Role of Atmospheric Pollution on Harmful Health Effects

A. Pietrodangelo[a], M. Bencardino[a], A. Cecinato[a], S. Decesari[b], C. Perrino[a], F. Sprovieri[a], N. Pirrone[a] and M.C. Facchini[b]

a. CNR, Institute of Atmospheric Pollution Research (IIA) Monterotondo St. (Roma), Italy

b. CNR, Institute of atmospheric sciences and climate (ISAC), Bologna, Italy

pietrodangelo@iia.cnr.it

ABSTRACT

Gaseous and particulate matter in ambient and indoor air has a key role on the increased morbidity or mortality observed in many clinical studies. Knowledge of the main toxicity patterns of atmospheric pollutants is still at an initial stage, especially as concerns particulate matter. This is mainly due to the varying size-distributions and chemical composition of PM10 and PM2.5 and to the many-sided toxicity mechanisms of ultrafine particles (UFPs). In this paper, recent findings on toxicity routes attributable to PM matter (i.e. the water-soluble organic fraction (WSOC), studied for the strong oxidative potential to biological tissues), to UFPs and to gases, are reviewed. Toxicity routes are discussed as evidence or hypothetical relationships between sources, diffusion paths, receptor sites and susceptible populations. Finally, strategic points are underlined which will be further developed in the "Pilot study for the assessment of health effects of the chemical composition of ultrafine and fine particles in Italy" project.

1. Introduction

Adverse health effects of atmospheric pollutants have been well documented in Europe and in other parts of the world. These include many diseases and an estimated reduction of a year or more in life expectancy for people living in European cities. There is also evidence of increased infant mortality in highly polluted areas. Concerns about these health effects have led to the implementation of regulations to reduce harmful air pollutants emissions and their precursors at international, national, regional and local levels. Further measures – while necessary to further reduce the health effects of air pollution – are becoming increasingly expensive. There is thus a growing need for accurate information on the health effect of air pollution to plan scientific, effective and well targeted strategies and reduce these effects. In July 2002 the European Parliament and the Council adopted the Decision 1600/2002/EC on the Sixth Community Environment Action Programme (Sixth EAP). This Programme sets out the key environmental objectives to be attained in the European Community, one of which (Article 2) is to establish ". a high level of quality of life and social well being for citizens by providing an environment where the level of pollution does not give rise to harmful effects on human health …"(1). The activities of the European Commission to implement the Sixth EAP currently take place within the Clean Air for Europe (CAFE) programme (2). This programme, launched in early 2001, aims at developing long-term, strategic and integrated policy advice to protect against significant negative effects of air pollution on human health and the environment. The World Health Organization (WHO) in support to the CAFE process, provided updated information on health effects

of air pollutants establishing the project "Systematic Review of Health Aspects of Air Quality in Europe" (3) in the course of which the current state of knowledge concerning health impacts of air pollution has been reviewed. The body of evidence of air pollution effects on health at the pollution levels currently common in Europe has been considerably strengthened by the contribution of both epidemiological and toxicological studies. The latter provide new insights into possible mechanisms to analyse the hazardous effects of air pollutants on human health and complement the large body of epidemiological evidence, showing, for example, consistent associations between daily variations in air pollution and some health outcomes. Exposure to ambient air pollution has been linked to a number of different health outcomes, starting from modest transient changes in the respiratory tract and impaired pulmonary function, to restricted activity/reduced performance, emergency room visits and hospital admissions and mortality. There is also increasing evidence of air pollution adverse effects not only on the respiratory system, but also on the cardiovascular system. This evidence stems from studies on both acute and chronic exposure. Short-term epidemiological studies suggested that a number of sources are associated with health effects, especially motor vehicle emissions, and also coal combustion. These sources produce primary as well as secondary particles, both of which have been associated with adverse health effects. If long-term exposure to a specific pollutant is linked to some health effects, cohort studies provide a basis to estimate chronic diseases and lifespan reduction in a given population. This is the case for mortality linked to PM long-term exposure. An expert group led by WHO – the Joint UNECE/WHO-ECEH Task Force on Health Aspects of Long Range Transboundary Air Pollution – recommended the use of risk coefficients from the American Cancer Society (ACS) study (4) to estimate the effects of chronic exposure to particulate matter (PM) on life expectancy in Europe. This study is the largest cohort study published in the scientific literature on the association between mortality and exposure to PM in air, and has involved 550,000 persons between 1982 and 1998. The risk estimates from this study were also used in the WHO Global Burden of Disease project (5). This project estimated that exposure to fine PM in outdoor air leads to about 100,000 deaths and 725,000 years of life lost each year in Europe. It is clear that there is a significant health risk associated with PM. It is also clear that there is a yet not known safe threshold for exposure but that there appears to be a linear relationship between exposure and risk. In addition, it has not yet been possible to identify with confidence which PM chemical constituents are primarily responsible for the different health effects. Therefore, even though the evidence on the relationship between exposure to different air pollutants and health effects has increased considerably over the past few years, there are still large uncertainties and important gaps in knowledge. These gaps can be reduced only by targeted scientific research. Areas in which such research is urgently needed include exposure assessment, dosimetry, toxicity of different components, biological mechanisms of effects, susceptible groups and individual susceptibility (taking into account gene–environment interactions), effects of mixtures versus single substances, and effects of long-term exposure to air pollution. The "Systematic Review" clearly demonstrated the need to set up a more

comprehensive air pollution and health monitoring and surveillance programme in different European cities. Air pollutants to be monitored include coarse PM, $PM_{2.5}$, PM_{1}, ultrafine particles, PM chemical composition, including elemental and organic carbon, and gases such as ozone, nitrogen dioxide and sulphur dioxide. The value of black smoke and ultrafine particles as indicators of traffic-related air pollution should also be evaluated. Furthermore, periodic surveillance of health effects requires better standardization of routinely collected health outcome data. The "Systematic Review" also showed the need of a system to maintain the literature database and develop meta-analysis to monitor research findings, summarize the literature on health effects and health impact assessment.

2. Air Pollution and Health

Ambient air pollution consists of a highly variable, complex mixture of different substances, which may occur in the gas, liquid or solid phase. Several hundred different components have been found in the troposphere, many of them potentially harmful to human health and the environment. The main sources of air pollution are transport, power generation, industry, agriculture, and heating. All these sectors release a variety of air pollutants – sulphur dioxide, nitrogen oxides, ammonia, volatile organic substances, and particulate matter – many of which interact with others to form new pollutants. These are eventually deposited and have a whole range of effects on human health, biodiversity, buildings, crops and forests.
Air pollution results in several hundreds of thousands of premature deaths in Europe each year, increased hospital admissions, extra medication, and the loss of millions of working days. The health costs for the European Union are huge. The pollutants of highest concern for human health are airborne particulates and ozone – indeed no safe levels have yet been identified for either of them. Nevertheless, the "Systematic Review" focused on three pollutants: particulate matter (PM), ozone and nitrogen dioxide, as requested by the CAFE Steering Group. This is not to imply that other substances do not pose a considerable threat to human health and the environment at the current levels present in Europe. It should also be mentioned that PM itself is a complex mixture of solid and liquid constituents, including inorganic salts such as nitrates, sulphates and ammonium and a large number of carbonaceous species (elemental carbon and organic carbon). Thus PM implicitly covers a number of different chemical pollutants emitted by various sources. The term 'particulate matter' (PM) is used to describe airborne solid particles and/or droplets. These particles may vary in size, composition and origin. Several different indicators have been used to characterize ambient PM. Classification by size is quite common because size governs the transport and removal of particles from the air and their deposition within the respiratory system, and is at least partly associated with the chemical composition and sources of particles. Based on size, urban PM tends to be divided into three main groups: coarse, fine and ultrafine particles. The border between the coarse and fine particles usually lies between 1 μm and 2.5 μm, but is usually fixed by convention at 2.5 μm in aerodynamic diameter ($PM_{2.5}$) for measurement purposes. The border between fine and ultrafine particles lies at about 0.1 μm. PM_{10} is used to describe particles with an aerodynamic diameter smaller than 10 μm. The particles

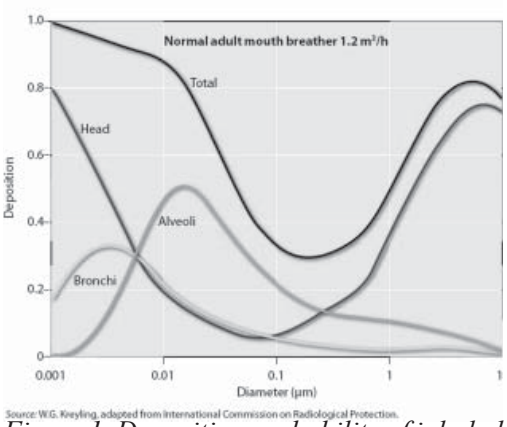

Figure 1. Deposition probability of inhaled particles in the respiratory tract according to particle size.

contained in the PM_{10} size fraction may reach the upper part of the airways and lung. Fig. 1 shows schematically where particles are deposited in the respiratory tract, depending on their size.

Smaller particles (in particular $PM_{2.5}$) penetrate more deeply into the lung and may reach the alveolar region. Ultrafine particles contribute only slightly to PM_{10} mass but may be important from a health point of view because of their large numbers and high surface area. They are produced in large numbers by combustion (especially internal combustion) engines.

As reported, (3) the most severe effects in terms of the overall health burden include a significant reduction, by a year or more, in average life expectancy linked to the long-term exposure to high levels of air pollution due to fine PM. Many studies have found that fine particles (usually measured as $PM_{2.5}$) have serious effects on health, such as increased mortality rates and emergency hospital admissions for cardiovascular and respiratory reasons. Thus there is good reason to reduce exposure to such particles. Coarse particles (usually defined as the difference between PM_{10} and $PM_{2.5}$) seem to have effects on, for example, hospital admissions for respiratory illnesses, but their effect on mortality is less clear. Nevertheless, there is sufficient concern to consider reducing exposure to coarse particles as well as to fine particles. Similarly, ultrafine particles are different in composition, and probably to some extent in effect, from fine and coarse particles. Nevertheless, their effect on human health have been insufficiently studied to permit a quantitative evaluation of health risks due to exposure to such particles.

As stated above, PM in ambient air has various sources. In targeting control measures, it would be important to know if PM from some sources or of a specific composition gave rise to special health concern due to their high toxicity. The few epidemiological studies that have addressed this important issue specifically suggest that combustion sources are particularly important for health. Toxicological studies have also pointed to primary combustion-derived particles as having a higher toxic potential. These particles are often rich in transition metals and organic compounds, and also have a relatively high surface area. By contrast, several other single components of the PM mixture (e.g. ammonium salts, chlorides, sulphates, nitrates and wind-blown dust such as silicate clays) have been shown to have a lower toxicity in laboratory studies.

Despite these differences found among the constituents studied in laboratory, it is currently not possible to quantify the contributions of the different sources and different PM components on the health effects caused by exposure to ambient PM.

2.1. Modelling approach on health impact

Health impact assessment allows to quantify the effects of exposure to an environmental hazard. It plays a central

role in assessing the potential health effects of different policies and measures, thereby providing a basis for decision-making. A detailed knowledge of several factors is required for any such assessment. Crucial information on exposure to air pollutants is provided by an integrated approach on ambient air quality monitoring and modelling study.

Air quality modelling, particularly the Integrated Assessment Modelling (IAM), is important in linking pollution levels to emission sources and integrating population data, findings from epidemiological studies, information about the formation and dispersion of fine particles in the atmosphere, assessment of current and future levels of emissions of fine particles and their precursors. In the frame of the UN-ECE Convention on Long-Range Transboundary Air Pollution (CLRTAP), and in the context of the Community Environmental policies of the EU Commission, the RAINS-Europe model provides one of the most relevant examples of successful application of Integrated Assessment Modelling (IAM). The RAINS model (6), developed at the International Institute for Applied Systems Analysis (IIASA), considers emissions of SO_2, NO_x, PM_{10}, $PM_{2.5}$, VOC and NH_3, provides deposition and concentration maps and addresses threats to human health posed by fine particulates (7). The assessment of fine particle health impacts is implemented through the Life Expectancy Reduction indicator (LER), defined as months lost attributable to $PM_{2.5}$ concentrations. Awaiting further refinements in the scientific disciplines, the quantitative implementation should be considered as preliminary and needs to be revised as soon as more substantiated scientific information becomes available. The Task Force on Health of the United Nations Economic Commission, when conducting the in-depth review of the RAINS approach for modelling health impacts of fine particles (TFH, 2003), noted "that some data suggested that different components that contributed to $PM_{2.5}$ mass might not be equally hazardous. In particular, the discussion focused on the role of the secondary inorganic aerosols (including nitrates and sulphates). It concluded that, due to the absence of compelling toxicological data about the active different PM components of a complex mixture, it was not possible to quantify the relative health impact importance of the main PM components at this stage". Therefore, it was recommended

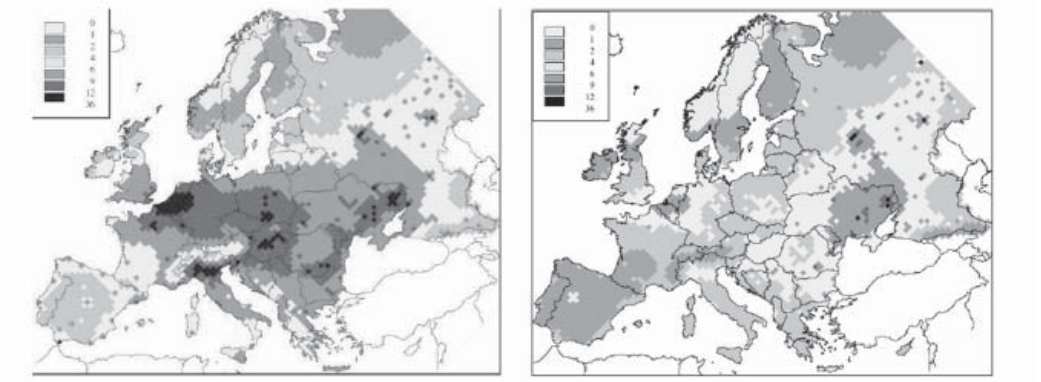

Figure 2. Changes in EU life expectancy loss in 2000 and in the interim objective in 2020 (Strategy) (9).

to relate health impacts to total mass of PM2.5 until more specific evidence becomes available (8). The methodology used in the RAINS model, at European and national scale, to estimate losses in life expectancy due to air pollution represents an initial implementation assessing the implications of present and future European policies to control exposure to particulate matter. In the Figure 2, an example of the changes in life expectancy loss in EU in 2000 and in the interim objective in 2020 (9) are reported. The impact assessment of the different policies is based on the analysis of a set of technological measurements with the RAINS model related to various emission reduction scenarios. The ambition level of the Strategy is based on a set of specific measurements which would need to be undertaken at Community and Member State level.

