

Project Progress Summary

Section 1: PROJECT IDENTIFICATION Information to be provided for project identification		NOT CONFIDENTIAL
Title of the project Chemical and biological characterisation of ambient air coarse, fine and ultrafine particles for human health risk assessment in Europe		
Acronym of the project PAMCHAR		
Type of contract Shared-cost RTD		Total project cost (in euro) 2,766,089 €
Contract number QLK4-CT-2001-00423	Duration (in months) 36 Months	EU contribution (in euro) 1,598,000 €
Commencement date 1 January 2002		Period covered by the progress report 1 January 2002 – 31 December 2004
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Key words (5 maximum - Please include specific keywords that best describe the project.). Particulate matter, chemical composition, cytotoxicity, genotoxicity, inflammation		
World wide web address http://www.pamchar.org		
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Objectives: The PAMCHAR project took a **systematic approach** in expanding the present knowledge on the physicochemical and toxicological characteristics of ambient air coarse (PM_{10-2.5}), fine (PM_{2.5-0.2}) and ultrafine (PM_{0.2}) particles. **Contrasting particulate pollution situations** in different geographical and seasonal conditions in Europe were investigated with advanced particulate sampling and analytical methods, and important biological end-points were investigated in well-established *in-vitro* and *in-vivo* test systems. Our ultimate aim was to identify **causative physicochemical characteristics** and chemical constituents of ambient air PM for **cytotoxic, proinflammatory and genotoxic responses** relevant to human cardiorespiratory health.

Results and Milestones:

1. PM sampling and characterisation (WP1-WP3): Substantial methodological developments were made before the actual sampling campaigns. The **Harvard high-volume cascade impactor (HVCI)**, used for large capacity, size-segregated sampling of ambient air PM, was tested in the laboratory and pilot studies and modified so that its collection efficiencies and 50% cut-off points at 2.5, 1 and 0.2 μm corresponded to validated low-volume impactors. **The feasibility of various source markers** (ions, elements, levoglucosan) analysed from PM_{10-2.5}, PM_{2.5} and 10-stage size-segregated PM samples (size 0.035-10 μm) was tested in a one-month pilot campaign in Helsinki. **The actual 7-week sampling campaigns at six urban background sites in Europe** were scheduled to include seasons of local public health concern due to high PM concentrations or findings in previously conducted epidemiological studies: Duisburg (autumn), Prague (winter), Amsterdam (winter), Helsinki (spring), Barcelona (spring) and Athens (summer). All six sampling campaigns used **the same sampling methods, standard operating procedures (SOPs)**, start-up training schemes and mid-campaign audit protocols. According to **chemical mass closure** (77-100% of mass identified), the major components in PM_{2.5} of all the campaigns were carbonaceous compounds (organic matter +elemental carbon), secondary inorganic ions and sea salt, whereas those in PM_{10-2.5} were crustal particles, carbonaceous compounds, sea salt and nitrate. However, **there were large differences between the sampling campaigns with regard to the relative contribution of these major components to PM_{2.5} and PM_{10-2.5} mass**. The PM_{2.5} samples had a lower endotoxin content and a higher PAH content than the PM_{10-2.5} samples. There were large differences between the campaigns in PAH, transition metal (Fe, Zn, Cu, V, Ni, Cd), As and endotoxin contents of both PM size ranges. Both the HVCI PM_{2.5-0.2} and PM_{10-2.5} samples induced **acellular hydroxyl radical production**, as investigated by electron spin resonance (ESR). Most often, there were relatively small differences between the two PM size ranges from the same sampling campaign, but larger differences were observed between the campaigns.

2. In-vitro toxicology (WP4-WP6): The PM extraction from the HVCI sampling substrates as well as some other procedures of sample preparation were validated, and the optimal duration (2-24 h) of PM exposure in cell culture was screened, in two pilot studies preceding the actual experiments with the six-city PM samples. In the **mouse macrophage cell line RAW264.7**, 24-h exposures to the Prague PM_{0.2} and PM_{2.5-0.2} samples (highest organic matter, PAH and As contents due to solid fuel combustion) caused the highest cytotoxicity and nitric oxide (NO) production, and practically no cytokine production. In contrast, the Barcelona PM_{2.5-0.2} sample (highest Na⁺, Mg²⁺, Cr, Cu, Ni and V contents) and the Athens PM_{2.5-0.2} sample (highest secondary organic ion, Ca²⁺ and Al contents) induced the highest proinflammatory cytokine productions (MIP-2>TNF α ≈IL-6). **The PM_{10-2.5} samples were much more potent inducers of cytokine production than the PM_{2.5-0.2} samples but the differences between the campaigns were smaller in this size range**. However, the Helsinki and Duisburg PM_{10-2.5} samples produced consistently smaller responses than the samples from the other four cities. In both the PM_{2.5-0.2} and PM_{10-2.5} size range, **the NO and cytokine responses as well as cytotoxicity were nearly totally caused by the water-insoluble fraction of PM samples from all the sampling campaigns**. Both the endotoxin antagonist polymyxin B and the metal chelator DTPA reduced the PM-induced responses. In the **human respiratory epithelial cell line A549**, the PM_{10-2.5} samples usually produced somewhat higher proinflammatory cytokine production (IL-8) than the PM_{2.5-0.2} samples with little difference between the sampling campaigns. Only the Prague PM_{2.5-0.2} sample (highest PAH content) caused significant DNA strand breakage (alkaline comet-assay) and high DNA adduct formation (³²P postlabelling).

