

Detection of Latex Allergens by Immunoelectron Microscopy in Ambient Air (PM₁₀) in Oslo, Norway (1997–2003)

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Abstract

The authors collected ambient air along two highways in Oslo to investigate the annual variations in particulate matter (PM₁₀) and the presence of latex as an outdoor allergen. PM₁₀ was monitored for a period of five years, during which time the use of studded winter tires was reduced. The presence of latex and of common aeroallergens was examined directly on the collection filters with immunoelectron microscopy visualized in a scanning electron microscope.

The annual variation in PM₁₀ was similar over the five years of sampling, with increased mass concentrations in winter. Statistical analysis indicated no major effect from the change to nonstudded tires. The most important factors influencing the PM₁₀ concentration were meteorological parameters like wind and rain. Immunolabeling of the filters showed latex as an outdoor allergen that adhered to carbon aggregates from vehicle emission. The results also indicated cross-reactive epitopes among the common allergens investigated, which for sensitized subjects may add to the risk of developing latex allergy.

Introduction

Urban atmosphere is known to contain combustion products, with the predominant source being traffic-related (Atkinson et al., 1999; Kunzli et al., 2000), as well as plant-derived biologically active particles that may induce allergy and asthma (Behrendt & Becker, 2001). Epidemiological studies suggest a correlation between exposure to particulate air pollution and several adverse health effects, including acute respiratory symptoms (Brunekreef, Dockery, & Krzyzanowski, 1995; Delfino, 2002; Pope, 2000; Schwartz, 2001). The acute respiratory ef-

fects may, in part, be caused by an allergic response to inhaled airborne particles that contain specific proteins or allergens (D'Amato, 2002; Glovsky, Miguel, & Cass, 1997; Polosa, 2001).

One of the so-called occupational allergies frequently described is latex allergy, which refers to allergy caused by natural rubber latex (NRL), either by direct contact with latex products or by inhalation of airborne allergens (Nettis, Colanardi, Ferrannini, & Tursi, 2002; Vandenplas, 1995). Latex is primarily recognized as an indoor allergen, but a major component of car tires is NRL,

and tire dust in urban air samples has been postulated to contribute to latex sensitization and asthma (Miguel, Cass, Weiss, & Glovsky, 1996; Williams, Akasawa, Dreskin, & Selner, 1996; Williams, et al., 1995). In Norway, studded tires have been used for decades because of the snow-covered and icy roads in winter. In 1997, however, the Norwegian government recommended the use of winter tires without studs to reduce particulate air pollution from road wear. In the winter of 2000–2001, 80 percent of the cars in the capital city, Oslo, were running on nonstudded winter tires. These tires, however, are said to contain more NRL to make them softer for improved road grip on snow and ice. Furthermore, softer tires wear more easily, releasing more rubber particles into the air that may be an additional source of latex in the environment.

Particulate air pollution is a complex mixture of solid particles and liquid droplets of different size, shape, and origin. The frequently measured particle size fraction that contains particulate matter with a mean aerodynamic diameter less than 10 micrometers (μm) (PM₁₀) is of special interest since these particles are inhalable. (Aerodynamic diameter is defined as the diameter of a spherical particle having unit density that has the same settling velocity as the particle in question.) This fraction can be divided into coarse, fine, and ultrafine fractions with diameters of >2.5 μm, <2.5 μm (PM_{2.5}), and <0.1 μm (PM_{0.1}),



The main collection site (Ring 2). The white arrow indicates the position of the field monitor in relation to the pavement and the road crossing.

respectively. Inhaled particles may be trapped by the mucociliary apparatus and removed from the lungs within 24 hours. Particles of 5 μm or less, however, are deposited in the alveolar region, and clearance may take days or months. Accumulation of particles may thus result in local exposures to high particle concentrations.