In the recent years, some European countries such as, among others, Italy, have tackled the issue of implementing the RAINS model at national level, introducing higher spatial resolution in similar models, pursuing the ultimate objective of a more adequate response to the need of evaluating, at national level, cost-effective policy measures to reduce air pollutant emissions, and consequently, the pressure on environment and human health. As a result, the RAINS-Italy model (10) as the national version of the RAINS-Europe model was defined considering as emission source areas either the nation as a whole or the 20 administrative Regions. In a recent work (11), the RAINS-Italy model

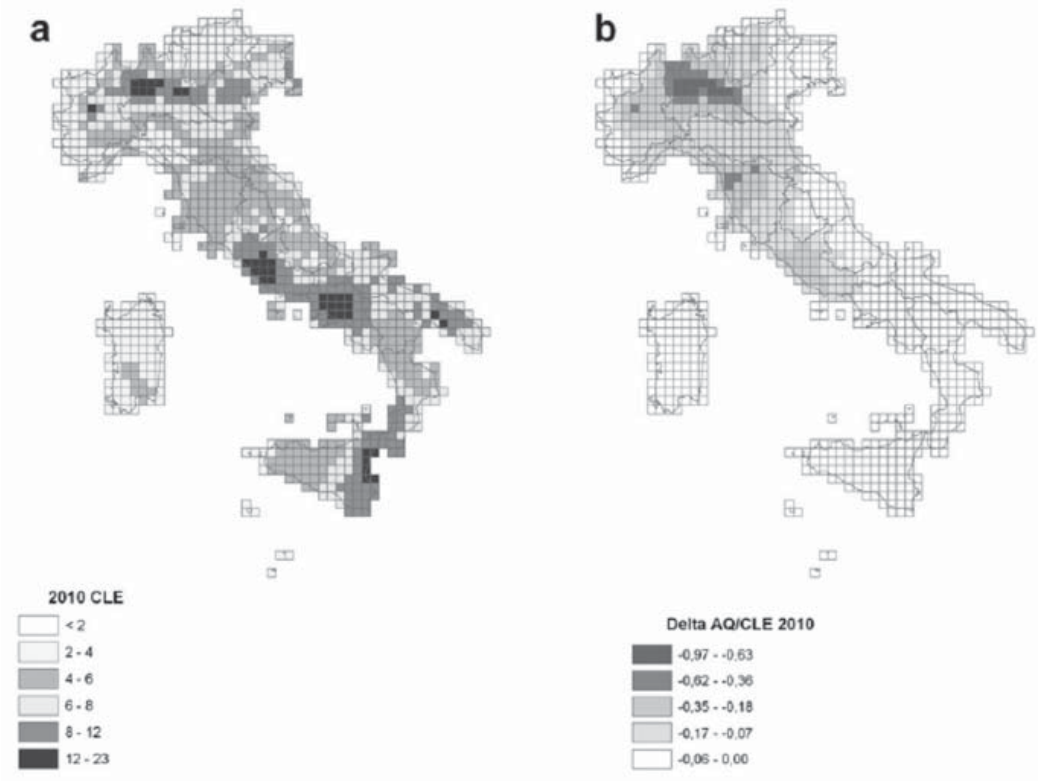

Figure 3. Losses in average Life Expectancy (months) attributable to $PM_{2.5}$ concentrations at 2010: a) CLE scenario; b) difference (months) between Air Quality Management Plan scenario and CLE scenario (11).

was used to assess the emission reduction strategies followed in the Regional Air Quality Management Plans (AQMPs) to meet environmental quality targets by means of Technical and Non-Technical Measures. Regarding health impacts (Figure 3a), the most important Italian metropolitan and industrial areas show an average Life Expectancy loss ranging between 12 to 23 months in the 2010-CLE scenario. This higher resolution map shows a better definition of the hot spots present in the urban areas of Turin, Milan, Rome and Naples, as well as in the industrial sites of eastern Sicily and Taranto, in the Apulia region (12). The above mentioned study showed that if compared to the 2010 CLE scenario, the 2010 AQMP scenario reduces PM_{10}, NO_x and SO_2 emission by 2.8%, 2.4% and 0.5%, respectively. Regions with a more effective AQMP reach higher PM10 yearly average concentrations reductions, with peaks of 7.5% in northern and central Italy, even if this is not sufficient to assure the compliance with air quality standard in 2010. Similarly the improvement in the average Life Expectancy loss indicator (Figure 3b) is of 1 month only in Lombardy (11).

2.2. Indoor vs outdoor air pollution

As it is the case for other air pollutants, the total exposure of an individual to suspended particulate matter (of whatever size) is the result of contributions from the two microenvironments, outdoor air and indoor air. The indoor air compartment can be further subdivided into homes, restaurants, car, buses and aircraft, workplaces etc. Consequently, in studies to detect and quantify the health effects of particles, attention must be paid that exposure is characterised adequately. Generally there are two different ways to obtain such characterization. One is by *measuring* total air exposure using personal sampling: the persons under study are provided each with a personal sampler that they have to carry on them or position as close to them as possible for 24 hours consecutively. Since this is cumbersome for a study participant, the following alternative can be used: total exposure is *modelled* taking into account the time spent in the various microenvironments (indoors and outdoors) and the concentrations observed in these microenvironments. Personal sampling provides a concentration level that represents the integration of all the concentration levels in all compartments visited by the studied person during the 24-h (or longer) measurement period and, thus, it cannot detect the individual contribution of any compartment. In contrast, the modelling process using the combination of the pollutant concentrations in the different microenvironments and the time spent therein permits to assess the contribution of total exposure to each of these microenvironments. This kind of source apportionment can be of great help to decide what measurements should receive priority in controlling pollutant concentrations. A recent publication on exposure to $PM_{2.5}$ describing the results of a model approach (13) stated that the "indoor-residential" microenvironment had the greatest influence on total exposure to $PM_{2.5}$, compared to the other microenvironments considered, namely outdoor and non-residential indoor (office, school, store, restaurant, bar, in-vehicle). It turned out that the outdoor compartment was responsible for a *direct* contribution of about 5% on average. Another 35% was due to an *indirect* contribution via infiltration of outdoor air into indoor spaces. Thus, about 60% of the total exposure to $PM_{2.5}$ could not be influenced by control measurements taken to reduce outdoor air $PM_{2.5}$ levels.

3. Inorganic air pollutants

3.1 Gaseous matter

Air pollution by inorganic gaseous matter was dealt with since the first major pollution events (i.e. the Great Smog of London, 1952; etc.) put on evidence the strict relationship between levels of chemical species in the ambient (and indoor) air their harmful effects on health and ecosystems. Among inorganic gases, carbon monoxide (CO) is one of the most common air pollutants. It has a low reactivity and a low water solubility and it is mainly released into the atmosphere as a product of incomplete combustion. CO is not only directly released in the air, but can also originate from the chemical reactions of organic air pollutants, such as methane. Its latency in the atmosphere is about three months. Since at moderate latitudes air masses travel for months and since the CO formation from organic air pollutants takes place everywhere in the atmosphere, a global background level of CO exists, ranging between 0.05 and 0.15 ppmv (0.06 and 0.17 mg/m^3) (14). It is estimated that about one-third of CO, including that derived from hydrocarbon oxidation, originates from natural sources. CO levels in busy city streets are higher than those present near highways, since the amount of CO emissions per kilometre strongly decreases with vehicle speed and also because ventilation in city streets is less. Ambient CO levels are usually highest in winter, because cold engines release much more CO than hot engines and also because the atmosphere tends to be more stable than in summer. It has to be reminded, however, that usually CO ambient levels do not exceed neither WHO guidelines for health protection nor the limits of the EU directives on air quality. Although CO is hardly removed from the air in atmospheric transport at continental level, long range transport does not lead to concentrations of concern at both rural and urban background level. Also at points of high traffic in large cities, levels exceeding legislation are only occasionally observed. Industrial areas may be affected by large CO emissions; however, when these emissions are released through high chimneys, local ambient concentrations show poor increases and do not pose risks for human health[14]. CO toxicity patterns are linked to its reaction with haemoglobin in the human blood to form carboxyhaemoglobin (COHb). The affinity of haemoglobin for CO is 200-250 times higher than for oxygen, and as a result this binding reduces the oxygen-carrying capacity of the blood and impairs the release of oxygen to extra vascular tissues. The most important variables determining the COHb level are CO in inhaled air, duration of exposure and lung ventilation. Physical exercise accelerates the CO uptake process.

The formation of COHb is a reversible process; however, the half-life elimination of COHb is much longer in the foetus than in the pregnant mother. The effects of CO exposure on cardiovascular disease have been studied for a long time (15). However, only limited information is available about the possible cardiac effects of gaseous pollutants at concentrations close to those present in ambient air. Apart from hazards due to high CO concentrations, other health effects seem to originate from the association between CO and other gaseous and particulate matter, especially exposure to exhausts from motor engines. Although attention has recently been focused on the cardiovascular effects of PM, few studies show evidence of the relationship between some cardiovascular diseases and the exposure of different populations to road

traffic exhausts. For example, experimental studies have demonstrated mild cardiac effects from both sulphur dioxide (SO_2) and ozone (O_3)(16). Other studies, where personal exposure to different pollutants has been investigated, have suggested that the estimated cardiac effects attributed to gases, including SO_2, are actually effects of other pollutants, specifically PM (17). At this stage of knowledge, however, it is difficult to differentiate between the effects of PM and those of gases because people are normally exposed to both types of pollutants at the same time. Because of these uncertainties, it seems prudent to further investigate both the effects that low concentrations of gaseous pollutants, alone or in combination with PM, might have on cardiovascular diseases, and the possibility that the associations with gaseous pollutants may actually reflect the effects of PM or some component that is not currently being studied for its health effects (16).

Anthropogenic sulphur dioxide (SO_2) results from the combustion of sulphur-containing fossil fuels (mainly coal and heavy oils) and the smelting of sulphur containing ores. Over the past years, however, there has been a net tendency towards emission reduction in Countries where low-sulphur fuels and emission control measures have been adopted. In addition, the source pattern has changed and moved from small multiple sources (domestic, commercial, industrial) to large single sources releasing SO_2 from tall stacks. Volcanoes and oceans are the major natural sources of SO_2. After being released in the atmosphere, sulphur dioxide is further oxidized to sulphate ($SO_4^=$) and sulphuric acid forming an aerosol often associated with other pollutants in droplets or solid particles having a wide range of sizes. SO_2 and its oxidation products are removed from the atmosphere by wet and dry deposition. Nowadays, it is also recognized that sulphate aerosols play an important cooling role in the radiative climate of the Earth through the phenomena of sunlight scattering in cloud free air and as cloud condensation nuclei. Sulphur dioxide is an irritant and when inhaled at high concentrations may cause breathing difficulties in people exposed to it. People suffering from asthma and chronic lung disease may be especially susceptible to the adverse effects of sulphur dioxide. Nevertheless adverse effects from high concentrations of SO_2 have been observed both on healthy people and asthma patients (18).

