European Collaboration in Research and Technology

COST Action 633:

Particulate Matter: Properties related to health effects

Particles and Health

– State of the Research and Policy Implications –

Public Conference

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Organizers: **Dr. Michael Riediker, IST Lausanne, Switzerland** [1)]
Prof. Dr. Regina Hitzenberger, University of Vienna, Austria [2)]

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[1)] Swiss COST 633 Delegate and Head of Research Group Particles and Health
Institut de Santé au Travail, IST, rue du Bugnon 19, CH-1005 Lausanne (Suisse/Switzerland)
Tél. +41-(0)21-314 74 21 / Fax +41-(0)21-314 74 20 / michael.riediker@hospvd.ch

[2)] Chair of COST 633 and a.o. Univ. Professor
Institute for Experimental Physics, University of Vienna, Boltzmanngasse 5, A-1090 Vienna (Austria)
Phone: +43-1-4277-51111 / Fax: +43-1-4277-9511 / regina.hitzenberger@univie.ac.at

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Size partitioning of airborne particles to compare their proinflammatory effect in airway epithelial cells

A. Baeza-Squiban[1]
K. Ramgolam[1], O. Favez[2], L. Martinon[3], H. Cachier[2], A. Person[4], A. Gaudichet[5], F. Marano[1] :
1. Laboratoire de Cytophysiologie et Toxicologie Cellulaire (LCTC), Université Paris 7, 75005 Paris,France
2 : Laboratoires des Sciences du Climat et de l'Environnement (LSCE),91191- Gif sur Yvette, France
3 : Laboratoire d'Etude des Particules Inhalées (LEPI), 11 rue Georges Eastman, 750013-Paris, France
4 : Laboratoire d'Hygiène de la Ville de Paris (LHVP) 11 rue Georges Eastman, 750013-Paris, France
5 : Laboratoire Interuniversitaire des Systèmes Atmosphériques (LISA), Université Paris 12, Créteil, France

Keywords: urban aerosols, fine and ultrafine fractions, lung-particle interactions, health effects of aerosols

Paris background aerosol is almost exclusively composed of fine (PM 2.5; PM 1) and ultra fine (PM 0.1) particles, originating mainly from combustion processes including traffic exhausts. Epidemiological and experimental investigations underlined the role of the aerosol size, in particular the ultra fine one.

The aim of the present study was to investigate which size-fraction of the urban particulate matter is the most relevant regarding to the biological effect considering the proinflammatory response of airway epithelial cells *in vitro*. This response is characterized by the release of mediators that would explains the inflammation observed in exposed subjects.

Human bronchial epithelial cells (16HBE) and primary cultures of nasal epithelial cells (HNE) that are the main target cells of airborne particles were exposed to the different size-fractions. The release of GM-CSF, a cytokine involved in allergic process was used as a proinflammatory biomarker of PM exposure and the cytochrome P450 1A1 (CYP1A1) that metabolizes xenobiotics, which activity was used as a biomarker of polyaromatic hydrocarbon (PAH) bioavailability.

Downtown Paris, four co-located 13-stage Dekati cascade impactors running in parallel were used to selectively collect particles from 30nm to 10µm on polycarbonate filters and were allocated to biological and physico-chemical (black carbon, particulate organic matter and water soluble organic compounds, major ions, PAH) investigations. 11 samplings were conducted in order to investigate whether the seasonal variability (summer and winter) and diurnal evolution related to photochemistry of the urban aerosol composition modulate the biological effects of some or all size-fractions.

In vitro biological assays were conducted with particles from pooled stages (1 to 3 representing ultra fine fraction [0.1-0.03µm], 4 to 7 the [1-0.1µm], 8 to 9 the [2.5-1µm] fine fraction and 10 to 13 the [10-2.5µm] coarse fraction). Particles were recovered from collection filters by brief sonications directly in the same volume of cell culture medium for each size-fractions. Two experimental strategies were used: cells were exposed for 24 hours either at isovolume of particles suspension in order to respect the proportion of the different size-fraction in the sampled-air volume or at isomass.