The present study focuses on the annual variation in mass concentration of PM_{10} and the detection of latex in these samples. Being small and too few in number, microbiological particles like viruses, bacteria, and molds, will not be detected in the SEM. Pollen grains, on the other hand, are $>10 \mu\text{m}$ in diameter, and do not belong to the collected fraction, but their allergens may be released and present. The detection of allergens in the PM_{10} fraction may thus help explain the increase in sensitization to common aeroallergens in urban areas.

The variation in PM_{10} and the presence of latex were investigated in relation to the change from studded to nonstudded winter tires. During periods of five years and two years, samples were collected along two highways, Ring 2 and Ring 3, respectively, in the urban area of Oslo. Since quantitative immunoassays of eluates require large amounts of PM_{10} , an immunogold labeling method was used to detect the allergens directly on the collection filters. In addition to latex, the presence of the most common aeroallergens was examined. The backscatter electron imaging (BEI) mode of a scanning electron microscope (SEM) was used to visualize the immunogold labeling of allergens, and secondary electron imaging (SEI) was used to examine the morphology of the particles.

Materials and Methods

Ambient-Air Sampling

Ambient-air samples, PM_{10} , were collected on filters at two of the highways running through Oslo, Ring 2 and Ring 3. The main sampling site was adjacent to a road crossing on Ring 2 (see photo above), where the traffic load was approximately 21,500 cars per 24 hours. The second site, Ring 3, had two more lanes, with approximately 54,000 cars per 24 hours, and the samples were collected adjacent to a toll road station. PM_{10} was collected from September 1997 to November 2002 at Ring 2 on a total of 266 filters. Air samples were collected on the same dates at Ring 3 from January 2000 until August 2001 on a total of 77 filters. The frequency of the sampling varied from every two weeks in summer to every week, or more often, in winter. The samples were collected for the same period of time, for 24 hours, every Tuesday to Wednesday, including two peak periods of traffic. For the 266 sample dates, the mean 24-hour meteorological parameters, like temperature and precipitation (rain/snow), were obtained from the Norwegian Meteorological Institute in Oslo, located close to and between the two collection sites. Incidences of strong wind on the sample dates were noted.

Low-volume ambient-air samples were collected on polycarbonate filters loaded in three-piece field monitors with 50-millimeter (50-mm) extension cowls (MHTS 025 AC, Millipore). The pore size of the collection filters was $0.4 \mu\text{m}$ with backing filters of $5 \mu\text{m}$ to ensure even distribution of particles. The field monitors were connected at Ring 2 to a Dymax 30 vacuum pump (C. Austen Pumps, United

Kingdom) with airflow of approximately 4 liters per minute (L/min) as measured with a mass flow meter (Aalborg Instruments). At Ring 3, an Airchek Sampler with constant airflow of 3 L/min (Model 224-PCXR8, SKC Ltd., United States) was used. The monitors were positioned 1.6 m above the ground, facing down at an angle of 45° to avoid collection of sedimented particles. The polycarbonate filters were equilibrated for 24 hours and weighed, both before and after sampling, with a microbalance (Mettler MT5). The equilibration, loading of filters, and weighing were carried out in a dust-free cabinet to ensure a stable environment, and the mean mass concentrations of PM_{10} were calculated and recorded in $\mu\text{g}/\text{m}^3$. Air filters were immunolabeled for allergens and studied in the SEM, or stored in endoport tubes in the freezer for later use.

Statistical Analysis

Since the data were not normally distributed, rank correlation (Spearman r_s), and non-parametric tests like Mann-Whitney (z) and Kruskal-Wallis (χ^2) were used to analyze the effect of meteorology on the variations in PM_{10} .

In addition, a multiple regression analysis was performed for the whole sampling period (1997–2002). The analyses were carried out separately for the winters (November 1 to April 30, $n = 141$) and the summers (May 1 to October 31, $n = 123$). The PM_{10} data were positively skewed and were therefore log-transformed ($\ln y$) to obtain a more normal distribution. Confidence intervals (CI) and β -values were back-transformed (e^x) to return to the original scale. The independent variables were all transformed to dummy variables (year, temperature, precipitation, dry weather, and wind) for easier interpretation of differences between parameters. The parameters with the largest number of data were used as references. The data for temperature were divided into three categories: <0 to $1^\circ\text{C} = 1$, 1 to $10^\circ\text{C} = 2$ and $>10^\circ\text{C} = 3$. For winter, the year 1998–1999, Temperature Category 1, and dry weather were used as references. For summer, the same year (1998) and same weather type were used, but the temperature category was 3. The software package SPSS version 10.0 was used for all analyses.