Oxidized nitrogen compounds (NO_2, NO_x, NO_y) and ozone (O_3) join common patterns in atmospheric formation chemistry, environmental fate and adverse effects on health and the ecosystem. NO is directly released by all combustion processes; once in the atmosphere, it reacts with oxygen and a number of other inorganic (e.g. O_3, OH radical, halogens) and organic (VOCs) gases to form NO_2, NO_3, HONO, HNO_3, PAN, nitro – PAH and other organic and halogen nitrates, in the gaseous or particulate phase. Ozone and oxidized nitrogen compounds are strongly oxidant and this aspect mainly characterizes their harmful health action. In particular, the oxidizing potential of these compounds is commonly referred to as "odd oxygen" (O_x) or "odd nitrogen" (NO_x), i.e families of chemical compounds that interconvert rapidly among themselves on time scales that are shorter than those necessary to form or destroy the family . Another family is that defined as "NO_z", which refers to the sum of NO_x oxidation products (19).

$$NO_x = NO + NO_2 \quad [1]$$

$O_x = \Sigma (O(^3P) + O(^1D) + O_3 + NO_2)$ [2]

$NO_z = \Sigma (HNO_3 + HNO_4 + NO_3 + 2NO_2O_5$ + PAN + other organic nitrate + halogen nitrate + particulate nitrate) [3]

Unlike some other compounds whose formation rates vary directly with the emissions of their precursors, O_3 differs in that its production changes nonlinearly with the concentrations of precursors. At the low NO_x concentrations found in most environments ranging from remote continental areas to rural and suburban areas, the O_3 net production increases with the increasing of NO_x. At the high NO_x concentrations found in downtown metropolitan areas especially near busy streets and roadways and in power plants, there is a net destruction of O_3 by titration reaction with NO. Between these two regimes is a transition stage in which O_3 shows only a weak dependence on NO_x concentrations. In the high NO_x regime, NO_2 scavenges OH radicals which would otherwise oxidize VOCs to produce peroxy radicals, which in turn would oxidize NO into NO_2. In the low NO_x regime, VOC oxidation generates, or at least does not consume, free radicals, and O_3 production varies accordingly. Sometimes the terms 'VOC-limited' and 'NO_x-limited' are used to describe these two regimes; also, the terms NO_x-limited and NO_x-saturated are used. The chemistry of OH radicals, that are responsible of the initiation of hydrocarbons oxidation, shows a behaviour similar to that of O_3 with respect to NO_x concentrations (19). These considerations introduce a high degree of uncertainty into attempts to relate changes in O_3 concentrations to precursors emissions. It should also be noted at the outset that in a NO_x-limited (or NO_x-sensitive) regime, O_3 formation is not insensitive to radical production or the flux of solar UV photons, but O_3 formation is more sensitive to NO_x. For example, global tropospheric O_3 is sensitive to CH_4 concentrations even if the troposphere is predominantly NO_x-limited. To get information about the O_3-NO_x-VOCs relationships and sensitivity, the ratio of summed VOC to NO_x concentrations determining whether conditions are NO_x-sensitive or VOC sensitive is not sufficient to describe O_3 formation, since other factors - i.e. the effect of biogenic VOCs (which are not present in urban centres in early morning) - and some important individual differences in VOCs ability to generate free radicals, have to be considered. The difference between NO_x-limited and NO_x-saturated regimes is also reflected in measurements of hydrogen peroxide (H_2O_2), another strong oxidant of ambient air. H_2O_2 formation takes place by self-reaction of photo chemically generated HO_2 radicals, so that there is large seasonal variation in H_2O_2 concentrations, and values in excess of 1 ppb are mainly limited to summer months, when photochemistry is more active (20). Hydrogen peroxide is produced in abundance only when O_3 is produced under NO_x-limited conditions. The transition from NO_x - limited to NO_x - saturated conditions is highly space and time dependent. In the upper troposphere, responses to NO_x additions from commercial aircraft have been found that are very similar to those in the lower troposphere. Moreover, the complex interplay between chemical and meteorological processes gives rise to uncertainties in understanding ozone formation. This is especially true for regions of complex topography. In coastal regions around the Mediterranean Basin, for instance, the combination of mountain and sea breeze re-circulations significantly affects ozone phenomenology. Ozone can also have very specific distributions

in mountain areas, and observed concentrations differ significantly between mountain peaks and valleys (20). Nitro-polycyclic aromatic hydrocarbons (nitro-PAHs) are generated from incomplete combustion processes through PAHs electrophilic reactions in the presence of NO_2 (21). Among combustion sources, diesel emissions have been identified as the major source of nitro-PAHs in ambient air. Direct emissions of nitro-PAHs in PM vary with the type of fuel, vehicle maintenance, and ambient conditions (22). In addition to being directly released, nitro-PAHs can also be formed from both PAHs gaseous and heterogeneous reactions with gaseous nitrogenous pollutants in the atmosphere. After formation, nitro-PAHs with low vapour pressures (such as 2NF and 2NP) immediately migrate to particles under ambient conditions; therefore harmful effects related to nitro – PAHs are better investigated in the organic fraction of particulate matter. An extended discussion on this topic is reported in par. 3.2.

Also in indoor environments NO_2 plays a key role in adverse health effects. It is indeed produced by NO reactions with ozone or peroxy radicals generated by indoor air chemistry involving O_3 and unsaturated hydrocarbons such as terpenes found in air fresheners and other household products (23). Nevertheless indoor NO_2 is also contributed by indoor – outdoor air exchange. The relationship between personal NO_2 exposure and ambient NO_2 can be modified by the indoor environment. For example, during the infiltration processes, ambient NO_2 can be lost through penetration and decay (chemical and physical processes) in the indoor environment, and the concentration of indoor ambient NO_2 is not just the ambient NO_2 concentration but the product of the ambient NO_2 concentration and the infiltration factor (F_{inf}, or α if people spend 100% of their time indoor). Indoor NO_2 is removed by gas phase reactions with ozone and assorted free radicals and by surface promoted hydrolysis and reduction reactions. The concentration of indoor NO_2 also affects PAN decomposition. These processes are important not only because they influence the indoor NO_2 concentrations to which humans are exposed, but also because some products of indoor chemistry may confound attempts to examine associations between NO_2 and health. As a matter of fact, NO_2 is an oxidant and lipid peroxidation is believed to be a major molecular event responsible for its toxicity. As a result, there has been considerable attention paid to NO_2 effect on the antioxidant defence system in the epithelial lining fluid and in pulmonary cells. Repeated exposure to indoor NO_2 at concentrations ranging from 0.04 to 33 ppm has been shown to alter low molecular weight antioxidants such as glutathione, vitamin E, and vitamin C, as well as some enzymes involved in cell oxidant homeostasis. NO_2 effects on structural proteins of the lungs have raised concern because elastic recoil is lost after exposure. It has been observed that the latter increases collagen synthesis. This, in turn, shows increases in total lung collagen which, if sufficient, could result in pulmonary fibrosis after longer periods of exposure. Such correlation has yet to be confirmed by in vivo studies involving NO_2 exposure; nevertheless some evidence shown in animal studies about asthma, emphysema and other lung diseases. Similarly to O_3, NO_2 is absorbed throughout the lower respiratory tract, but the major delivery site is the centriacinar region, i.e, the junction between the conducting and respiratory airways in humans and animals (21).

Ozone is a strong oxidant, and as such can react with a wide range of cellular components and biological materials: damage can occur to all parts of the respiratory tract. The time pattern of these changes in the respiratory system, as determined in laboratory animals as well as in epidemiological investigations, is complex. During the first few days of exposure, inflammation occurs and then persists at an attenuated level. At the same time, epithelial hyperplasia progresses, and reaches a plateau after about one week of exposure. When the exposure ceases, these effects slowly disappear. In contrast to this, interstitial fibrosis increases slowly and can persist even when exposure ceases. In a large number of controlled human studies, significant impairment of pulmonary function has been reported. Field studies in children, adolescents, and young adults have indicated that pulmonary function decrements, similarly to those observed in controlled studies, can occur as a result of short term exposure to ozone concentrations in the range of 120-240 $\mu g/m^3$ and higher. In comparison with adults, children have a higher intake of ozone and other air pollutants. This is due to a higher basal metabolic rate, resulting in a higher breathing volume per minute and a higher breathing frequency. Furthermore, their respiratory tract is still under development until the age of six and a half, and it is therefore more susceptible to the inflammatory effects of ozone. Children's immune systems are not yet fully developed and are generally under bigger stress. For these and other reasons, children are at higher risk when exposed to ambient ozone concentrations. Hospital admissions for respiratory causes and exacerbation of asthma are observed both in exposures to ambient ozone (and co-pollutants) and in controlled exposures to O_3 alone. Other groups at risk are those people exercising outdoors during evening hours or whenever ozone concentrations tend to be highest (e.g. in photochemical smog events). Due to the irritant nature of ozone, capable of inducing airway inflammation and bronchoconstriction, asthma patients are deemed to be at enhanced risk from exposure to ozone and photochemical smog, because inflamed airways contribute to the pathogenesis and exacerbation of the disease and to morbidity and mortality for asthma. Results from recent epidemiologic studies have suggested that ozone might have serious cardiovascular effect (24 and references therein). Although a large number of toxicity animal studies have been performed on respiratory and other effects of NO_2, O_3 and other gaseous pollutants on metabolic and physiological functions (body weight, hepatic, renal, brain, etc.), results are often affected by serious limitations, due to both the necessary animal-to-human extrapolation of concentration-response data and the fact that controlled exposures to a single pollutant alone provide incomplete information. Human clinical studies attempt to recreate in laboratory the atmospheric conditions of ambient pollutant atmospheres, paying great attention to concentrations, duration, timing, and other conditions which may impact responses. These studies allow the measurement of health symptoms and physiological markers resulting from air breathing. This carefully controlled environment allows researchers to identify responses to individual pollutants, to characterize exposure-response relationships, to examine interactions among pollutants, and to study the effects of other variables such as exercise, humidity, or temperature. Susceptible populations, including individuals with acute and chronic respiratory and cardiovascular

diseases, can participate with appropriate limitations based on subject comfort and protection from risk. Endpoint assessment has traditionally included symptoms and pulmonary function, but more recently a variety of markers of pulmonary, systemic, and cardiovascular function have been used to assess pollutant effects. It is reasonable to consider, however, that human clinical studies have limitations. For practical and ethical reasons, studies must be limited to relatively small groups, to short durations of exposure, and to pollutant concentrations that are expected to produce only mild and transient responses. Findings from the short-term exposures in clinical studies may provide limited insight about the health effects of chronic or repeated exposures. Moreover, the choice of previous- and after-exposure time lags for the observation of health effects is critical in assessing the role of a pollutant in toxicity events. Many studies have shown that NO_2 has a fairly consistent, immediate effect on health outcomes, including respiratory hospitalizations and mortality. Several studies also observed significant NO_2 effects over longer cumulative lag periods, suggesting that in addition to single-day lags, multiday lags should be investigated to fully capture a delayed NO_2 effect on health outcomes. Finally it should be kept in mind that, although many biochemical changes are not necessarily toxic manifestations of the pollutant per se, such changes may anyway impact the metabolism and toxicity of other chemicals in humans and animal species (21).

The EU regulates the main harmful inorganic gaseous pollutants by the EC legislation of the Air Quality Framework (25). Other legally binding Protocols have been established since the 1979 Geneva Convention on Long Range Transboundary Air Pollution (LRTAP) (26). Guideline levels aimed at health and environment protection have been set by WHO and other institutions, too, to be used for impact assessment. The first edition of the WHO "Air quality guidelines for Europe (AQG)" was published in 1987 (27). To determine critical or guideline levels, quantitative relationships between the pollutant exposure and its studied effect are needed. However, any such relationships have a certain degree of uncertainty, and the data necessary to produce them are often scarce. Therefore, the establishment of guideline values, such as levels at which acute (or chronic) effects on public health or ecosystems are likely to be not relevantly harmful, impose the support of biological, clinical and epidemiological evidence, often not available or inadequate. Different legal tools aimed at protecting and improving the health and quality of ecosystems from air pollution have been used in recent years. The 1992 fifth action programme of the European Commission (EAP) on the environment recommended "the establishment of long-term air quality objectives" for many inorganic gaseous pollutants (CO, NO_2 and NO_x, SO_2, O_3). The list of key requirements, also includes the need for "studies to analyze the effects [on health ant ecosystems] of the combined action of various pollutants or sources of pollution and the effect of climate on the activity of the various pollutants examined". Under the 5th EAP the Air Quality Framework was established, within which the 96/62/EC Directive and the following four Daughter Directives have been adopted. This law establishes limits and threshold values for SO_2, NO_2 and NO_x under the 99/30/EC, CO under the 2000/69/EC and O_3 under the 2002/03/EC for EU Member States. In the 6th EAP, further steps have been taken toward health

/ environment protection by the Clean Air for Europe (CAFE) programme. The CAFE is conceived as a process based on technical analysis and policy development to achieve the adoption of a Thematic Strategy on Air Pollution. The major elements of the CAFE programme are outlined in Communication COM(2001)245 (2). The programme, launched in early 2001, aims at the development of a long-term, strategic and integrated policy advice to protect against the significant negative effects of air pollution on human health and the ecosystem. Within this process, the 2008/50/EC Directive on ambient air quality and cleaner air for Europe has been adopted and will enter into force as from 11 June 2010, when the Directives 96/62/EC, 1999/30/EC, 2000/69/EC and 2002/3/EC shall be repealed.

3.2 Composition and size distribution of the inorganic fraction of suspended PM

3.2.1 Inorganic fraction of suspended PM

The history of air pollution is very long, and since its very first occurrence - smoke from heating and cooking activities in prehistoric dwellings - particles have been addressed as one of the most important issues. Pollution from combustion sources and specifically suspended particles have been responsible for the most relevant pollution disasters (e.g. Mause Valley, Belgium, 1930, the Big Smoke, London, 1952), which led to increasing efforts towards pollution monitoring, the understanding of main pollution processes, political awareness and, finally, regulations.

At European level, pollution from particulate matter (PM) has been first addressed by the First Daugther Directive (1999/30/EC) to the Air Quality Framework Directive (1996/62/EC); recently, a new Directive (2008/50/EC, published in June 2008) summarised most of the existing legislation on ambient air and introduced some new requirements. As far as PM is concerned, the First Daugther Directive addressed only PM_{10}, setting limits for its annual average concentration (40 $\mu g/m^3$) and the number of exceedances (35 per year) of the 50 $\mu g/m^3$ daily concentration limit. Air quality limits also for PM2.5 were introduced only by the recent Directive 2008/50/EC (25 $\mu g/m^3$, with a 20% margin of tolerance that will be reduced to zero on 1st January 2015). In addition to the measurement of PM mass concentration, Directive 2008/50/EC also includes the measurement of $PM_{2.5}$ chemical composition in background sites, listing a number of components that must necessarily be determined in each $PM_{2.5}$ sample (sulphate, nitrate, chloride, sodium, ammonium, potassium, calcium, magnesium). This new issue is related to the increasing awareness of the complexity of this "pollutant", which is a mixture of thousands of different chemical species, each one with its own properties and possibly its own environmental and health effects.

Unlike gaseous pollutants, where the concentration is generally sufficient to define the system, for the atmospheric aerosol many parameters have to be defined. Physical parameters include the geometric and aerodynamic diameter, shape (spheres, fibres, etc.), phase (solid, liquid, mixture of both), density, electrical charge, hygroscopicity etc. and are necessary to understand particles behaviour in the atmosphere as well as inside the respiratory system. The most complex issue in aerosol characterisation, however, is its chemical composition, which includes a variety of components, whose determination requires a variety of analytical techniques.