3. In-vivo toxicology (WP7-WP8): The **dose-relationships** (1, 3 and 10 mg/kg intratracheally) and **time-relationships** (10 mg/kg with 4-, 12- and 24-h follow-ups after exposure) of PM responses were tested in **intratracheal exposures of healthy C57BL/6J mice**. Most often, the PM_{10-2.5} samples produced a somewhat stronger and longer-lasting increase of neutrophils in bronchoalveolar lavage fluid (BALF) than the PM_{2.5-0.2} samples. The cytokine concentrations (IL-6>TNF α >KC) increased profoundly with the samples of both PM size ranges at 4 h after exposure. The PM_{0.2} samples had practically no effect on the cell number, protein or cytokines in BALF. The 4-h cytokine concentrations in BALF, produced by the PM_{2.5-0.2} samples, correlated highly with the increased cell number observed 12 h after exposure as well as with the same cytokine responses observed *in vitro* in the mouse macrophage cell line RAW264.7. In the repeated dose study (10 mg/kg 3 times in a week), there were signs of stronger inflammatory responses than after a single dose. The histopathological examination of the lungs showed that the Athens PM_{10-2.5} and PM_{2.5-0.2} samples caused somewhat more extensive lung inflammation than the corresponding samples from the other cities. **A hypothesis of genotoxicity and other biological responses in relation to metal and PAH contents of PM samples was addressed in intratracheal exposures of spontaneously hypertensive rats.** Pilot studies in two transgenic mice strains and their wild-type strain (C57BL/6J) did not show an increased sensitivity to the genotoxic effects of ambient air PM, and a larger species turned out to be more feasible for the planned work. The PM_{2.5-0.2} samples from Duisburg and Prague as well as the PM_{10-2.5} samples from Prague and Barcelona were chosen for a single dose study (7 mg/kg with a 24-h follow-up after exposure) in the compromised rat strain. Exposure to a PM sample with a high metal or PAH content resulted in elevated lactate dehydrogenase (LDH), and protein levels as well as increased neutrophil numbers in BALF compared to a PM sample with a low metal or PAH content. **The blood coagulation factor fibrinogen** was also increased more by PM samples with a higher metal or PAH content. In the lung epithelial cells of PM-exposed rats, no PAH-DNA adducts were detected, whereas DNA strand breakage in these target cells correlated significantly with neutrophilic inflammation in BALF.

4. Human relevance (WP9-WP10): An **open workshop** (50 participants) was arranged on 20 October 2004 in connection to the 3rd Annual AIRNET Conference in Prague in order to present and discuss the PAMCHAR results. The consistent results on strong inflammatory responses to the Athens and Barcelona PM_{10-2.5} and PM_{2.5-0.2} samples, *in vitro* in the mouse macrophage cell line and *in vivo* in the mouse lung, suggest an involvement of this mechanism and PM characteristics in the stronger PM₁₀ mass concentration based risk estimates of short-term mortality and morbidity observed in Mediterranean hot climate in the epidemiological **APHEA studies**. The *in-vivo* connection between lung inflammation and genotoxicity in compromised rats suggests the former mechanism possibly being important also in the increased **cancer risk** observed in some US epidemiological studies on long-term PM exposures. The high organic matter, PAH and As contents as well as the high genotoxicity and cytotoxicity of the Prague PM_{2.5-0.2} and PM_{0.2} samples suggest increased carcinogenic and non-carcinogenic health risks in communities with **heavy solid fuel consumption for residential heating**. Thus, our findings support the results of a recently published epidemiological **intervention study from Dublin** that showed a remarkable decrease in cardiorespiratory mortality after a replacement of coal with natural gas in residential heating.

Benefits and Beneficiaries: A new concept of harmonized sampling and in-depth chemical, source and toxicological characterisation of size-segregated particulate samples was developed. This concept and the well-established *in-vitro* and *in-vivo* models of inflammation, genotoxicity and cytotoxicity are recommended for **future integrated study approaches on ambient air PM** involving epidemiologists, toxicologists, aerosol scientists and exposure modellers. The new supportive toxicological information to the present epidemiological knowledge should facilitate the development of new policies for reduction of ambient air PM associated health effects in Europe.

Future Actions: A total of 5 original scientific articles, 4 extended abstracts and 21 short conference abstracts have been published or are in press on the PAMCHAR results. Another 13 original articles and 3 PhD-theses will be finalised by the end of 2007. The project results are presented in international and national meetings, and they are also communicated to the EC DG-Environment and the national and local authorities in the participating countries. Other stakeholders, media and the public are also informed on the key findings. The SOPs on the field campaign methods, procedures and preparation of the HVCI samples for cell and animal experiments will be published on the project website (www.pamchar.org), where a list of publications is also periodically updated. The PAMCHAR concept and experience will be offered for future collaborative studies in Europe.

