability to account for seasonal allergic rhinitis may have confounded the association between PEFR and air pollution levels in susceptible children. During data analysis, we attempted to correct for child hay fever by controlling for parental history of atopy. It is possible that although parental history of atopy has been shown to be a risk factor for childhood allergic rhinitis, (Graif, Garty et al. 2004) it was not a sufficient indicator of hay fever in these children.

Another possible contributing factor could result from the use of mini-Wright peak flow meters to assess PEF. The portable peak flow meters are considered less

precise than timed forced expiratory volumes measured by spirometry. (Giannini, Paggiaro et al. 1997) The absence of complete spirometry is a potential limitation of the study, however Neas et al. (Neas, Dockery et al. 1995) reported on a similar population based cohort in Uniontown Pennsylvania. They observed high correlations between PEFR measurements and FEV_1 values obtained via spirometry. Furthermore, the correlation between both PEF and ΔPEF with external measures of bronchial hyperreactivity would suggest reasonable reliability.

The short duration of the measurement periods, prevented controlling for any potential training effect in the use of the peak flow meters. Previous researchers have documented that inconsistencies due to training effect are observed for the first 2-3 days of measurement. (Siersted, Hansen et al. 1994; Enright, Sherrill et al. 1995) The inability to correct for any potential training effect is an accepted limitation of the study.

A common practice in studies analyzing the relationship between outdoor pollutants and lung function is to construct multiple statistical models with varying lag day exposures and to report those associations which are the strongest. While the exact temporal relationships between exposures to air pollutants and potential physiologic inflammatory responses are not known, this practice increases the potential of detecting a chance association. Maintaining the relationship as a 5-day moving average minimizes the risk of obtaining an erroneous association based upon model selection. Furthermore, studies have observed a larger and more precise estimate regarding the effect of air pollution exposure on lung function using the 5-day average instead of shorter periods of exposure. (Pope and Dockery 1992; Gold, Damokosh et al. 1999)

In addition, the use of ΔPEF may have limited the potential to detect changes between a child's morning and afternoon PEF rates. In measuring the effect of maternal smoking on childhood lung function (Frischer, Kuhr et al. 1993), investigators involved in the current study employed the percentage ratio of the amplitude above the mean (AVAM) of daily PEFR. (%amplitude/mean = (abs(PEF_{PM}-PEF_{AM}))/meanX100) as a measure of bronchial hyperreactivity. The AVAM model is conceptually based on an asthmatic model where morning peak flow rates are thought to be disproportionately affected by ambient air pollution levels in comparison to afternoon and evening rates. In such models, an increase in the calculated PEFR variability (AVAM) is thought to correspond to increased BHR. This phenomenon is based on a physiologic understanding of airway hyperreactivity in relation to diurnal variations serum cortisol levels. The end result of this relationship is an observed increase in susceptibility to airway insults in morning hours when cortisol levels are at their nadir in the bloodstream. Although this model has been employed successfully in populations with known BHR (asthmatics), its applicability to non-asthmatic populations is less clear. In the current study population, we observed that many of the children had evening PEFR that were consistently lower than their morning PEFR. The authors concede that the observed decrements in afternoon PEF may simply reflect a more accurate response to ambient air pollution given increased exposure to outdoor environments. Furthermore, there may have been a greater degree of exposure misclassification in regard to morning PEF secondary to the anticipated increase in time spent indoors during night-time and early morning hours. Irrespective of the potential etiology, the authors do not feel that the relative decrements in PEF_{PM} observed in the current study would accurately be reflected in the AVAM

model. Given these findings, we would further caution future researchers who might consider employing this model in non-asthmatic populations.

Another potential factor which could have limited our ability to detect effects of air pollution on lung function was the assumption of an unstructured covariance structure in the statistical models. Given the perceived correlation between each individual child's daily PEFR measurements, not accounting for this correlation would have resulted in increased sampling error for the β-coefficients. Typically, this would be expected to bias the hypothesis toward the null association. We attempted to correct for correlation and thus improve precision of the β-coefficient estimates by fitting the model to a first order autoregression structure. Upon analysis, these models did not allow convergence of the matrix. We suspect this was due to the relatively short time period for the repeated PEFR measurements and the lack of individual measurement variability with respect to time.

To our knowledge, there are very few epidemiological studies investigating the adverse health impacts of trace metal exposure. One report investigating the association of various transition metals with adverse respiratory outcomes indicated a positive association between ambient iron, nickel, and zinc concentrations with daily mortality (Burnett, Brook et al. 2000) in adults. Another report studied the association between trace metal PM_{10} speciation and PEF. (Roemer, Hoek et al. 2000) The authors reported no significant effect between iron, nickel, zinc, vanadium, sodium, lead or silicon with ΔPEF_{PM} or ΔPEF_{AM}.

We are unaware of any published study which has attempted to analyze specific health outcomes with the individual speciation of $PM_{2.5}$. In a recent study conducted in Michigan, Batterman et al. (unpublished) reported that weaker correlations were observed

between two sites for trace metals than for gaseous air pollutants. Their speciation analysis of $PM_{2.5}$ also concluded that the contribution of various $PM_{2.5}$ species was more dependent on specific site location and local pollutant sources than was observed for other pollutants. The absence of a clear association between various trace metals and PEFR in the current study could indicate that at the levels reported there is no significant health impact. It is also possible that the variation between communities was insufficient to detect any potential health impact. With the exception of platinum, this hypothesis is supported by similar variation between mean $PM_{2.5}$ concentrations and the mean concentrations of the transition metals. The large protective association seen for platinum may be explained by the large discrepancy between Crumstadt and Bickenbach and the other communities. A similar discrepancy was observed with cobalt between Michelstadt and the other communities respectively.

. In conclusion, this longitudinal study of 70 children in Southwestern Germany failed to demonstrate consistent associations between air pollutants and childhood lung function as measured by changes in morning and afternoon peak expiratory flow rates. The results provide additional evidence for reductions in childhood lung function associated with exposures to low level ambient NO_3^- and strong acid aerosol concentrations. The inability to demonstrate adverse affects of particulate matter on PEF support previous studies in which no clear associations were established in a healthy population. The significant association observed for antimony suggests further research into health effects of various transition metal species is warranted. The inability to demonstrate consistent relationships supports previous assertions that future research

endeavors investigating associations regarding PM and its speciation should target susceptible children with chronic respiratory complaints.

APPENDICES

APPENDIX A

PEAK FLOW MEASUREMENT DATA SHEET ENGLISH TRANSLATION

PEAK-FLOW-MEASUREMENT from to Code Number

Directions: Take a deep breath and take mouthpiece in your mouth
Put lips tightly around the mouthpiece
As strong and as fast as possible blow into the mouthpiece
Do this 3 times and record the highest of the 3 results in the table below

Important: Please do under supervision of a parent
Please stand while blowing
After each trial reset meter to 0
If on a certain day you did not do the measurement make a diagonal line through that day on the table

Please fill in:
Height of child |_|_|_| cm
Weight of child |_|_|.|_| kg

How often, during the week of measuring, was your child in a room with second hand (passive) smoke?
Daily
A few times a week
Once
Never

The results must be recorded by the parent (not the child)

Date										
7-9 AM										
4-7 PM										
Please mark if your child experienced any of the following symptoms for each day										
Coughing/Shortness of breath at night or in the morning	o	o	o	o	o	o	o	o	o	o
Coughing during exertion or with exposure to cold air	o	o	o	o	o	o	o	o	o	o
Wheezing sounds	o	o	o	o	o	o	o	o	o	o
Medications:	o	o	o	o	o	o	o	o	o	o

If yes which ones: _____

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