The knowledge of health effects caused by inhalation of atmospheric particles has been improved a lot during the last decade and there is no doubt that particles can be harmful to human health. PM is associated with a wide variety of both acute and chronic cardiovascular and respiratory effects. Acute effects include increased hospital admittance for respiratory disease or premature mortality for cardiovascular disease, while chronic effects include a number of diseases leading to longevity reduction. The increase in respiratory and cardiovascular morbidity and mortality is in the order of a few percent for a PM increase of 10 $\mu g/m^3$ (28-34).

The study of the link between particulate matter and health is extremely complex and poses many problems, including the difficulties in assessing the role of particle size and particle composition, in quantifying the real exposure and understanding the biological mechanisms that are responsible for the effects, in evaluating the impact of the different sources and, last but not least, in detecting the real concentration and composition of the atmospheric aerosol.

The size of atmospheric particles varies among five orders of magnitude, from a few nanometres to hundreds of micrometers. The size of the aerosol influences its lifetime in the atmosphere (and thus the spatial range of influence of any single source) as well as its pathway inside the human body. Basically, the atmospheric aerosol consists of three modes, which are closely linked to their formation mechanism: the coarse mode, predominantly mechanically generated (e.g. by erosion and by re-suspension), the accumulation mode, produced by condensation from vapours and coagulation from smaller particles, and the nucleation mode, which includes particles smaller than 0.1 μm originating from combustion processes (e.g. vehicle exhausts, biomass burning). Natural aerosol, originating from the sea and the soil, is mostly in the coarse mode and is generally considered as less harmful than anthropogenic aerosol, generated by combustion sources, found mainly in the fine mode and able to penetrate deeply in the respiratory tree. The harmful role of nanoparticles, able to reach the alveoli and to be directly transported inside the body cells, is still a matter of debate. The chemical composition of an atmospheric particle depends on its source as well as on its "story" from the time of its emission or formation to the time when it reaches the receptor (e.g. the human body). Some particles are directly released from their source into the atmosphere (primary PM), but the characterisation of any emission source is quite complex, as it generally changes with time and operative conditions. In addition, once formed, particles often undergo chemical and physical transformations and for this reason what is measured at the receptor may be also very different from what is released at the source. Even more difficult to trace are the other particles formed in the atmosphere as a result of chemical reactions between gaseous compounds or gas-to-particle conversion; of particular relevance, in this framework, is the oxidation of biogenically released VOCs.

Information about PM sources can be obtained by analysing their chemical composition. Only a limited number of compounds constitute more than 1% of the overall PM mass: a few metals (Al, Si, Fe), the main anions and cations (chloride, nitrate, sulphate, carbonate, sodium, ammonium, potassium, magnesium and calcium), elemental carbon and organic material. This last category is the most important, as it generally represents 20 – 60% of PM,

but, unlike the other main components, it is not a single chemical compound but it is constituted by many hundreds of compounds, none of which constitute more than 1% of the total PM mass. Although the determination of the listed compounds is in most cases sufficient to obtain the mass closure (i.e the sum of the single components equals the gravimetric mass), the determination of micro-components is generally necessary to obtain a picture of PM sources and effects (35-45). Although quite complex, the determination of most inorganic PM components has been one of the targets of field research during the last 10-20 years. By determining inorganic PM macro-components it is possible to trace natural sources (sea-spray, desert dust, local crustal components) and to measure the contribution of secondary compounds (ammonium sulphate and ammonium nitrate); inorganic micro-components, on the contrary, may be of help in determining the contribution of anthropogenic sources, e.g. dust re-suspension and industrial sources (46-48). Much less understood and quantified is the organic fraction, as the chemical analysis is generally able to identify no more than 15-20% of total organic mass.

Once we are able to measure PM concentration and to determine its chemical composition, we need to clarify the link between concentration and exposure. This is a critical point in the scientific studies about health effects, as the reference PM values are generally those measured by local Protection Agencies, i.e. outdoor values sometimes taken at traffic hotspots, while people generally spend most of their time in indoor environments, including homes, working places and vehicles, and only a small part of their time outdoors. Considerable work is still needed to develop models able to simulate the behaviour of individuals in indoor microenvironments. Also, we need information about the composition of indoor PM, that may greatly differ from the composition of outdoor particles (49-56). For example, in indoor environments we may be exposed to much more particles produced by peculiar sources such as domestic wood burning or cooking than to particles emitted by traffic sources.

As a consequence of the many difficulties arising when relating PM concentration to the results of epidemiological studies, the scientific community is now trying to find a relationship between health effects and individual chemical components. This attempt requires the availability of long time series of PM composition study and is still in its infancy. The other pathway to elucidate the link between PM and health effects is the study of the PM toxicological effects, that is the specific mechanisms that lead PM to cause the observed health impacts. These studies include animal models, human exposure during occupational activities and experimental exposures. The mechanisms of PM effects on human health are still quite uncertain, but given the variety and degree of observed health associations, it is likely that more than one of them are involved. Basically, particles entering the tissue cells may cause inflammation; researchers increasingly find that reactive oxygen compounds (in the PM or produced by stimulated cells) play a role. Because of the presence of particles in the respiratory tract, changes in the respiratory function may occur. Particles in the blood may increase viscosity, causing thrombosis or myocardial infarct. Of course, individuals with pre-existing deficiencies of the cardiovascular or respiratory system may suffer more severe effects (56-61).

It is clear that a more integrated approach

is needed to get insights into PM health effects. In particular, the link between health effect and PM component and size and the biological specific mechanism of its action requires further combined interdisciplinary studies.

3.2.2 Transition (heavy) metals

In contrast to gaseous specific compounds such as benzene or carbon monoxide, the assessment of metal and metalloid compounds in ambient air is complicated by the fact that different species with considerably differing toxicity and/or carcinogenic potency may be encountered. Therefore, to fully evaluate the health effects, it is important to know which compounds do occur in the environment or at least which compounds form the main constituents. In ambient air, metals, metalloids and their compounds are mainly encountered as part of particulate matter. They may be present in the non soluble, non stoichiometric mixture phase (for example as spinels) or as soluble ionic compounds (salts). To a lesser extent and under certain environmental conditions, gaseous forms (i.e, organometallic compounds) may or may not be adsorbed by particles. In respect to their effects on the environment and on human health, these compounds can be characterized by other parameters, such as water solubility (extended to solubility in biological fluids), particle size distribution, morphology and specific surface area, and chemical heterogeneity of their particles (for example, a metal compound encapsulated in another aerosol or surface enrichment of volatile compounds), or the concentration of metals and metalloids in the particles ultimately contacting target tissues in the human body. All parameters mentioned will influence bioavailability and possible effects. In addition, metal and metalloid containing substances can undergo various chemical and physical transformations in the atmosphere on their way from the source to a possible receptor. For example, As (III) compounds may be oxidized to As (V). Unfortunately, analytical methods normally identify only the elements present in atmospheric particles, since a specific analysis is extremely difficult in the concentration range occurring in ambient air (typically several ng/m^3). In addition, the state of oxidation may change during sampling. Consequently, information on the concentration of different compounds in ambient air is very limited at present. Another possibility to gain some insight into them is to analyze which compounds are emitted by the most important natural (i.e, weathering processes) and anthropogenic sources.

Some metals are naturally found in the body and are essential to human health. Iron, for example, prevents anaemia, and zinc is a cofactor in over 100 enzyme reactions. They normally occur at low concentrations and are known as trace metals. In high doses, they may be toxic to the body or produce deficiencies in other trace metals; for example, high levels of zinc can result in copper deficiency, another metal required by the body. Heavy metals (HMs) (or toxic metals) are trace metals with a density at least five times that of water. As such, they are stable elements (meaning they cannot be metabolized by the body) and bio-accumulative (passed up the food chain to humans). These include: mercury (Hg), nickel (Ni), lead (Pb), arsenic (As), cadmium (Cd), aluminium (Al), platinum (Pt), and copper (Cu) (the metallic form versus the ionic form required by the body). Heavy metals have no function in the body and can be highly toxic. Once liberated into the environment through air, drinking water,

food, or countless human-made chemicals and products, heavy metals are taken into the body via inhalation, ingestion, and skin absorption. If heavy metals enter and accumulate in body tissues faster than the body's detoxification pathways can dispose of them, a gradual build-up of these toxins will occur. High concentration exposure is not necessary to produce a state of toxicity in the body, as heavy metals accumulate in body tissues and, over time, can reach toxic concentration levels. Human exposure to heavy metals has risen dramatically in the last 50 years as a result of an exponential increase in the use of heavy metals in industrial processes and products. Today, chronic exposure comes from mercury-amalgam dental fillings, lead in paint and tap water, chemical residues in processed foods, and "personal care" products (cosmetics, shampoo and other hair products, mouthwash, toothpaste, soap).

The effects of Heavy Metal toxicity studies confirm that heavy metals can directly influence behaviour by impairing mental and neurological functions, influencing neurotransmitterproductionandutilization, and altering numerous metabolic body processes. Systems in which toxic metal elements can induce impairment and dysfunction include the blood and cardiovascular system, detoxification pathways (colon, liver, kidneys, skin), endocrine (hormonal)system, energy production pathways, enzymatic, gastrointestinal, immune, nervous (central and peripheral), reproductive, and urinary systems. Breathing heavy metal particles, even at levels well below those considered nontoxic, can have serious health effects. Virtually all aspects of animal and human immune system functions are compromised by the inhalation of heavy metal particulates. In addition, toxic metals can increase allergic reactions, cause genetic mutation, compete with "good" trace metals for biochemical bond sites, and act as antibiotics, killing both harmful and beneficial bacteria. For the most toxic HMs, atmospheric concentrations for Pb, As, Cd, Ni and Hg in ambient air have been regulated by European Commission directives (Directive 1999/30/EC from 22 April 1999 for Pb; Directive 2004/107/EC from 15 December 2004 for As, Cd, Ni and Hg). For Pb, an annual limit value of 0.5 g m^{-3}, entered into force 1.1.2005, has been set. The pertaining As, Cd and Ni Target Values are, otherwise, reported in Table 3.1.

Table 3.1 Target values for As, Cd and Ni. 2004/107/EC

Pollutant	Target Value (1)
Arsenic	6 ng m^{-3}
Cadmium	5 ng m^{-3}
Nickel	20 ng m^{-3}

(1) For the total content in the PM_{10} fraction averaged over a calendar year

Specifically, from chronic arsenic exposure, the greatest dangers are lung and skin cancers and gradual poisoning, most frequently derived from living near metal smelting plants or arsenic factories. Arsenic toxicity has been recognized for centuries, and hair shows significant correlation with its intake. As can be released to the atmosphere from metal transformation, fuel combustion and the use of pesticides.

In the air, As exists predominantly absorbed on particles, and is usually present as a mixture of arsenate (As(+V)) and arsenite (As(+III)), except in areas of arsenic pesticide application or biotic activities, where organic species are predominant (27,62). Recent data display a wide range of As concentrations in atmospheric particulate matter, for samples collected

at various sites in Spain (ranging from 13 to 144 mg kg^{-1} for a rural area and an industrialised area, respectively) (63). All studies performed in the Mediterranean basin (64-65) agree with an enrichment of As in the atmosphere as shown by (66).

Cadmium is an element that is naturally found in the earth's crust. Cadmium is often found as part of small particles present in air. Cadmium has many uses in industry and consumer products, mainly batteries, pigments, metal coatings, and plastics. Cadmium can enter the environment in several ways. It can enter the air from the burning of coal and household waste, and metal mining and refining processes. Cadmium attached to small particles may get into the air and travel a long way before coming down to earth as dust or in rain or snow. Cadmium does not break down in the environment but can change into different forms. Most cadmium stays where it enters the environment for a long time. Cadmium has no known good effects on health. Breathing air with very high levels of cadmium severely damages the lungs and can cause death. Breathing lower levels for years leads to a build-up of cadmium in the kidneys that can cause kidney disease. Other effects that may occur after breathing cadmium for a long time are lung damage and fragile bones. Workers who inhale cadmium for a long time may have an increased chance of getting lung cancer. The greatest danger from chronic nickel exposure is lung, nasal, or larynx cancers, and gradual poisoning from accidental or chronic low-level exposure, the risk of which is greatest for those living near metal smelting plants, solid waste incinerators, or old nickel refineries. Nickel combined with other elements is naturally found in the earth's crust, in all soils, and it is also released from volcanoes. Nickel is the 24^{th} most abundant element, and in the environment it is found primarily in the form of oxides or sulphides. Nickel is also found in meteorites and in lumps of minerals on the bottom of the ocean, and it is known as sea floor nodules. The earth's core is believed to contain large amounts of nickel. Nickel is released into the atmosphere during nickel mining and by industries that convert scrap or new nickel into alloys or nickel compounds or by industries that use nickel and its compounds. It is also released into the atmosphere by oil-burning power plants, coal-burning power plants, and trash incinerators. The nickel that comes out power plants' stacks is attached to small particles of dust that settle to the ground or are transported in the air by rain. It will usually take many days for nickel to be removed from the air. If the nickel is attached to very small particles, removal can take longer than a month. Given nickel's ability to cause contact dermatitis, and its observed perturbation of immunoglobulin levels, elevated hair levels may serve as an indicator of possible immune dysfunction, as well as a potentially useful marker of cardiovascular problems.

Pb is one of the most released HMs in the Mediterranean atmosphere. Traffic remains the main source at global scale (67), but its relative importance varies from region to region. In Europe, the phasing out of alkyl-leads in gasoline resulted in a decrease in atmospheric lead concentrations. As a result, industrial emissions (lead smelting and steelworks) became predominant in Europe and discernable from traffic emissions at continental scale (68). The annual emission of Pb in the Mediterranean region has been estimated to be about 1.1 10^4 Mg yr^1 in 2005 by (69). Lead is a known neurotoxin (it kills brain cells), and excessive blood lead levels in children have been linked to learning disabilities,

attention deficit disorder, hyperactivity syndromes, and reduced intelligence and school achievement scores.