When cells are exposed to an isovolume of particles suspension, the highest GM-CSF secretion was induced by PM1-0.1 that is the most important fraction in Paris background aerosol (up to 71% of the total PM10 mass). With a cell exposure at an isomass of particles, GM-CSF secretion was significantly induced by fine and ultra-fine particles with a dose-dependent increase from 1µg/cm² (5µg/mL) to 10µg/cm², without inducing any cytotoxicity. Whatever the season or diurnal sampling, the finer the aerosol fraction, the higher the GM-CSF secretion was, whereas coarse particles displayed no or fewer effect. Moreover, endotoxins were not involved in the ultrafine particle-induced GM-CSF secretion whereas they partially contributed to the fine particle ones as assessed by the use of endotoxin neutralizing recombinant protein. Considering PAH bioavailability, PM1-0.1 from winter samples induced the higher CYP1A1 activity whereas the CYP 1A1 activity increases as the size decreases with summer samples.

Chemical analyses enlightened the major presence of carbonaceous species in Paris aerosols especially in the ultra-fine and fine fractions where PAH are also predominant (90% in these fractions).

To conclude, we observed that the proinflammatory response of bronchial epithelial cells *in vitro* was closely related to particle size with ultrafine particles exhibiting the highest effect.

This work was supported by ADEME, the french environmental agency, under the PRIMEQUAL grant n° 0462C0056.

PM and NO_2 at urban sites with different traffic exposure: curb site measurements in Flemish cities

M. Van Poppel[1], E. De Dekker[2], L. Int Panis[1], N. Bleux[1], M. Spruyt[1] and Patrick Berghmans[1]
[1] *VITO, Flemish Institute for Technological Research, Belgium*
2 Environmental Department – City of Ghent, Belgium

Road transport is known as one of the main sources of urban air pollution, especially PM and NOx. Recently, studies have reported associations between residential proximity to busy roads and a variety of adverse respiratory health outcomes in children. Moreover, it was shown that traffic related pollution (PM, NOx and black carbon) is associated with respiratory symptoms in children.

The knowledge that PM may induce adverse health effects is an incentive for progressive cities to evaluate local air quality and to look for actions to be taken. Before cost effective measures can be taken, the actual air quality of the city has to be assessed in order to identify hot spot locations.
Existing air quality measurement networks measure air pollution at different sites and give an 'average value' for the surrounding area. However, most of these measurement stations are not located at hot spot locations e.g. close to busy roads. In Flanders, the daily average limit value for PM_{10} was exceeded more than 35 times in 17 of 31 monitoring sites in 2005.

Some of the larger Flemish cities have implemented innovative transport and mobility policies in the past decade. Before supplemental cost effective measures can be taken, the actual air quality of the city has to be assessed in order to identify hot spot locations. The aim of this study is to assess the air quality at urban (curb site) locations.

PM_{10} daily average values were measured during 3-4 weeks at 6 locations representing different 'typical' traffic locations: e.g. ring road, access road, parking route, local traffic. NO_2 was measured at all locations.

At the background location lowest concentrations were measured for all parameters. Differences in PM_{10} and NO_2 were observed between the different locations. It seems that NO_2 is more sensitive to traffic exposure than PM_{10}. This is due to the higher background and background variation of PM_{10}. At the bus location, highest NO and NO_2 values were measured. Highest concentrations of PM_{10} and black carbon are measured at the ring location. However some trends could not be explained.

Formation of sulphate in concentrated solutions of salts

Axel Berner[1], Janja Turšič[2] and Regina Hitzenberger[1]

Chemical reactions in concentrated solutions of salts and electrolytes in general contribute to the transformation of atmospheric species. Laboratory experiments demonstrate that SO_2 is oxidised in solutions of salts (e.g. NaCl, $NaNO_3$), at relative humidity of 75% to 90% in air, in the absence of light. (This formation of SO_2 oxidation is enhanced by transition metals (e.g. Mn) and by NH_3). Under similar conditions formation of SO_2 is also observed on size segregated samples of atmospheric aerosols.