Immunolabeling

The authors labeled the ambient-air samples directly on the collection filters by punching out 3-mm disks from the filters. The filter specimens, backed on nickel grids by the aid

of conductive carbon tabs (Ted Pella, Inc., Redding, California), were washed and incubated with antibody, upside down, on 10- μ L droplets on parafilm. To block the free binding sites, the filter specimens were incubated with 1 percent bovine serum albumin (BSA) in buffer for 15 minutes before incubation overnight with the appropriate antibody in a humid atmosphere, at 4°C. The specimens were washed in buffer for 15 minutes $\times$ 4 before labeling for 90 minutes with the secondary antibody conjugated to 30-nanometer (30-nm) colloidal gold particles. After they were washed and dried, the specimens were coated with a thin layer of carbon and mounted on stubs with the aid of carbon tabs, before examination in the SEM.

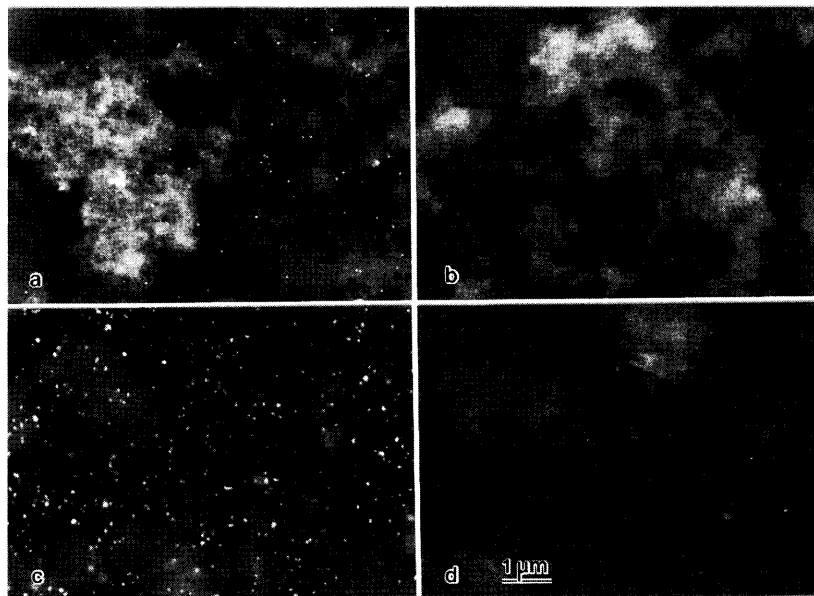
From September 1997 to January 1999, two PM₁₀ filters were labeled each month with a monoclonal antibody to latex. After that, filters were selected at random and labeled to a total of 50 filters. In addition, more than 100 filters from every season were labeled for the common aeroallergens using both monoclonal (MoAbs) and polyclonal antibodies (PoAbs) to birch, timothy, cat, and dog.