3.2.3. Mercury (Hg) and impact on human health

Although mercury is an element found in nature and as such it will always be present in the environment, human activities have significantly increased global atmospheric mercury deposition since pre-industrial times. A significant increase in mercury emissions in the atmosphere occurred during the industrial revolution due to fossil fuel combustion and other human activities. Mercury is today a severe and chronic pollution problem in the environment. It is released into the atmosphere from a variety of anthropogenic (i.e, cement production, waste incineration, power generation facilities, smelters) (70-71) and natural sources (i.e, volcanoes, crustal and vegetation degassing, oceans) (72-74) in different chemical and physical forms.(75). In the troposphere, the most important forms are gaseous elemental mercury (Hg^0), divalent reactive gaseous mercury, $Hg^{(II)}$, which consists of various oxidised compounds, and particle-bound Hg (Hg-p), which consists of various Hg compounds. It should be noted that information on the speciation/fractionation of these different chemical and physical forms is largely operationally defined. Conversions between these different forms provide the basis of Hg's complex distribution pattern on local, regional and global scale. Hg cycles in different environmental compartments depends on the rate of different chemical and physical mechanisms (i.e, dry deposition, wet scavenging) and meteorological conditions as well as on the anthropogenic variables that affect its fate in the global environment. Experimental field data and model estimates indicate that anthropogenic mercury emissions are at least as great as those from natural sources, and contribute to the global atmospheric pool. A threefold increase of mercury deposition since pre-industrial times was in fact observed from the analysis of lake sediments, ice cores and peat deposits in both hemispheres (74-79). Recent studies have highlighted that in fast developing countries (i.e, China, India) mercury emissions are rapidly and dramatically increasing due mainly to a sharp increase in energy production from coal combustion. Recent emission estimates highlighted that the Asian emissions are considered to have a global impact.

Evidence shows that an increase in ambient air levels of mercury is linked to an increased load of toxic mercury in ecosystems (80). The atmospheric input of this element in aquatic and terrestrial ecosystems is driven by particle dry deposition and wet scavenging by precipitation mechanisms (81-83). The most important from a toxicological point of view are the metallic forms. In fact, the impact of mercury on human health and the environment depends on several mechanisms, which, in turn, depend on the toxicokinetic of its major chemical forms present in different environmental media including elemental mercury (Hg^0), inorganic mercury (i.e, $HgCl_2$) and organic mercury (i.e, methylmercury). This toxicokinetic mechanisms include absorption, distribution, metabolism and excretion. Therefore, according to the relevant chemical form of mercury, the combination of these mechanisms will determine the risk associated to the human exposure. For instance, the absorption of Hg^0 vapour occurs rapidly through the lungs, but it is poorly absorbed from the gastrointestinal tract.

Once absorbed, elemental mercury is readily distributed throughout the body, it crosses both placental and blood-brain barriers (84-86) Elemental mercury is oxidised to inorganic divalent mercury by the hydrogen peroxidase-catalase pathway, present in most tissues. The distribution of absorbed elemental mercury is limited by the oxidation of elemental mercury into mercuric ion as the mercuric ion has a limited ability to cross the placental and blood-brain barriers. Once elemental mercury crosses these barriers and is oxidised to mercuric ion, its return to the general circulation is impeded, and mercury can be retained in brain tissue. The elimination of elemental mercury occurs via urine, faeces, exhaled air, sweat and saliva. The excretion pattern depends on the extent to which elemental mercury has been oxidised to mercuric mercury (87-90). Absorption of inorganic mercury through the gastrointestinal tract varies with the particular mercuric salt involved and decreases with its increasing solubility and can reach even 20% (91). Available data indicate that absorption of mercuric chloride from the gastrointestinal tract results from an electrostatic interaction with the brush border membrane and limited passive diffusion. Increases in intestinal pH, high doses of mercuric chloride causing a corrosive action, a milk diet and increases in pinocytotic activity in the gastrointestinal tract have all been associated with increased absorption of inorganic mercury. Inorganic mercury has a limited capacity of penetrating blood-brain and placental barriers. There is some evidence indicating that mercuric mercury in the body following oral exposure can be reduced to elemental mercury and excreted via exhaled air. Because of the relatively poor absorption of orally administered inorganic mercury, most ingested doses in humans are excreted through the faeces. Methylmercury is rapidly and extensively absorbed through the gastrointestinal tract (92). Absorption information following inhalation exposure is limited. Epidemic of mercury poisoning following high-dose exposures to methylmercury in Japan and Iraq demonstrated that neurotoxicity is the most worrisome health effect when methylmercury exposure occurs to the developing foetus. Dietary methylmercury is almost completely absorbed into the blood and distributed to all tissues including the brain. It also readily passes through the placenta to the foetus and foetal brain. Methylmercury in the body is considered to be stable and it is only slowly demethylated to form mercuric mercury in rats. It has a relatively long biological half-life in humans (44-80 days) and it is excreted through faeces, breast milk and urine.

4. Organic air pollutants

4.1 Volatile organic compounds

The atmosphere is formed by a restricted number of macro-components, namely gaseous nitrogen, oxygen, argon, carbon dioxide, solid particulate matter and water; the latter exists as vapour, or in liquid and solid forms. Thousands of micro-components are dispersed in the gas phase and/or participate to aerosol composition [93]. Although occurring often at very low levels (even below one part-per-trillion), they nevertheless intervene in the physics and chemistry of the atmosphere, heavily influencing our life. Among the micro-components, a key role is played by hydrocarbons and their derivatives, cumulatively called "volatile organic compounds" (VOC), which are in gas form. Congeners of VOC occur in particulates as adsorbed on soot or dissolved in water

drops and crystals.

In its chemical structure, VOC include: *i)* aliphatic hydrocarbons (linear, branched and cyclic); *ii*) *arenes* having at least one aromatic group (Ar), namely benzene, alkylbenzenes and some polyaromatics (naphthalene); *iii)* alcohols (ROH) and ethers (ROR_1); *iv)* carbonyls, comprising aldehydes (RCHO) and ketones (RCR_1O), *v)* carboxy-acids (e.g. formic, HCOOH) and phenols (ArOH); *vi)* organic halogenides; *vii)* nitrogen, sulphur and phosphorus compounds; *ix)* heterocyclic and *x)* mixed functionality types (94-96).

Widely varying in concentration, structure and properties, different classifications have been proposed for VOC. One worth of mention distinguishes four groups of substances according to major aftermaths induced onto the environment, and their chemical formulas. They are:

i) gases promoting the Earth warming (greenhouse effect VOC); *ii)* compounds responsible for the stratospheric ozone hole; *iii)* hydrocarbons promoting (or involved in) the tropospheric ozone and secondary particulate generation (photochemical smog); and *iv)* toxic compounds. At this regard, it seems worth to remark that:

- The global Earth warming has been overall associated with carbon dioxide and water vapour. Nevertheless, it is well known that other compounds promote this phenomenon, e.g. methane, nitrogen protoxide, sulphur hexafluoride, and chlorofluorocarbons (freons or CFC) (97-98). The worldwide use of these substances and/or their release into the environment as by-products of human activities caused a strong increase in their respective atmospheric loads, and long, expensive and concerted actions must be launched to control and remediate global warming.
- The "ozone hole" first observed on stratosphere Antarctica has been associated with CFC, which capture the solar light and start a reaction chain with those transforming molecular oxygen (O_2) into ozone (O_3). The variety and intensity of CFC use, combined with their long lifetime, caused their accumulation in the air and transport by winds in remote regions. This phenomenon leads to an increased ultra-violet radiation that reaches the ground, causing in particular an increase in skin tumours. This is the reason why CFC have been banned and replaced by other chemicals characterized by shorter lifetimes; nevertheless, they have lower heat capacities and higher prices, so their true use gains place with difficulty (99-100).
- At ground level, ozone represents a sanitary risk for humans and causes damages to crops and materials, thanks to its strong oxidant potency. Ozone is primarily generated in reactions involving oxygen, nitrogen oxide and dioxide, and "active" sunlight (l <430 nm) (93). The natural ozone background regulated and limited by them. Nevertheless, this equilibrium is modified by VOC that trigger processes leading to the formation of ozone without NO_2 consumption. Thus, the ozone concentration can largely increase. A lot of other oxygen-containing compounds are generated in the form of molecules (carbonyls, carboxy-acids, phenols, epoxides) and free radicals (OH, RO, RO_2, HO_2); when oxidized, hydrocarbons easily condensate giving raise to nuclei around which nano-particles are formed. Not all hydrocarbons participate equally to the photochemical smog formation. Methane, short-chain alkanes and benzene are quite non-reactive,

similarly to acids and ketones. By contrast, alkenes and alkylbenzene have high photochemical ozone formation potentials (101). A special role is played by isoprene and terpenes (biogenic hydrocarbons), which are very reactive vs. OH and NO_3 radicals as well as vs. O_3; they can induce high airborne concentrations of O_3 in rural areas (forests, crops). Through nano-particle and oxidant formation, reactive VOC indirectly affect the air quality.

Some VOC have been recognized as toxicants for their acute and/or chronic effects and have been included in the priority list of pollutants (102). For instance, benzene is known as tumour promoter, similarly to many halogen-substituted hydrocarbons (e.g. bromoform, methyl tetrachloride). Numerous VOC are carcinogenic (butadiene, diazomethane), mutagenic (chloroform) or induce cough, skin, eye and noose sensitization, throat irritation (aldehydes, organic halogenides), faint, loss of knowledge (tetrachloroethylene, methanol, xilene), diarrhoea, liver and kidney damages (aniline); some of them are poisonous (ethylene oxide, camphor, methanol, monomethyl mercury), or psycho-active inducing euphoria, depression, headache (methyl bromide, ethanol). Acids, bases and strong solvents are also caustic (trichoroacetic acid; dimethylamine; methanol, acetone, chlorobenzene).

As far as the above mentioned features of VOC have been clarified, dedicated legislations have been issued to preserve the environment and health (103-112). Regulations refer to industrial and car emissions, power and heat production, agriculture, waste management, materials, food, open air, indoor and work places.

4.2. Organic particulate matter

The organic fraction of particulates is well known, yet much remains not understood. Thousands of chemical substances have been identified in airborne and emission particles. They include n-alkanes and non-polar aliphatic or alkylbenzenes, polynuclear aromatic hydrocarbons (PAH) and the corresponding alkyl-, nitro-, amino-, carbonyl-, oxy-, sulphur- and aza-derivatives, halogen- and phosphorus-containing pesticides, phthalate esters, acids and phenols, alcohols and nitriles (113-115). Most of these substances show scarce hydro-affinity, whilst short-chain, mixed functionality acids are water soluble. Finally, polymers and macromolecules, often of biogenic origin, contribute to the bulk (116). Several studies have been carried out to elucidate chemical composition, with two main objectives in mind: to draw an indirect toxicity parameterization of emissions or environment, to solve the biogenic/anthropogenic origin of particulates and evaluate the relative contribution of their sources (117-121). Finally, the detection of reactive compounds and their corresponding by-products has allowed to put in evidence the oxidation capacity of the atmosphere, deriving from both the presence of oxidants (ozone, free radicals, nitrogen oxides) and light (122). This seems particularly important in the case of reactions involving gaseous substances that are converted into particles (secondary pollution), and whenever the degradation products are much more toxic than their parent compounds (116). Despite extensive and prolonged efforts made in this field, the complete characterization of organic fraction of particulates is far from being reached. It has been demonstrated that the organic fraction accounts for 10 to over 80% of total airborne particulates.

This variability widely depends not only on the environmental contour investigated (locality, emission sources impact, orography, meteorology, indoor or outdoor), but also on the approach adopted to measure it. In particular, very different results are found if the sole "organic solvent extractable fraction" is considered, the water soluble fraction is taken in account, or optical automatic methods are adopted. Otherwise, the WSOC of aerosol contains many different compounds that, to date, are poorly characterized. Experimental evidence suggest that these compounds are at least partly responsible for the main oxidizing and toxicant properties of urban and non-urban aerosols (56,116). Unlike elemental carbon, which is generally associated with the presence of humans (the sole exceptions consist of forest spontaneous fire and volcano emissions), the organic fraction of particulates has a twin origin, i.e. biogenic and anthropogenic. That can be easily explained through an example. Linear alkanes globally account for a few percents of organic particulate matter. Their group presents two well distinct composition behaviours. The saw-tooth distribution dominated by long-chain odd homologues ($C_{29}H_{60}$, $C_{31}H_{64}$ or $C_{33}H_{68}$) is typical of biogenic sources (e.g. high vegetation), whilst the mono-modal (or bell shaped) distribution characterizes anthropogenic emission like motor vehicles; in this case, the maximum centred at $C_{19}H_{40} \div C_{25}H_{52}$. With the exception perhaps of macromolecules and acid compounds having many hydrophilic groups (carboxyl-, hydroxyl-, carbonyl-, epoxy-), organic substances are, individually, micro-components of soot accounting for parts per million down to parts per trillion of the particulate mass. Together, they form a mixture often adhering to the surface, while the particle core is made of elemental carbon and inorganic elements. The polarity and size of molecules influence the hydro-affinity of particles, their growth capacity and then their time life in the atmosphere. Most organic compounds are neutral, although exhibiting different polarities; basic species are a few (they include aromatic amines and aza-PAH), whilst a lot are acidic (phenols, carboxy-acids), even when lypophilic. This variety is of environmental concern, since its medium- and long-term toxicity is strictly dependent on the polarity features of these elements. Studies conducted in Italy and abroad have demonstrated that organic fraction is a major contributor to particulate toxicity. In

Table 5.2. UFPs/NPs natural and anthropogenic sources.