For SO_2 the highest formation rates observed in the laboratory are to the order of 0.01 mol of newly formed sulphate per mol of salt and per hour. When these data hold for the atmospheric sulphate aerosol, the formation rate would become 1% sulphate per hour. Consequently the excess of sulphate formed on a sample leads to a 4.6% increase of the aerosol sulphate concentration for an 8h sampling period, and to a 13.5% increase for a 24h sampling period, at constant sampling conditions including the aerosol.

Conclusions and suggestions:

- Chemical reactions in concentrated electrolytic solutions contribute to the transformation of atmospheric aerosol material and precursors, inorganic and organic. Long term sampling can therefore lead to considerable sampling artefacts due to reactions on or in the aerosol material. (Artefacts during sampling.)
- The transformations so far observed are slow. It is the question whether there are also fast reactions that change the composition of the aerosol, regarding the reactive species, upon humidification in the lung. If so, water is probably among the reactants. (Artefacts on humidification.)

[1]: Experimentalphysik, Fakultät für Physik, Universität Wien, A-1090 Wien, Strudlhofgasse 4, Österreich.
[2]: National Institute of Chemistry, Hajdrihova 19, SI-1000 Ljubljana, Slovenia.

Translocation of particles, effects, and cellular interplay after exposure to fine particles and nanoparticles in an epithelial airway model

Peter Gehr, Barbara Rothen-Rutishauser, Christian Mühlfeld and Fabian Blank

Division of Histology, Institute of Anatomy, University of Bern, CH 3000 Bern 9, Switzerland

So far, little is known about the interaction of nanoparticles with lung cells. Using a triple cell co-culture model of the human airway wall composed of epithelial cells (EC), macrophages (MP) and dendritic cells (DC) we studied the interaction of these cells with polystyrene particles (PSP) of different sizes (1 µm, 0.2 µm, and 0.078 µm) as well as with different nanoparticle (NP) types, i.e. gold (AuNP; 0.025 µm) and titanium dioxide (TiO_2NP; 0.02-0.03 µm) particles using laser scanning microscopy (LSM) and energy filtering transmission electron microscopy (EFTEM). The cellular pro-inflammatory response was determined by measurements of the tumor necrosis factor-α (TNF-α) in the supernatants.

By quantification of fine PSP and nano PSP inside the different cell types we found 6-9 times more 1µm and 0.078 µm particles within the cells than 0.2 µm particles. Furthermore the amount of particles which translocated into the cells was dependent on the cell type. The distribution of the different particles among the different cell types was compared using a contingency table analysis, and we could show that NP had different translocation characteristics than larger particles. TiO_2NP were detected as single particles without membranes as well as in membrane bound agglomerations. AuNP were found inside the cells as free particles only. We measured a 2-3 fold increase of TNF-α in the supernatants after applying 1µm PSP and AuNP, but not with nano PSP and TiO_2NP.

We found that the translocation of NP into cells was different from that of fine particles. We postulate that the size and material of the NP did not influence penetration into the cells but their localisation inside, i.e. free in the cytoplasm or membrane-bound, as well as their potential to induce cellular responses was different. Single NP which were not membrane-bound may have penetrated the cells by a mechanism which is different from endocytosis. This mechanism which is still unknown is likely to lead to a toxic effect because free NP may enter mitochondria or the nucleus.

Using LSM and digital image restoration MP and DC were found to interact with each other upon exposure to fine particles (1 µm). The two cell types may form a fine transepithelial network of interacting cell processes with which particles deposited on the epithelial layer can be transported to the base of the epithelial layer. This is postulated to be a mechanism by which particulate antigen can be translocated through the epithelial layer.

Particles and Health in Europe

Matti Jantunen, KTL-Environmental Health, Kuopio, Finland

When unspecified ambient air fine particulate matter (PM2.5) turned out to be arguably the biggest environmental pollution risk to human life, it first (1993) raised surprise, was then deemed scientifically unexplainable until the accumulating evidence before and after 2000's established this as a broadly accepted fact.