Antibodies and Gold Conjugates

The authors used a MoAb (LAM-2) produced against the 23-kilodalton (23-kD) latex polypeptide Hev b 3 (Lu, Kurup, Hoffman, Kelly, Murali, & Fink, 1995) to label the PM₁₀ samples. Monoclonal antibodies to the birch pollen allergen *Betula alba verrucosa* (*Bet v 1* [clone 2E10, CS2/135, Allergopharma J. Ganzer, Hamburg, Germany]), and timothy (*Phleum pratense*, *Phl p 1* [IG 12] and *Phl p V* [Bo1]) were applied. The two MoAbs to timothy grass pollen were both hybridoma supernatants, used without dilution, and were kindly donated by Dr. A. Petersen of the Division of Biochemical and Molecular Allergology, Research Center Borstel, Germany. Two rabbit anti-sera to timothy and birch were purchased from Pharmacia Upjohn, (former Pharmacia AS, Norway branch). Monoclonal antibodies to cat, *Felin domesticus* (*Fel d 1* [clone 6F9 A4-H]), and dog, *Canis familiaris* (*Can f 1* [clone 6E9F9]), were provided by M. Chapman of the University of Virginia. The MoAbs were used in concentrations of between 1 and 3 μ g/mL, whereas the PoAbs were diluted 1:500 or 1:1,000. The relevant gold probes, goat anti-mouse IgG-30 nm and goat anti-rabbit IgG-30 nm (BioCell Research Laboratories, Cardiff, United Kingdom) were applied in 1:40 dilution. The buffer used was 0.1N ammonium acetate with 1 percent BSA, pH 7.2.

FIGURE 1

Backscatter Electron Micrographs of Ambient-Air Samples from the Racetrack



These images show moderate labeling of the carbon aggregates with the anti-latex (LAM-2) (Micrograph a) and no labeling with an irrelevant antibody (IgG) (Micrograph b). Latex gloves show strong labeling with LAM-2 (Micrograph c) and nonlatex gloves show no labeling (Metin) (Micrograph d). Magnification: 10,000 $\times$.

Controls

Since Norway has no tire-manufacturing plants, a pilot study was performed indoors in a go-cart racetrack to check that the MoAb to latex (LAM-2) labeled a latex protein that originated from car tires. Sampling was performed when no cars were running, when cars were running for half the sampling time, and when cars were running for all sampling hours (24 hours) (Figure 1a). The same sampling method was used as for the ambient-air sampling done outdoors. The hall was equipped with an air-filtering system that excluded most particles from the outside. All samples were immunolabeled with anti-latex antibody (LAM-2).

Anti-human serum albumin (anti-HSA) (clone ZMHSA, Zymed, San Francisco) and several other irrelevant antibodies (to different bacterias) of IgG class were used as specificity controls for the antibodies under investigation (Figure 1b). The buffer replacing the antibody was used as specificity control for the gold probes. A positive control for the latex antibody (LAM-2) was disks (3 mm) punched out of a brand of latex glove (Safeskin Corp., Boca Raton, Florida) (Figure 1c). One brand of synthetic glove, (Metin, Medical Technology AS Glomfjord, Norway) served as negative control for LAM-2

(Figure 1d). The punched-out glove disks were backed on nickel grids, as above, and labeled.

Scanning Electron Microscopy

The authors visualized gold labeling with atomic number contrast using backscatter electrons (BEI mode) in the SEM (JSM 840, Jeol Ltd., Japan). The microscope was operated at 15 kiloelectron volts (keV), with the anode in the highest position, and with a working distance of 7 mm (Namork, 1991). Secondary electron imaging (SEI mode) was used for better visualization of the morphology of the ambient-air particles. Immunolabeling is not a quantitative method, but was in this study judged as weak, moderate, and strongly labeled with reference to the positive control being strongly labeled (Figure 1c). The authors judged the density of labeling during microscopy for at least 10 different areas on each specimen (primary magnification 7,000 $\times$).