	Anthropogenic	
Natural	**Unintentional**	**Intentional (NPs)**
Gas-to-particle conversion	Internal combustion engines	Controlled size and shape, designed for functionality
Forest fires	Power plants	Metals, semiconductors, metal oxides, carbon, polymers
Volcanoes (hot lava)	Incinerators	Nanospheres, -wires, -needles, -tubes, -shells, -rings, -platelets
Viruses	Jet engines	Untreated, coated (nanotechnology applied to many products: cosmetics, medical, fabrics, electronics, optics, displays, etc.)
	Metal fumes (smelting, welding, etc.)	
	Polymer fumes	
	Other fumes	
	Heated surfaces Frying, broiling, grilling	
	Electric motors	

fact, up to 90% of total carcinogenic and/or mutagenic potency of particulates results to be associated to the corresponding organic extracts, although the substrate can act as promoter of synergic effects. Two main aftermaths can be detected, the former acute (cyto-toxicity, causing cell death) and the latter chronic (cell damage, inducing carcinogenicity, mutagenicity, teratogenicity). Both of them generally appear as associated to neutral polar and acidic compounds taken as pools, and a number of very strong toxicants have been identified among them, including nitro-lactones and dioxins (123-124). By contrast, non-polar components are not toxic, although can exalt the potencies of active species. Despite that, the true contribution provided by each primary toxicant to environmental toxicity cannot be evaluated, since the interaction with the matrix is neglected in terms of synergic/ antagonistic actions, and with respect to net exposition of humans. At this regard, two points must be taken in account. First, due to combination of its nature and airborne concentration, to ambient conditions (temperature and humidity), to soot concentration and characteristics, each organic species partitions between gas and particulate (125). Thus, their interaction with human body is variable. Secondly, the total load of organic compounds is distributed among the particle size fractions, with the general tendency to accumulate into the ultra-fine and fine particles (126-127). It is worth remarking that the strongest toxicants (e.g. PAH, chlorinated hydrocarbons, nitro-compounds) accumulate into the ultra-fine and fine particles, while components with less pronounced potencies are more equally distributed. Very polar components, deriving from the oxidation of gaseous emissions, act as condensation nuclei, triggering the formation of nano-particles. These latter are considered, as a consequence of their number and size, the main cause of air toxicity and an emerging "hot issue" for environmental safety preservation. Thus, the association of organic species to them has heavy environmental consequences.

The importance of the organic component of particulates is well depicted by legislation issued to preserve health, and especially the health of workers (103-106, 128-130). In fact, a set of organics is listed by WHO (UNEP) among the most important toxicants; these include a dozen of persistent pesticides, polychlorinated dioxins, furans and biphenyls. On the other hand, PAH are quoted in European Union Directives concerning air quality or integrated pollution prevention and control. The target value of 1 nanogram per cubic metre of air, calculated as yearly average, has been established for benzo(a)pyrene, kept as an index of aerosol carcinogenicity. European and Italian legislations require to measure also other seven relevant carcinogenic PAH. Toxicants like dioxins and furans are also quoted somewhere, however non target or limit values are provided.

In terms of future actions, certainly the role played by organics in particle generation and accumulation, in troposphere properties (e.g. radiance, heat absorption, albedo, water condensation, global warming), human health and environmental preservation has a key importance. Concern would be ascribed also to "new" pollutants like polyfluorinated acids, pesticides and plastic surrogates, psychotropic substances and poly-functionalized species (oxyacids, polycarboxylic acids, which are suspected to affect our world.

4.3. Research in organic air pollutants: the past, the present and the future

Organic substances are at the core of a series of studies conducted by CNR with the aim of clarifying the features and dynamics of the environment, in the frame of international and national programmes. Special attention has been paid to the composition of the atmosphere, the effects induced by anthropogenic activities and by natural events and sources, the kinetics of pollutants in the presence of oxidants and light, the assessment of mobile and stationary emissions and pollution sources, particulate generation and properties, and sanitary relevance of pollution. According to recent developments, some items seem to deserve better analysis: the clean (green) energy generation, the chemistry of radicals, the air/sea, air/ice and air/soil interactions, the influence on meteorology and climate, the strict relationships between chemical composition and toxicity. As concerns this latter, interesting issues have been identified in nanoparticles, water-soluble organics, organo-metallic compounds and psychotropic substances. Finally, the economic, social and legislative aspects cannot be neglected in view of life quality promotion.

5. Particles in the ultrafine (UFP) and nano-size fractions

5.1 Sources and formation of ultrafine particles

Ultrafine particles are the dominant contributors of particle numbers in the PM2.5 fraction. Particles with dimensions < 100 nm are defined as nano-sized and ultrafine particles (NSPs, UFPs), according to their manufactured or environmental / biological origin. In particular, NSPs are called 'ultrafine' particles (UFPs) also by toxicologists, 'Aitken and nucleation mode' particles by atmospheric scientists and 'engineered nanostructured materials' by materials scientists(132). The simplest examples of naturally occurring ultrafine particles are those found in the biological tissues of organisms. For example, biogenic magnetite is a natural NSP found in many animal species. Other nanosized materials, including fullerenes, are naturally originated from combustion processes such as forest fires and volcanoes. Sources of ambient UFPs are either primary or secondary. In urban environments, the dominant contributors of primary UFPs are combustion products found in motor vehicle exhausts, which are usually black carbonaceous soots with particle dimensions 200–300 nm. Atmospheric oxidation of gas-phase primary exhaust species can produce lower vapor pressure compounds that readily condense onto existing particles and produce secondary mass. Particle composition and size can also evolve due to interaction/reaction of gas- and liquid- or solid-phase species at the particle surface or in the bulk solution, as well as through coagulation of existing particles. In some conditions, photochemical oxidation of gas-phase species can directly produce new UFP (133). Most atmospheric UFPs are usually <50 nm and evidence has been found that the particle count distribution peaks at 20–30 nm at roadsides with heavy traffic[134]. Diesel-exhaust particles (DEP) are major components of $PM_{2.5}$. Although installing a diesel particle filter (DPF) can reduce the number of larger particles in the exhaust, nanoparticles are produced during DFP regeneration. Environmental or atmospheric UFPs contain semivolatile alkanes that originate from fuels and lubricants (135). In particular, primary exhaust emissions of particles consist

mainly in a mixture of elemental carbon and organic compounds, and traces of heavy metals and sulphur. Tire wear also contains carbonaceous materials, while brake wear is rich in heavy metals[136]. Moreover, particle hygroscopic growth measurement techniques have been used to show that ultrafine particles may show two separate modes of ‘‘less’’ and ‘‘more’’ hygroscopic particles (137). The less hygroscopic particles are those that exhibit little or no growth when exposed to a high relative humidity (RH) (typically 80–90%) and are thought to be mainly composed of hydrophobic chemicals such as water-insoluble organic compounds and soot, and must be attributed to primary emissions from traffic and other combustion sources. More hygroscopic particles grow by a larger factor (e.g, 1.38–1.69 for 35–265 nm particles at 90% RH) and have been shown to contain inorganic chemicals such as nitrate, sulphate, sodium and potassium and sometimes organic carbon as well. The more hygroscopic particles can result from the conversion of gaseous compounds into particles, or from the modification and oxidation of pre-existing particles. Such chemical and physico-chemical processes, named “secondary”, may occur even far from the emission sources, and may influence the concentrations of ultrafine and fine particles outside urban areas. It has been shown that the nucleation of ultrafine particles from condensation of reactive gases is responsible for the increase of ultrafine particle number concentrations in many rural areas, including polluted and natural sites. The hygroscopic behaviour of the aerosol is an important quality, as this will determine how they interact with clouds (138), which, in turn, affects the lifetime of the particles in the atmosphere. Conversely, components of manufactured or engineered nanoparticles vary, depending on the type of product. In table 5.1 (136) main differences between environmental and manufactured UFPs are reported.

Table 5.1. Differences between environmental and manufactured nanoparticles

	Environmental nanoparticles	Manufactured nanoparticles
Size	≤50 nm (≤100 nm: ultrafine)	1–100 nm (biomedical nanoparticles can be larger than 100 nm)
Dimensions	Two or three dimensions are on the nanoscale	Three dimensions are on the nanoscale (nano-objects with one and two nanoscale dimensions are called nanoplates and nanofibers, respectively)
Name	Nanoparticles, fibrous nanoparticles	Nano- : particles, spheres, tubes, rods, fibers, wires, ropes, sheets, eggs, liposomes, dendrimers, etc.
Components	Carbon soot, hydrocarbons (alkanes), heavy metals, sulfur	Carbon (fullerene, nanotube), metal oxide (TiO2, ZnO), CdSe, metalloids, transition metals, polymers

More recently, even smaller particles in the nucleation mode with peak diameters around 4 nm have been observed. Humans have been exposed to airborne NSPs throughout all their evolutionary stages. Nevertheless, such exposure has increased dramatically over the last century due to anthropogenic sources. These can be classified as unintentionally or intentionally produced, depending on weather UFPs represent a sub-product or the major product coming from an anthropogenic source. Although the mass of UFPs in ambient air is very low, approaching only 0.5–2.0 μg/m^3 at background levels, it can increase several-fold during high pollution episodes or on highway. In urban areas motor vehicle particle emissions are a dominant pollution source, where more than 80% of particle number concentrations are found in the

ultrafine size range. However, very little information can be obtained about particle number from particle mass measurements, and as current air quality standards are mass and not particle number-based, this means that the greater proportion of motor vehicle particle number emissions are not controlled or regulated (141). The latter case is represented by nanotechnologies. Main natural, unintentional and intentional sources of NSPs are reported in table 5.2 (141).

5.2 Role of UFPs and nano-particles as atmospheric toxicants

Inhalation of particulate matter leads to pulmonary inflammation and reduction in lung function (142) with secondary systemic effects or, after translocation from the lung into the circulation, to direct toxic effects on cardiovascular function (143) and on the coagulation pathway thus contributing to the onset of coronary events (144). Through the induction of cellular oxidative stress and proinflammatory pathways (144), particulate matter augments the development and progression of atherosclerosis (145).

The main factor of these adverse health effects seems to be combustion-derived nanoparticles that incorporate reactive organic and transition metal components. An important source of these particles is new diesel cars with oxidizing converters, such as modern taxis in North Europe. Many epidemiological, human clinical, and animal studies showed that ultrafine particles (UFPs) penetrate deeply into the lungs initiating an inflammatory response leading to respiratory diseases and may be absorbed directly into the circulating blood, causing cardiovascular diseases[146]. Recent studies highlighted the importance of identification of susceptible sub-populations and mechanisms of involved effects. Several chronic clinical conditions are good candidates to define the susceptible population to the acute effects of UFP, while elevated levels of oxidatively altered biomolecules are important intermediate endpoints that may be useful markers in hazard characterization of particulates. Overall, despite the increasing amount of data provided by both laboratory and field studies, the nature of the fraction of aerosol particles responsible for health effects is still a matter of debate. The issue is of importance, because the different constituents of the aerosol exhibit distinct sources and emission/formation processes (147-148). Therefore, linking toxicological and epidemiological impacts of atmospheric particulate matter to chemical composition is a key for the evaluation of effective pollution abatement strategies (149-150).

The potential role of UFPs as strong toxicant species of ambient air derives also from the following considerations (151):

1. smaller particles have a greater total surface area per unit of mass than larger particles; thus, for a given mass, smaller particles may present a larger surface area for interacting with airway tissue or for transporting toxic material associated with the particle surface into the airways.
2. In vitro studies suggest that ultrafine particles may not be as effectively phagocytosed (ie, ingested for removal) as larger particles by cells of the innate immune response.
3. On the basis of size, models predict that a higher proportion of ultrafine particles of ~ 20 nm than of larger particles reach the air-exchanging alveolar region of the lung. On the basis of mass, however, more larger particles than smaller particles reach this lung region.
4. When particles have been instilled intra-

tracheally into animals, on the basis of mass, ultrafine particles were more effective than fine particles in inducing airway inflammatory responses.

5. In some recent studies ultrafine particles appeared to move rapidly out of the airways and into the circulation.

Similarly to gases, when inhaled, specific sizes of UFPs are efficiently carried by diffusional mechanisms in all regions of the respiratory tract. The greater surface area per mass compared with larger-sized particles of the same chemistry renders NSPs more active biologically. This activity includes a potential for inflammatory and pro-oxidant, but also antioxidant, activity, which can explain early findings showing mixed results in terms of toxicity of NSPs to environmentally relevant species (150). It has not been well investigated whether nanoparticles are responsible for pulmonary and extrapulmonary health effects of $PM_{2.5}$, although the fine particles are reportedly associated with mortality from cardiovascular diseases (149). Nanoparticles can permeate through tissue walls, translocate to other tissues from the deposition sites, and cause cardiovascular dysfunction. However, we do not have a clear answer as to how far nanoparticles have a more distinctive toxicological aspect and are more toxic than larger particles (151).

The extraordinarily high number concentrations of NSPs per given mass will likely be of toxicant significance when these particles interact with cells and sub-cell components. Likewise, their increased surface area per unit mass can be of key importance. The small size facilitates uptake into cells and transcytosis across epithelial and endothelial cells into the blood and lymph circulation, to reach potentially sensitive target sites such as bone marrow, lymph nodes, spleen, and heart. Access to the central nervous system and ganglia via translocation along axons and dendrites of neurons has also been observed. NSPs penetrating the skin distribute via uptake into lymphatic channels. Endocytosis and biokinetics are largely dependent on NSP surface chemistry (coating) and in vivo surface modifications (151).

The importance of surface area becomes evident when considering that surface atoms or molecules play a dominant role in determining bulk properties; the ratio of surface to total atoms or molecules increases exponentially with decreasing particle size, as reported in table 4.3 (141).