Time series studies in the US, Europe (APHEA) and elsewhere have provided most of the current evidence. Yet, American cohort studies, the Harvard six cities study and the ACS study, have provided the most used PM mortality estimates. Interventions such as the Utah Valley Steel Mill strike and the Dublin coal ban of 1990, have added compelling evidence about the causal relationship between ambient PM and mortality.

Combining epidemiological, experimental and clinical findings from around the world has created a model of the complex pathways from PM exposure to CV mortality. ROS formation is a key mechanism. Probably primary combustion and transient metals particles are the most harmful, secondary particles and primary soil mineral particles less harmful, and sea-salt particles harmless.

PM2.5 mass and secondary particles are smoothly distributed over large areas, and ambient air monitors reflect exposures well. Exposure to traffic particles is elevated for those living close to busy traffic, and for all while in transit. Most of the exposure to particles of outdoor origin takes place indoors, because this is where ca. 90 % of the time is spent. Relative to outdoor concentrations, indoor exposure levels are 10 … 75 % lower due to filtration of the building envelopes and ventilation. Consequently ambient air monitoring overestimates population exposure to and underestimates the dose/response of PM2.5 of outdoor origin, and (ii) may both over- and underestimate individual exposures to traffic particles.

Searching for the mechanisms of interaction between nanoparticles and lung-cells

M Kalberer[1*], M Geiser[2*], M Savi[2], D Lang[2], A Gaschen[1], M Fierz[3], M Ryser[4], J Ricka[4]

[1] Department of Chemistry and Applied Biosciences, ETH Zurich, 8093 Zurich, Switzerland
[2] Institute of Anatomy, University of Bern, 3012 Bern, Switzerland
[3] Institute for Aerosol- and Sensor Technology, FHNW, 5210 Windisch, Switzerland
[4] Institute of Applied Physics, University of Bern, 3012 Bern, Switzerland
* presenting authors

Up to about 50% of atmospheric nanoparticles are composed of organic material but this highly complex organic mixtures are chemically only poorly characterized. The chemical composition, the surface chemistry and number of inhaled particles are likely important factors involved in adverse health effects associated with exposure to these particles. Therefore, potentially harmful aerosol components should be carefully monitored when studying the interaction of particles with the lung. Additionally, laboratory experiments investigating particle-lung interactions need to accurately reproduce the complex interaction between nanoparticles and the lung surface in order to identify the health relevant processes.

We developed an aerosol deposition chamber to expose lung-cell cultures to particles ≤ 1 μm in diameter using a conditioned airflow and mimicking closely the particle deposition conditions in the lung. In this new deposition chamber particles of different chemical composition are deposited highly efficient, reproducible and very uniformly onto the entire cell culture, a key aspect if cell responses are quantified in respect of the deposited particle dose for low-concentration atmospheric particles.
Online analyses of the lung cells, e.g. ciliary beat frequency, indicative of the defense capability of the cells, are complemented by off-line biochemical, physiological and morphological cell analyses. The suitability of the chamber for cells was successfully tested on lung epithelial cells and macrophages using (inert) particles of different sizes.

On-going research will investigate the cell responses to oxidized and atmospherically aged particles with organic and inorganic components.

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Relationship between ambient air pollution and daily mortality in the Urban Area of Katowice – comparison on two periods 1994-1995 and 2001-2002.

M. Kowalska[1], J.E.Zejda[1], L. Ośródka[2] , K. Klejnowski[3], E. Krajny[2], M. Wojtylak[2]

[1]Department of Epidemiology, Medical University of Silesia, Katowice, Poland
[2] Institute of Meteorology and Water Management, Katowice, Poland
[3] Institute of Environmental Engineering Bases of PAS, Department of Air Protection, Zabrze, Poland

Urban Area of Katowice is a heavily industrialized area of Poland with population about 2 millions inhabitants and total number of deaths about 20 000 per year. Air pollution levels in this region remain high, especially during the heating season, because of a dominant method of energy production and heating of houses, based on coal combustion.