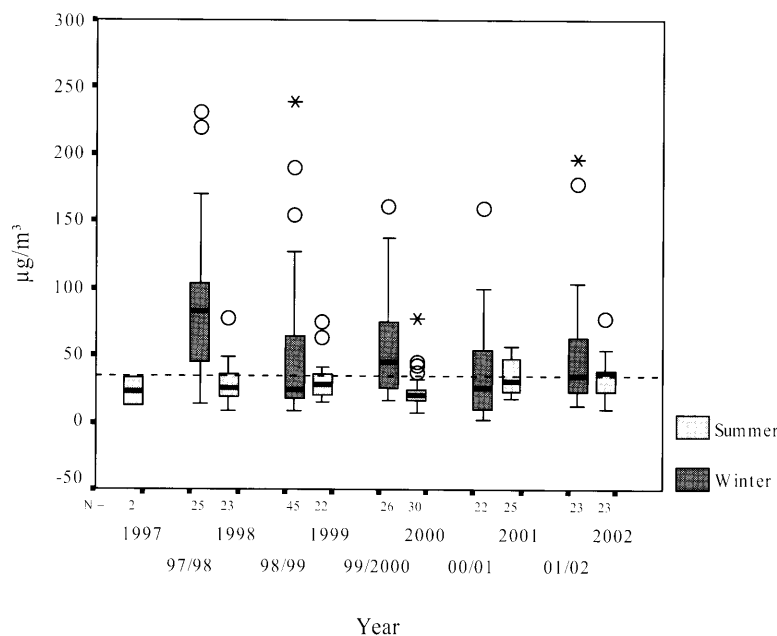
Results

Mass Concentrations of Ambient Air and the Effect of Meteorological Parameters

The 24-hour mean mass concentrations of PM₁₀, for winters and summers, as recorded

FIGURE 2

Box Plot of PM₁₀ Mass Concentrations (24-Hour Mean) for Each Year, Collected at Ring 2 (1997–2002)



The data are plotted separately for winters and summers, showing the 2.5, 25, 50, 75, and 97.5 percentiles. The horizontal broken line represents the recommended national guideline of 35 µg/m³. *N* = number of filters. Outliers (o) and extreme (-) values resulted from sampling during dry weather and wind.

from September 1997 to November 2002 at Ring 2, are plotted in a box-plot (2.5, 25, 50, 75 and 97.5 percentiles) (Figure 2). The horizontal dotted line at 35 µg/m³ (24-hour mean) indicates the national PM₁₀ guideline for outdoor air quality at which level health effects begin to appear (recommended by the Norwegian Pollution Control Authority). Studded tires are used in the period from November 15 until the first Monday after Easter (April 20 in 1998, April 12 in 1999, May 1 in 2000, April 23 in 2001, and April 8 in 2002).

In general, the same annual variation in PM₁₀ was observed during the five years of sampling, with significantly higher concentrations in winter than in summers ($z = 3.78$, $p < .000$) (Figure 2). Of the winter and summer PM₁₀ samples, 51 percent and 31.5 percent, respectively, exceeded the national guideline of 35 µg/m³. In addition, the percentiles indicated in Figure 2 show higher variation in mass concentrations during winters.

Analysis found the correlation between pooled PM₁₀ data for the five years and temperature (°C) to be weak but significant ($r_s = -0.18$, $p < .01$), showing a tendency for the

PM₁₀ concentration to increase with decreasing temperatures. There was, however, found to be a highly significant effect from other meteorological parameters such as precipitation (rain/snow), dry weather, and wind ($\chi^2 = 98.4$, $df = 2$, $p < .000$). Furthermore, post hoc tests between pairs of the three types of weather showed significant differences. Medians for PM₁₀ concentrations collected on days with precipitation, dry weather, or wind were 20.14 µg/m³, 36.8 µg/m³, and 66.1 µg/m³, respectively.

Multiple regression analysis was conducted separately for winter and summer to further examine the annual variation in PM₁₀ concentration and the effect of meteorological parameters. When "year" only was used as an independent variable, the PM₁₀ for the first winter (1997–1998) was found to be significantly different from the PM₁₀ for the reference year (1998–1999), with a ratio of 1.37 (95 percent CI: 1.36–3.11, $p = .001$). This difference was diminished, however, by adjusting for the meteorological parameters (ratio = 1.15, 95 percent CI = 1.01–2.10, $p = .05$). The model was highly significant ($R^2 = .52$) and showed that precipitation, dry weather, and

wind were the significant predicting factors. The ratio for wind versus dry weather was 1.25 (95 percent CI = 1.33–2.84, $p = .001$) and for precipitation versus dry weather was 0.58 (95 percent CI = 0.27–0.45, $p < .000$). In this model, the temperature was not a significant predictor variable. The same analyses of the summer data showed no significant differences between any of the five years. Meteorological parameters other than temperature were significant predictor variables in the summers ($R^2 = .33$). The PM₁₀ ratio for wind versus dry weather was 1.29 (95 percent, CI = 1.16–1.94, $p = .002$) and for precipitation versus dry weather was 0.70 (95 percent, CI = 0.61–0.84, $p < .000$).