Table 5.3. Particle number and particle surface area per 10 μg/m³ airborne particles.

Particle diameter (μm)	Particle no. (cm^{-3})	Particle surface area ($\mu m^2/cm^3$)
5	153,000,000	12,000
20	2,400,000	3,016
250	1,200	240
5,000	0.15	12

There are many debates about the dose-metric which best describes the toxicity of manufactured nanoparticles. The most commonly accepted dose metric is probably the surface area. Particle shape (e.g. fibrous or spherical), chemical composition, and the chemistry of the particle surface, including the zeta-potential, are also important factors that determine the toxicity of nanoparticles. It has been reported that the carcinogenic potency and toxicity of asbestos (151) largely depend on fiber length. Fibrous titanium dioxide particles have been shown to be much more cytotoxic than spherical nanosize titanium dioxide particles to alveolar macrophages (149). Special attention should be paid to

fibrous nanoparticles, because fiber length may be predominant metric determining the toxicity of biopersistent fibrous nanoparticles

5.3 Innovative techniques of chemical–physical characterization of the UFP fraction

A major limitation of traditional impaction and membrane technologies is that the detection limits of the laboratory analysis instrumentation necessitate the collection of a large enough mass of sample for analysis, so temporal resolution is limited to greater than several hours for ambient samples. Also, the sample may be affected by evaporation or condensation of semi-volatile components during or after sample collection and chemical reactions may take place within the sample itself or between oxidants in the sample gas and the collected particles, affecting the results (148). The size resolution of these instruments is generally poor to moderate because of the limitations of aerodynamic particle separation and the need to increase sampling times and analyze a larger number of samples with increasing size resolution. Nevertheless, examples exist of nano-size impactors (e.g. NanoMoudi by MSP Corporation, USA) and thermophoretic precipitators (152) which allow single particle collection aimed at analysis of UFPs chemical composition, i.e. by scanning transmission electron microscopy (STEM).

As a matter of fact, anyway, the size distribution of ultrafine particles is expected to evolve rapidly in urban air and such knowledge is essential to the evaluation of human exposure. Differential mobility analyzers (DMA) and other particle evolution / growth measurement techniques have been used since recent years to evaluate the behaviour of airborne UFPs in the ambient air, especially at urban sites. In example (153), combined the use of the scanning mobility particle spectrometer (SMPS), electrical low pressure impactor (ELPI) and TEM techniques to characterize the evolution of the particle size, morphology and composition distribution during dispersion of traffic – related emissions at Birmingham (UK). The ELPI (Dekati, Finland) measures real-time particle concentration and size distribution and the SMPS (TSI, USA) measures particle size distributions with a high resolution. Combined use of these two instruments enhances the quality of particle size distribution measurements. Measurement size range of the ELPI is 30-10000 nm, 50% cut size and the SMPS is 9.6-352 nm. The analytical problem posed by the wide range of atmospheric PM sizes is dynamic range. A 1 nm diameter particle might have a mass of 10-21 g (zeptograms), whereas a 10 mm particle a mass of 10 ng. Reliable sampling and accurate chemical composition determination of a single nanogram particle is a tough analytical challenge; for a zeptogram particle, it is nearly impossible. In addition to PM size, composition and mass loading, many other physical and chemical properties are of interest, and should be preferably measured simultaneously. Research on climate changes, i.e, needs to correlate size and chemical composition with a particle's ability to scatter and absorb radiation from the infrared to the near ultraviolet; health scientists hypothesize that a particle's surface area and surface composition may be a key to understanding how it interacts with lung tissue to affect pulmonary functions and transfer chemicals into the blood stream (152); finally, the physical phase (liquid or solid), surface area, and surface composition can strongly affect the interaction of atmospheric trace gases with airborne particles, impacting the chemical composition of both the gaseous

and condensed phase components of the atmosphere.

Real-time instruments that measure physical properties such as particle number densities, mass loadings, and particle mobility or aerodynamic size distributions have been available since recent years. However, real-time instruments that characterize the chemical composition of atmospheric PM, ideally as a function of particle size, are a more recent development (153). Some near real-time PM chemical composition instruments, operating with measurement cycles of 10–60 min, can characterize the average PM content of one or more key PM constituents for an ensemble of particles in a size range defined by the sample collection system. Examples include the particle into-liquid sampler (PILS) that utilizes automated ion chromatography to quantify average major anion or cation PM content or an automated carbon analyzer to determine the water-soluble organic carbon (OC), instruments based on particle collection followed by thermal decomposition and gas phase chemiluminescence or absorption spectroscopy allow for semi-continuous measurements of sulfate and nitrate (153 and references therein). However, the universal nature of mass spectrometric detection for atomic and molecular species makes this technique eligible as most comprehensive and sensitive to characterize the chemical content of atmospheric PM. Over the past decade, several research groups have made major strides in adapting mass spectrometric techniques to meet this challenge and three major directions of evolution of mass detection techniques can be currently identified. One major theme involves the use of lasers to both vaporize and ionize individual atmospheric particles sampled into a mass spectrometer's source region. This class of instruments focuses on single particle measurements. A second class of aerosol mass instruments uses thermal vaporization of individual or collected particles followed by various ionization techniques. Separation of the vaporization and ionization steps enables quantitative detection of PM chemical composition and mass loading. In addition, the simplicity of thermal vaporization allows the use of a variety of ionization techniques that will produce less sample fragmentation than traditional electron impact (EI) methods, such as chemical ionization techniques(154). However, the most widely used technique is thermal vaporization aerosol mass spectrometer (AMS), which was designed and developed at Aerodyne Research, Inc. (ARI). The initial version of the ARI AMS was designed to measure the real-time non-refractory (NR) chemical speciation and mass loading of fine aerosol particles with aerodynamic diameters between <50 and 1000 nm as a function of particle size155. The original ARI AMS utilizes a quadrupole mass spectrometer (Q) with EI ionization and produces ensemble average data of particle properties. Later versions employ time-of-flight (ToF) mass spectrometers and can produce complete mass spectral data for single particles (153).

6. Future perspectives and developments in the framework of the "pilot study for the assesment of health effects of the chemical composition of ultrafine and fine particles in Italy" project

Despite the increasing amount of data provided by both laboratory and field studies, the nature and role of aerosol particles responsible for health effects, as well as of gaseous mixtures especially in urban areas, is still a matter of debate. In the former case the issue is of importance also because the different constituents of

the aerosol exhibit distinct sources and emission/formation processes. Therefore, linking toxicological and epidemiological impacts of atmospheric particulate matter to chemical composition is a key for the evaluation of effective pollution abatement strategies.

Results of size-segregated aerosol chemical analyses for Italian stations have already been published during the last ten years and are available in the peer-reviewed literature and in project reports (40). These data generally refer to sparse measurements employing multi-stage impactors in both urban (e.g, Bologna, Catania, Rome) and rural/background sites (e.g, Monte Cimone); information on the inorganic and organic composition of ultrafine to coarse particles have been retrieved by chemical determinations of size-segregated fractions.

Nevertheless, an increasing series of data on the aerosol chemical composition and size-distribution has been provided by short-term intensive field studies performed in the frame of national and European research projects(46). During these experiments, state-of-the-art instrumentation has been deployed for aerosol characterization.

In this direction, the "Pilot study for the assessment of health effects of the chemical composition of ultrafine and fine particles in Italy" project, recently approved by CNR, will combine the results of two advanced activities in the field of atmospheric ultrafine particles composition and their toxicological properties, carried out by CNR-ISAC and CNR-IIA, with two new advanced health studies carried out by CNR-IFC and CNR-IBIM, aimed at exploring short-term effects due to air pollutants exposure in subjects with pre-existent arrhythmia and lung diseases.

As far as CNR-ISAC and CNR-IIA are concerned, in the project a collection of all these data will be performed and a data-base of size-resolved chemical compositions of the aerosol classified according to site characterization and sampling period will be provided as outcome. The data-bases emerging from the above activities will be further integrated and the results evaluated to derive conclusions on the available knowledge on the size-segregated chemical composition of the aerosol in the different environments explored. In this view, a further step forward will be also to identify systematic behaviours in the contributions of inorganic and organic chemical constituents as a function of particles size, and depending on site classification (urban, sub-urban, rural, marine, high-altitude, etc.), as well as to provide a summary of the constituents of ultrafine particles. This will help interpreting clinical and epidemiological observations under an enhanced awareness of the behaviour of particulate pollutants in different environments. Finally, a comparison with published results of analogous measurements performed in other European countries will be carried out, to identify singularities to be further investigated in the future. New directions of research in the field of understanding impact routes of ambient and indoor air on health relate to selected species showing a precise toxicant action and to their size-segregated behaviour in aerosols. In this view, research activities will be run concerning either the characterization or the toxicant and reactive behaviour of the water-soluble organic fraction of PM. Besides transition metals and PAHs, on which some peer-reviewed literature already exist, the chemical analysis of fine particulate samples has shown that even in urban areas the water-soluble fraction of the aerosol contains large amounts of

poorly-characterized organic compounds (WSOC, "water-soluble organic carbon"), in contrast to the paradigm of many toxicological studies which attributes the organic-soluble and water-soluble fractions of the aerosol to organic and inorganic compounds, respectively. On the contrary, recent findings point to WSOC as a major agent for aerosol toxicity and oxidizing properties (56,116). Although a number of bioassays have been adopted in previous studies to provide fast and sensitive measurement of the aerosol chemical reactivity, mechanistic pathways for toxicity were not established. Relevant bioassays will be thus tested, for the scopes of evaluating the oxidative potential of airborne aerosol in humans and animals. Among possible bioassays, those will be selected which are sensitive to reactive oxygen species, like superoxide and hydrogen peroxide. The latter species, indeed, can be produced in biological liquids and tissues by organic compounds via redox reactions. Such tests include for instance those based on dithiothreitol (DTT) consumption rate, or employing dichlorofluorescin (DCFH) (56,156). The key importance of testing these methods to provide, i.e, an optimal application to the analysis of the water-soluble organic extracts of ambient aerosol samples is a matter of evidence. Finally, a state-of-the-art analytical technique like the Aerosol Mass Spectrometry (AMS) will be employed for the quantitative mass determination of UFPs. This is at date an obligate step towards enhancing the knowledge about responsibilities of the atmospheric pollution on health impacts. Indeed, the AMS is currently the only technique providing unique information on the short-term processes controlling the concentration and composition of ultrafine particles and their interaction with larger particles. Moreover, the data analysis of the emerging results from AMS will allow comparison with the more consolidated outcomes from available measurements by multi-stage impaction methods.

In summary, by examining the priorities for the evaluation of upcoming research activities of CNR for linking atmospheric aerosols composition and properties to their health effects, at least two specific key issues can already be addressed and dedicated to a) ultrafine particles and b) WSOC.

Keywords: PM, UFP, WSOC, AMS, oxidizing potential.

References

1. Decision No. 1600/2002/EC of the European Parliament and of the Council of 22 July 2002 laying down the Sixth Community Environment Action Programme. Official Journal of the European Communities, 2002;242:1–15.
2. The Clean Air for Europe (CAFE) programme: towards a thematic strategy for air quality. Brussels, European Commission, 2001 (COM(2001)245).
3. WHO Regional Office for Europe. Health aspects of air pollution with particulate matter, ozone and nitrogen dioxide: report on a WHO working group, 2003 Jan 13-15; Bonn, Germany. WHO Regional Office for Europe Available from: URL: http://www.euro.who.int/document/e79097.pdf
4. Pope CA. Lung cancer, cardiopulmonary mortality, and long-term exposure to fine particulate air pollution. J Am Med Assoc 2002;287:1132–1141.
5. Burden of Disease project. 2004 May 20.
6. Available from: URL: http://www3.who.int/whosis/menucfm?path=evidence,burden
7. Amann M, Cofala J, Heyes C, Klimont Z, Schöpp W. The RAINS model: a tool for assessing regional emission control strategies in Europe. Pollut Atmos 1999