The aim of the study was to analyze the current relationship between daily total and specific mortality and daily PM_{10}, SO_2 average area concentrations in the ambient air and to compare the estimated relative risk associated with the levels of pollution, obtained seven years apart. Both time-series analyses (1994/5 and 2001/2) were performed by the same method (Poisson regression model) in the same area. The effect of air pollution (either SO_2 or PM_{10} in a model) on daily count of mortality (total and cardiorespiratory deaths) was controlled for meteorological variables and season effect.

The comparison of current estimates with the findings obtained in the past suggests that the magnitude of the risk is similar for both periods. The range of the relative risk ratio for total mortality end-points related to a 10 $\mu g/m^3$ increase of PM_{10} concentration in total population of Katowice amounts: 1.007 in 1994/95 and 1.003 in 2001/02. The range is close to the value published by WHO in 2004. Sulphur dioxide is the predominant air pollutant associated with day-to-day variation in mortality, in the Urban Area of Katowice.

This project was supported by the State Committee for Scientific Research, Poland (Grant: COS/87/2006)

Assessing long-term exposure to traffic-related air pollution in a large Swiss Cohort (SAPALDIA)

L.-J. Sally Liu, Dirk Keidel, Armin Gemperli, Marianne Hazenkamp, Christian Schindler, and the SAPALDIA team. Institute of Social & Preventive Medicine, University of Basel, Switzerland

While evidence on acute health effects related to traffic exhaust is accumulating, little is known about long-term effects of traffic-related air pollution in the general population. This study, as part of the Swiss cohort study of air pollution and lung function in adults (SAPALDIA), investigated spatial variation of traffic-related NO_2 within and between 8 areas in Switzerland in 1993 and 2002. A dispersion model of 200x200 m resolution was used to predict NO_2 concentrations at residences of 9651 subjects in 1990 and 2000, respectively. In 1993 and 2003, weekly passive NO_2 measurements were collected outside 60 homes per area. This paper compared the dispersion model results for NO_2 with home outdoor measurements. Predictions for home outdoor NO_2 were refined by incorporating the dispersion model predictions in a land-use regression model that included local parameters from the geographic information system (GIS), meteorological variables, and interaction terms between the GIS and meteorological parameters. Our results indicate that the dispersion model alone does not predict NO_2 well within cities, with an R^2 ranging between 0 and 0.75 depending on area. Our hybrid models incorporating the dispersion predictions as the background layer, GIS parameters to enhance the local characteristics, and meteorological variables to add temporal dynamics, perform very well with an R^2 ranging between 0.77 and 0.96 for all areas. These individual exposure estimates provide powerful tools in health effect assessment for traffic-related exposure.

Health effects of air pollution in a large Swiss Cohort (SAPALDIA)

T. Rochat*, L.-J. S. Liu, S. Downs, E. Zemp, L. Bayer-Oglesby, D. Felber Dietrich, M.W. Gerbase, J.M. Gaspoz, N. Probst-Hensch, U. Ackermann-Liebrich, P. Leuenberger, Christian Schindler, and the SAPALDIA team. (*Pulmonary Division, University Hospitals of Geneva)

The SAPALDIA cohort (Swiss Air Pollution And Lung Diseases in Adults) consists of 9'651 randomly selected subjects from 8 different sites representative of various conditions of urbanization and climate of Switzerland. The participants (age 18 – 60 at baseline) underwent detailed health questionnaire and spirometry in 1991 and were re-examined in 2002. In addition a sample of 1837 participants ≥ 50 years of age had 24h ECG recording in 2002.

First results indicated significant association of spirometry results and respiratory symptoms with local, annual mean concentration of PM10 and NO2 obtained at local monitoring stations of the eight regions. Assessment of exposure to traffic exhaust by distance from home to the next main road also showed significant association with respiratory symptoms. Finally, modellization of individual exposure will allow to estimate the change of PM10/NO2 concentration experienced by each participants from 1991 to 2002 as well as individual cumulative exposure over the same period of time. These data will make it possible to look for association with cardio-respiratory parameters such as pulmonary function tests, respiratory symptoms, and heart rate variability.