The median PM₁₀ concentrations sampled at Ring 2 and Ring 3 on the same dates were 25.8 µg/m³ and 43.4 µg/m³, respectively. The correlation between the concentrations sampled at the two sites was significant ($r_s = .63$, $p < .000$).

Scanning Electron Microscopy and Immunolabeling

The SEM of the air filters showed the characteristic morphology of carbon particle aggregates of all sizes up to 10 µm, imaged with secondary electrons (Figure 3a). On days with strong wind or after spring-cleaning of the streets, particles of higher elemental composition than carbon were observed in the BEI mode. Increase in mass concentration of PM₁₀ also was registered after days with strong wind. Lower mass concentrations were recorded, however, when sampling was conducted during precipitation, and fewer and smaller carbon aggregates were observed in the SEM. No characteristic debris from tires or pollen grains was observed.

The gold probe used in immunolabeling for allergens was visualized as white spots in the BEI mode (Figure 3b, Figure 3c, Figure 3d, Figure 1a, and Figure 1c). Figures 3a and 3b show the same carbon aggregate imaged with secondary electrons and backscatter electrons, to visualize the surface morphology and the immunolabeling, respectively. A comparison of Figure 3a and Figure 3b makes it evident that the gold probe labels only the carbon particles and aggregates and not the background filter. The PM₁₀ filters labeled with the MoAb LAM-2 showed moderate labeling of the carbon aggregates (Figure 3b). No major differences in labeling strength of LAM-2 were visible among months, seasons, or years. The MoAbs to the timothy and birch allergens showed weak to moderate

labeling with anti-*Phl p I* (Figure 3c), independent of the time of year, and no labeling with anti-*Phl p V* (Figure 1d) or *Bet v I*. The PoAbs, however, labeled the filters irregularly through the year, and some even in winter. The MoAbs to *Fel d I* (cat) and *Can f I* (dog), gave weak to moderate labeling and no labeling, respectively. The antibodies that showed positive labeling of the carbon aggregates on the PM₁₀ filters also labeled the surface of the latex glove.

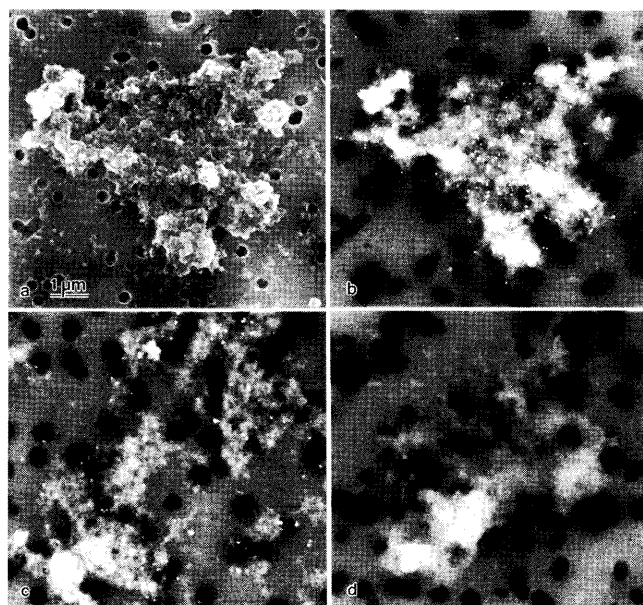
Observations in the SEM of the three control specimens collected in the indoor racetrack showed that all filters contained carbon aggregates with increasing mass concentrations for sampling with no cars running to sampling with cars running half the sampling time and sampling with cars running at all sampling hours. Consequently, immunolabeling using the anti-latex antibody LAM-2 labeled the carbon aggregates with increasing intensity. The MoAb LAM-2 was considered to label a latex protein from car tires, since cross-reactive proteins from outside were unlikely to be present in the indoor racetrack with filtered air conditioning (Figure 1a). The latex glove surface, serving as positive control for latex, always labeled strongly with LAM-2 (Figure 1c). Labeling of the carbon aggregates and latex glove surface with different irrelevant antibodies and replacement of the antibody with buffer always produced negative results (Figure 1b). Labeling of the synthetic glove also was negative for all the antibodies tested (Figure 1d).