Assessment of Diesel exhaust particulate exposure and surface characteristics in association with levels of oxidative stress biomarkers

Jean-Jacques Sauvain[1], Ari Setyan[1], Michael Riediker[1], Michel J. Rossi[2], Michel Guillemin[1]

[1] Institute for Occupational Health Sciences, Rue du Bugnon 19, CH-1005 Lausanne, Switzerland
[2] Swiss Federal Institute of Technology, Air and Soil Pollution Laboratory, CH-1015 Lausanne, Switzerland

Keywords: Diesel exhaust, Aerosol sampling, Aerosol size distribution, Aerosol-surface reactions, Health effects of aerosols.

Exposure to PM_{10} and $PM_{2.5}$ (particulate matter with aerodynamic diameter smaller than 10 μm and 2.5 μm, respectively) is associated with a range of adverse health effects, including cancer, pulmonary and cardiovascular diseases. Surface characteristics (chemical reactivity, surface area) are considered of prime importance to understand the mechanisms which lead to harmful effects. A hypothetical mechanism to explain these adverse effects is the ability of components (organics, metal ions) adsorbed on these particles to generate Reactive Oxygen Species (ROS), and thereby to cause oxidative stress in biological systems (Donaldson *et al.*, 2003). ROS can attack almost any cellular structure, like DNA or cellular membrane, leading to the formation of a wide variety of degradation products which can be used as a biomarker of oxidative stress.

The aim of the present research project is to test whether there is a correlation between the exposure to Diesel Exhaust Particulate (DEP) and the oxidative stress status. For that purpose, a survey is conducted in real occupational situations where workers are exposed to DEP (bus maintenance yard).

Different exposure parameters have been considered:
- particulate number, size distribution and surface area (SMPS);
- particulate mass - $PM_{2.5}$ and PM_4 (gravimetry);
- elemental and organic carbon (coulometry);
- total adsorbed heavy metals - iron, copper, manganese (atomic adsorption);
- surface functional groups present on aerosols (Knudsen flow reactor). (Demirdjian *et al.*, 2005)

Several potential biomarkers of oxidative stress (8-hydroxy-2'-deoxyguanosine and some aldehydes) have been determined either in urine or serum of volunteers.

Results obtained during the sampling campaign in several bus depots indicated that the occupational exposure to particulates in these places was rather low (40-50 $\mu g/m^3$ for PM_4). Size distributions indicated that particles are within the nanometric range. Surface characteristics of sampled particles varied strongly, depending on the bus depot. They are usually characterized by high carbonyl and low acidic sites content.

Among the different biomarkers which have been analyzed within the framework of this study, 8-hydroxy-2'-deoxyguanosine mean levels in urine have been observed to increase significantly ($p<0.05$) during two consecutive days of exposure for non/old-smokers (n=15).

Correlations between the exposure parameters and the formation of ROS by-products have to be done in order to know if the observed biological effect can be related to particulate exposure.

This project is financed by the Swiss State Secretariat for Education and Research. It is conducted within the framework of the COST Action 633 "Particulate Matter – Properties Related to Health Effects".

Demirdjian B., Rossi M. J. (2005). *Atmos. Chem. Phys. Discuss.*, 5, 607 – 654.
Donaldson K., Stone V., Borm P. J., Jimenez L. A., Gilmour P. S., Schins R. P., Knaapen A. M., Rahman I., Faux S. P., Brown D. M., MacNee W. (2003). *Free Radical Biol. Med.*, 34, 1369-1382.

Research needs for Health Effects of Air Pollution – CONCAWE recommendations for FP7

Jan Urbanus, Technical Coordinator Health Issues, CONCAWE
(the oil companies' European organisation for environment, health and safety)

An open international workshop was convened in January 2007 to debate research needs for the EU in the field of health effects of ambient air pollution in the wider context of environment and health. Following a series of scientific updates workshop participants produced in facilitated discussion sessions a set of recommendations as follows:

Strategic research direction:
- improved coordination of research in this area
- better and more frequent synthesis and interpretation of research findings, using methodologically more rigorous approaches to evaluate the weight of scientific evidence
- for large, long-term projects available funding from different sources (EU, national, institutional) needs to be linked

Identified priorities for specific studies included:
- European prospective cohort analysis, with exposure assessment on the individual level including such features as biomarkers
- assessment of public health impacts of traffic emissions
- refinement of particulate matter metric(s) to measure health impacts
- elucidation of mechanisms of toxic action
- development of toxicity screening approaches for emissions to air
- improvements in cost-benefit analytical tools

Improvements to the science-policy interface:
- research to track impacts of implemented policy measures ('accountability research')
- improvements in interdisciplinary exchanges
- linkage of research project to time windows of expected policy needs

Workshop proceedings will be published and will form the basis for further work programmes.

Overview on the activities of COST633 Working Group 3: source apportionment methods EU

M. Viana[1]*, X. Querol[1], T.A.J. Kuhlbusch[2], A. Miranda[3], M. Vallius[4], A. Kasper-Giebl[5], S. Szidat[6], W. Winiwarter[7], R.M. Harrison[8]

[1]Institute of Earth Sciences "Jaume Almera", Barcelona, Spain (*mviana@ija.csic.es)
[2]IUTA e.V. Duisburg, Germany
[3]University of Aveiro, Aveiro. Portugal
[4]National Public Health Institute, Kuopio, Finland
[5]Institute for Chemical Technologies and Analytics, Vienna, Austria
[6]University of Bern, Bern, Switzerland
[7]Austrian Research Centers, Vienna, Austria
[8]The University of Birmingham, Birmingham, UK

The focus of COST Action 633 Working Group 3 (WG3) is directed towards the evaluation of the availability and application in COST633 member countries of emission inventories, dispersion models, source apportionment methods and integrated assessment models. To this end, a compilation of meta-data among the COST633 member countries was carried out by means of questionnaires. The results from the questionnaires may be seen as an overview on the dispersion modelling and source apportionment activities in Europe before the year 2006. In the case of source apportionment methods, a total of 47 publications were reported from 7 countries based on 10 different methodologies. The evaluation of the resulting data showed a broad range of PM10 and PM2.5 sources across Europe, as well as the different criteria adopted to interpret the nature of such sources. Achieving comparable results was identified as one of the major challenges in Europe. The relevance of sources such as regional background contributions road dust or shipping emissions, some of them unobserved in the reported studies, was evidenced.

Release of particulate material into the atmosphere: quality considerations of PM emission inventories

W. Winiwarter

Austrian Research Centers – ARC, A-1220 Vienna, Austria

Transfer of material into the atmosphere is a key factor influencing atmospheric concentrations of trace constituents. Emission inventories provide the information needed to understand and quantify this process. In contrast to inventories of gaseous pollutants, which are well-established, inventories of particulate matter (PM) suffer from a low level of reliability. While considerable efforts for improvements are underway, the major obstacles are specific properties of PM. PM is not a unique substance, but an internal and/or external mixture of a multitude of compounds from very different origin, different effects on substrates, and different size. Quantification of emission rates is hampered by their strong dependencies on measuring conditions (temperature, lapse time) and process state (start-up vs. equilibrium). Successful efforts to abate emissions have lead to a situation where emissions very strongly depend on the technology of PM abating equipment used – for which information often is not available on an aggregate level. Furthermore, abatement measures have very different effects on different particle size classes, and typically rather influence larger size fractions. Emission rates, e.g. in domestic heating from small installations, are strongly determined by national and regional habits, making it difficult to identify appropriate emission factors and use them for a larger domain. Information on fugitive emissions is sparse, i.e. suspension of particles due to wind shear, loading processes or other mechanical forces which are not contained in an exhaust air duct. This concerns activities in industry and agriculture, but also motorized vehicle traffic in addition to exhaust (Diesel) particles. Comparing independent efforts in assessing emissions, here national data (from Austria) to the trans-national estimates of IIASA's GAINS model, allows to better understand and quantify an inventory's significance. Methodological improvements and harmonization efforts currently under way in Europe will focus activities and allow for more reliable PM inventories in the near future.