Discussion

The sampling conditions used in this study have previously been found to collect carbon aggregates of sizes up to 10 μm (PM₁₀), with the majority of particles (60–80 percent), in the fine-particle fraction PM_{2.5} (Ormstad, Gaarder, & Johansen, 1997). This finding is in agreement with results from another study reporting high correlation between PM₁₀ and PM_{2.5} (Peters et al., 2000). Hence, the majority of the particles will be deposited in the alveolar region, resulting in accumulation of particles for up to several months. Furthermore, the geometry of the monitor and the low-volume sampling were found to collect ambient and not sedimented particles. To obtain samples relevant for human exposure, the monitor was placed at a height equal to the breathing zone of a person with average height.

FIGURE 3

Scanning Electron Micrographs of PM₁₀ Collected on Filters, Immunogold-Labeled for Allergens



Micrographs a and b show the same carbon aggregate labeled with anti-latex (LAM-2); Micrograph a is imaged with secondary electrons to enable observation of the morphology (note the single carbon particles of approximately 50 nm [arrows]). Micrograph b is imaged with backscatter electrons to enable visualization of the immunogold labeling. In the last two micrographs, carbon aggregates labeled with antibodies to timothy pollen *Phl p I* (Micrograph c) and *Phl p V* (Micrograph d), imaged with backscatter electrons, show moderate-to-weak labeling and no labeling, respectively. Magnification: 10,000 $\times$.

The statistical analysis verified the recorded (Figure 2) and observed influence of meteorological parameters on the annual variation in PM₁₀. Since the PM₁₀ concentrations for winter and summer were found to be significantly different, the data were analyzed separately to get an indication of the effect of a decrease in the use of studded tires. The regression analysis, however, showed that dry weather, precipitation, and wind were the only significant predicting factors in the present model. The lower predicting value for the model used on the summer data, 33 percent versus 52 percent in winter, is probably due to the much more stable weather at this time of year (Figure 2).

Since the observations in SEM of the filters from Ring 2 and Ring 3 showed mainly carbon particles and aggregates and since both sampling sites were alongside highways, their origin was concluded to be mainly vehicle emissions. This conclusion was in agreement with the higher traffic load and the 70 percent higher PM₁₀ concentration recorded at Ring 3,

as compared with that recorded at Ring 2. The significant correlation between the two sites sampled on the same dates could in part be explained by equivalence in meteorology, since the air distance between the two sites was only 2 kilometers (km). The increase in mass concentrations registered when sampling was conducted during wind correlated with the increase in particles with higher elemental composition than carbon, observed in the BEI mode. These particles were likely to be caused by resuspension of sedimented particles (Tittanen, Timonen, Ruuskanen, Mirme, & Pekkanen, 1999) originating from erosion of road material. This was supported by the fact that the outliers and extreme values in Figure 2 were sampled during strong wind; periods of cold, dry weather (as opposed to precipitation); or a combination of the two. Although temperature was not a highly significant predictor, the increase in PM₁₀ recorded when sampling was conducted during cold, dry weather in winter could in part be explained by high atmospheric pres-

sure followed by a low-lying inversion layer leading to little exchange of air.

The positive labeling observed with LAM-2 on the PM₁₀ filters from the highways Ring 2 and Ring 3 suggests latex is an outdoor allergen. Many of the latex proteins are soluble proteins and may be released from tires, tire debris, or wear. The latex allergens were shown to be adhering to the carbon aggregates, probably by charge, thereby becoming ambient in the same way as the latex allergens are carried by starch powder from gloves indoors. There was no indication, however, of the softer winter tires generating more latex in the ambient air, since no major difference in labeling strengths with LAM-2 was observed with respect to seasons. Furthermore, since no rubber particles were recognized on the filters, they were deduced to belong to the sedimented fraction and not to the PM₁₀.

Cross-reactivities caused by defense-related proteins like plant profilins are responsible for the pollen-fruit syndrome and similarly the latex-fruit syndrome (Ganglberger, et al., 2001; Kurup, Kelly, Elms, Kelly, & Fink, 1994; Perkin, 2000; Yagami, 2002), and for the cross-reactivities between latex and pollen (Fuchs et al., 1997; Merret, Merret, & Kekwick, 1999). The irregular labeling with the pollen antibodies in this study might therefore be caused by homologies in epitopes of plant profilins, since the presence of pollen allergens was not expected in late winter in Norway. The cat and dog allergens, on the other hand, are present in dander and saliva of the animals, which may become airborne and attach to the carbon particles in the ambient air, giving a positive labeling by *Fel d I*. Although both cat and dog allergens have been found to adhere to carbon particles in house dust in previous studies (Ormstad, Namork, Gaarder, & Johansen, 1995; Ormstad, Johansen, & Gaarder, 1998), the con-

centration of the dog allergen *Can f I* might have been too low outdoors to give a positive labeling. The labeling of the latex glove surface by antibodies to timothy, cat, and dog allergens could be explained by the fact that latex gloves are processed products of NRL. Chemical treatment of NRL or vulcanization for tire production is likely to produce residual and neo-allergens in the finished products that may cross-react with a number of proteins (Kurup & Fink, 2001).

Traffic exposure not only adversely affects lung function in asthmatics but also may promote airway sensitization to common allergens (D'Amato, 2002; Polosa & Salvi, 1999). Recent studies suggest that traffic exposure is unlikely to constitute an important determinant of latex allergy because the levels are too low (Miguel, Cass, Glovsky, & Weiss, 1999; Vermeulen, Doekes, & Kromhout, 2000). It has been suggested, however, that latex-allergic hyperactivity during pollen season may have as a provoking factor other allergens not recognized as latex because the doses inhaled are too low to produce acute reactions (Pham & Baldo, 1995). In line with this hypothesis, a mutual booster effect of timothy grass pollen and latex sensitization in vivo has been demonstrated, augmenting reactions in patients already allergic to plant allergens (Mahler et al., 2000). Plant profilins are responsible for a significant number of pollen-related allergies and are important pan-allergens. Since environmental pollutants stimulate defense responses in plants, they may induce pan-allergens. Latex and its cross-reactive proteins, found in the present investigation to be carried by carbon aggregates from vehicle emissions, may therefore potentiate the effect; this observation may help explain the increase in sensitization to common airborne allergens in urban areas.

Conclusions

The method of immunolabeling directly on the collection filters has proved useful in studies of traffic-related air pollution and allergens. SEM observations have shown mainly carbon aggregates, from vehicle emissions, that increase in mass and mass variation during winter. The statistical model used in the study reported here did not indicate any significant effect on PM₁₀ from a decrease in use of studded winter tires from 1997 to 2002. The meteorological parameters of precipitation, dry weather, and wind were found to be the only significant predicting factors for the PM₁₀ concentration.

Immunolabeling showed latex to be an outdoor aeroallergen that adhered to the carbon aggregates. No annual or seasonal variation in labeling density was present to indicate that the softer winter tires liberated more latex to the environment. Since carbon aggregates have a very large surface-to-volume ratio, they could be regarded as a depot of aeroallergens inhaled into the immune system, which implies an increased risk for previously sensitized people. Predisposed and allergic subjects might therefore be advised not to take part in outdoor activities in episodes of cold, dry periods with high air pollution.

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