December.
8. Amann M, Johansson M, Lükewille A, Schöpp W, ApSimon H, Warren R, et al. An integrated assessment model for fine particulate matter in Europe. Water Air Soil Pollut 2001;130:223–228.
9. Amann M, Cofala J, Heyes C, Klimont Z, Mechler R, Posch M, et al. RAINS REVIEW 2004. The RAINS Model. Documentation of the Model Approach Prepared for the RAINS Peer Review 2004; IIASA, Laxenburg, Austria.
10. Amman M, Berttok I, Cofala J, Gyarfas F, Heyes C, Klimont Z, et al. Baseline Scenarios for the Clean Air for Europe (CAFÉ) Programme; International Istitute for Applied Systems Analysis: 2005; Laxemburg, Austria.
11. Vialetto G, Contaldi M, De Lauretis R, Lelli M, Mazzotta V, Pignatelli T. Emission scenarios of air pollutants in Italy using integrated assessment models. Pollut Atmos 2005;185,71–78.
12. D'Elia I, Bencardino M, Ciancarella L, Contaldi M, Vialetto G. Technical and Non Technical Measures for Air Pollution Emission Reduction: the Integrated Assessment of the Regional Air Quality Management Plans through the Italian National Model. Atmos Environ 2009;43:6182-6189.
13. Pignatelli T, Bencardino M, Ciancarella L, D'Elia I, Racalbuto S, Vialetto G et al. Comparative and qualitative analysis of impact scenarios developed by RAINS_Europe and RAINS_Italy, in the perspective of downscaling. In: Anderssen RS, Braddock RD, Newham LTH, editors. MODSIM09. Proceedings of the 18th World IMACS Congress and International Congress on Modelling and Simulation. 2009 Jul 13-17; Cairns, Australia. 2321-7.
14. Burke JM, Zufall MJ, Özkaynak H. A population exposure model for particulate matter: case study results for PM2.5 in Philadelphia, PA. J Exposure Anal Environ Epidemiol 2001;11:470-489.
15. Position Paper on Ambient Air Pollution: Carbon Monoxide. European Commission, DG Environment, 1999.
16. Raub JA, Mathieu-Nolf M, Hampson NB, Thom SR. Carbon monoxide poisoning — a public health perspective. Toxicology , 2000, 145, 12000, 1 – 14.
17. Peters A, Dockery DW, et al. Particulate Air Pollution and Nonfatal Cardiac Events. Health Effects Institute (HEI) Report, 2005, Vol. 124.
18. Sarnat HB. CNS malformations: Gene locations of known human mutations. European Journal of Paediatric Neurology, 2005, 9, 6, 427-431.
19. Position Paper on Ambient Air Pollution: Carbon Monoxide. European Commission, DG Environment, 1997.
20. EPA (United States Environmental Protection Agency). Integrated Science Assessment for Oxides of Nitrogen — Health Criteria. Annexes. Report. 2008, EPA/600/R-08/072.
21. Position Paper on Ozone. Ad-Hoc Working Group on Ozone Directive and Reduction Strategy Development of the European Commission, DG Environment, 1999.
22. HEI Air Toxics Review Panel. Mobile-Source Air Toxics: A Critical Review of the Literature on Exposure and Health Effects. Health Effects Institute (HEI) Report, 2008, Vol. 16.
23. Zielinska B. Atmospheric transformation of diesel emissions. Experimental and Toxicologic Pathology, 2005, 57, 1, 31-42.
24. Carslaw N. A new detailed chemical model for indoor air pollution. Atmos. Environ. 2007, 41, 6, 1164-1179.
25. Martuzzi M, Mitis F, Iavarone I et al. Health impact of PM10 and Ozone in 13 italian cities. WHO (World Health Organization Regional Office for Europe), ISBN 92 890 2293 0 WHOLIS number E88700.
26. Council Directive 96/62/EC of 27 September 1996 on ambient air quality assessment and management. Official Journal L 296 , 21/11/1996 P. 0055 – 0063.
27. UNECE Convention on Long-range Transboundary Air Pollution (LRTAP).

Website: http://www.unece.org/env/lrtap/.
28. World Health Organization (WHO). Health aspects of air pollution. Report E83080, 2004.
29. Forastiere F, Stafoggia M, Picciotto S, Bellander T, D'Ippoliti D, Lanki T, von Klot S, Nyberg F, Paatero P, Peters A, Pekkanen J, Sunyer J, Perucci CA. A case-crossover analysis of out-of-hospital coronary deaths and air pollution in Rome, Italy. Am J Respir Crit Care Med 2005; 172; 1549-1555.
30. Von Klot S, Peters A, Aalto P et al. Health Effects of Particles on Susceptible Subpopulations (HEAPSS) Study Group. Ambient air pollution is associated with increased risk of hospital cardiac readmissions of myocardial infarction survivors in five European cities. Circulation, 2005; 112; 3073-3079.
31. Laden F, Neas, L.M, Dockery, D.W, Schwartz J. Association of fine particulate matter from different sources with daily mortality in six US cities. Envir. Health. Persp, 2000; 108; 941-947.
32. McCreanor J, Cullinan P, Nieuwenhuijsen M.J et al. Respiratory effects of exposure to diesel traffic in persons with asthma. N. Engl. J. Med, 2007; 357; 2348-2358.
33. Lippmann M, Frampton M, Schwartz J et al. The U.S. Environmental Protection Agency Particulate Matter Health Effects Research Centers Program: A Midcourse Report of Status, Progress, and Plans. Envir. Health. Persp. 2003; 111; 1074-1092
34. Russell A.G and Brunekreef B. A focus on particulate matter and health. Environ. Sci. Technol. 2009; 43; 4620 – 4625.
35. Samet J.M, Dominici F, Curriero F.C, Coursac I, Zeger S.L. Fine particulate air pollution and mortality in 20 US cities, 1987-1994. New Eng. J. Medicine, 2000; 343; 1742-1799.
36. Astolfi M.L, Canepari S, Catrambone M, Perrino C and Pietrodangelo A. Improved characterisation of inorganic components in airborne particulate matter. Environ. Chem. Letters, 2006; 3; 186-191.
37. Baltensperger U, Dommen J, Alfarra M.R et al. Combined determination of the chemical composition and of health effects of secondary organic aerosols: The POLYSOA project. Journal of Aerosol Medicine and Pulmonary Drug Delivery, 2008; 21; 145 – 154.
38. Canepari, S, Perrino, C, Olivieri, F, Astolfi, M. L. Characterisation of the traffic sources of PM through size-segregated sampling, sequential leaching and ICP analysis. Atmos Environ, 2008; 42; 8161-8175.
39. Chan, Y.C, Simpson, R.W, McTainsh, G.H. and Vowles, P.D. Characterisation of chemical species in PM2.5 and PM10 aerosols in Brisbane, Australia. Atmospheric Environment 1997; *31; 3773-3785.*
40. Fabiani R, De Bartolomeo A, Rosignoli P, Morozzi G, Cecinato A, Balducci C. Chemical and toxicological characterization of airborne total suspended particulate and PM10 organic extracts. Polycyclic Aromatic Compounds, 2008; 28; 486-499.
41. Matta, E, Facchini M.C, Decesari S, Mircea M, Cavalli F, Fuzzi S, Putaud J-.P,. Dell'Acqua A. Mass closure on the chemical species in size-segregated atmospheric aerosol collected in a urban area of the Po Valley, Italy. Atmos. Chem. Phys. 2003; 3; 623 - 637.
42. Perrino C, Canepari S, Cardarelli E, Catrambone M, Sargolini, T. Inorganic costituents of urban air pollution in the Lazio region (Central Italy). Environ. Monit. Assess. 2007; 128; 133-151.
43. Perrino C, Canepari S, Catrambone M, Dalla Torre S, Rantica E, Sargolini T. Influence of natural events on the concentration and composition of atmospheric particulate matter. Atmospheric Environment 2009; 43; 4766–4779.
44. Putaud, J.P, Raes F, Van Dingenen R. et al. A European aerosol phenomenology-2 : chemical characteristics of particulate matter at kerbside, urban, rural and background sites in Europe. Atmospheric

Environment, 2004; *38; 2579-2595.*
45. Querol, X, Alasturey A, Ruiz C.R, et al. Speciation and origin of PM10 and PM2.5 in selected European cities. Atmospheric Environment. 2004;*38; 6547-6555.*
46. Vecchi R, Marcazzan G, Valli G. A study on nighttime–daytime PM10 concentration and elemental composition in relation to atmospheric dispersion in the urban area of Milan (Italy). Atmospheric Environment. 2007; 41; 2136–44.
47. Canepari, S, Pietrodangelo A, Perrino C, Astolfi M.L, Marzo M.L. Enhancement of source traceability of atmospheric PM by elemental chemical fractionation. Atmospheric Environment, 2009; 43; 4754–4765.
48. Snyder, D. C, Schauer, J. J, Gross, D. S, Turner, J. R. Estimating the Contribution of Point Sources to Atmospheric Metals using Single-Particle Mass Spectrometry. Atmospheric Environment, 2009; 43; 4033-4042.
49. Viana M,. Kuhlbusch T.A.J, Querol X, et al. Source apportionment of particulate matter in Europe: A review of methods and results. Aerosol Science 2008; 39; 827–849.
50. Branis M, Safranek J, Hytychova A. Exposure of children to airborne particulate matter of different size fractions during indoor physical education at school. Building and Environment, 2009; 44; 1246–1252.
51. Chan A.T, Chung M.W. Indoor–outdoor air quality relationships in vehicle: effect of driving environment and ventilation modes. Atmospheric Environment, 2003; 37; 3795-3808.
52. Chao C.Y.H, Tung T.C. An empirical model for outdoor contaminant transmission into residential buildings and experimental verification. Atmospheric Environment, 2001; 35; 1585-1596.
53. Poupard O, Blondeau P, Iordache V, Allard F. Statistical analysis of parameters influencing the relationship between outdoor and indoor air quality in schools. Atmospheric Environment, 2005; 39; 2071-2080.
54. Rudel R.A, Perovich L.J. Endocrine disrupting chemicals in indoor and outdoor air. Atmospheric Environment, 2009; 43; 170-181.
55. Saliba N.A, Atallah M, Al-Kadamany G. Levels and indoor–outdoor relationships of PM10 and soluble inorganic ions in Beirut, Lebanon. Atmospheric Research, 2009; 92; 131-137.
56. Tippayawong N, Khuntong P, CNitatwichit C, Khunatorn Y, Tantakitt C. Indoor/outdoor relationships of size-resolved particle concentrations in naturally ventilated school environments. Building and Environment, 2009; 44; 188–197.
57. Biswas S, Verma V, Schauer J, Cassee F, Cho A, and Sioutas C. Oxidative potential of semi-volatile and non volatile particulate matter (PM) from heavy-duty vehicles retrofitted with emission control technologies. Environ. Sci. Technol, 2009; 43; 3905 – 3912.
58. Binkovà B, Vesely D, Veselà D, Jelinek R, Sram R.J. Genotoxicity and embryotoxicity of urban air particulate matter collected during winter and summer period in two different districts of the Czech Republic. Mutation Research Genetic Toxicology and Environmental Mutagenesis 1999; 440; 45-58.
59. Calderón-Garcidueñas L, Solt A.C, Henríquez-Roldán C, Torres-Jardón R, Nuse B, Herritt L, Villarreal-Calderón R, Osnaya N, Stone I, García R, Brooks D.M, González-Maciel A, Reynoso-Robles R, Delgado-Chávez R, Reed W. Long-term air pollution exposure is associated with neuroinflammation, an altered innate immune response, disruption of the blood-brain barrier, ultrafine particulate deposition, and accumulation of amyloid beta-42 and alpha-synuclein in children and young adults. Toxicol Pathol, 2008; 36; 289-310.
60. Geller M.D, Ntziachristos L, Mamakos A, Samaras Z, Schmitz D. A. , Froines J.R, Sioutas C,. Physicochemical and redox characteristics of particulate matter (PM) emitted from gasoline and diesel passenger cars. Atmospheric Environment

2006; 40; 6988-7004.
61. Risom L, Møller P, Loft S. Oxidative stress-induced DNA damage by particulate air pollution. Mutation Research, 2005; 59; 119–137.
62. Rückerl R, Ibald-Mulli A, Koenig W, Schneider A, Woelke G, Cyrys J, Heinrich J, Marder V, Frampton M, Wichmann H.E, Peters A. Air pollution and markers of inflammation and coagulation in patients with coronary heart disease. Am J Respir Crit Care Med, 2006 15; 173; 432-441.
63. Duker A.A, Carranza E.J.M, Hale M.: Arsenic geochemistry and health. Environ Int 2005;31: 631641.
64. Sanchez-Rodas D, Sanchez de la Campa A.M, de la Rosa J, et al. Arsenic speciation of atmospheric particulate matter (PM10) in an industrialised urban site in southwestern Spain. Chemosphere 2007;66:14851493.
65. Thomaidis N.S,. Bakeas E.B, Siskos P.A. Characterization of lead, cadmium, arsenic and nickel in PM2.5 particles in the Athens atmosphere, Greece. Chemosphere 2003;52:959966.
66. Kanias G.D, Viras L.G, Grimanis A.P. Source identification of trace elements emitted into Athens atmosphere, Relation between trace elements and tropospheric ozone. J Radioanal Nucl Chem 2004;260:509518.
67. Güllü G, Dogan G, Tuncel G. Atmospheric trace elements and major ion concentrations over the eastern Mediterranean Sea: Identification of anthropogenic source regions. Atmos Environ 2005;39:63766387.
68. Pacyna J, Pacyna E.G. An assessment of global and regional emissions of trace metals to the atmosphere from anthropogenic sources worldwide. Environ Res 2001;9:269298.
69. Flament P, Bertho M.L, Deboudt K, Véron A, Puskaric E. European isotopic signatures for lead in atmospheric aerosols: a source apportionment based upon $^{206}Pb/^{207}Pb$ ratios. Sci Total Environ 2002;296:3557.
70. Pirrone N, Costa P, Pacyna J.M. Past, current and projected atmospheric emissions of trace elements in the Mediterranean region. Water Sci Technol 1999;39:17.
71. Pirrone N, Allegrini I, Keeler G.J, Nriagu J.O, Rossmann R, Robbins J.A. Historical Atmospheric Mercury Emissions and Depositions in North America Compared to Mercury Accumulations in Sedimentary Records. Atmos Environ 1998;32:929-940.
72. Pirrone N, Costa P, Pacyna J. Past, Current and Projected Emissions of Trace Elements in the Mediterranean Basin. Water Sci Technol 1999;39:1-7.
73. Lindqvist O, Johansson K, Aastrup M, Andersson A, Bringmark L, Hovsenius G, et al. Mercury in the Swedish environment - recent research on causes, consequences and corrective methods. Water Air Soil Pollut 1991;55.
74. Pirrone N, Costa P, Pacyna J.M, Ferrara R. Atmospheric mercury emissions from anthropogenic sources in the Mediterranean Region. Atmos Environ 2001;35:2997-3006.
75. Sprovieri F, Hedgecock I.M, Pirrone N. An Investigation of the Origins of Reactive Gaseous Mercury in the Mediterranean Marine Boundary Layer Atmos Chem Phys Discuss 2009;9:24815-24846.
76. Pacyna E.G, Pacyna J.M, Pirrone N. European emissions of atmospheric mercury from anthropogenic sources in 1995. Atmos Environ 2001;35:2987-2996.
77. Bindler R, Renberg I, Appleby P.G, Anderson N.J, Rose N.L. Mercury accumulation rates and spatial patterns in lake sediments from west Greenland: a coast to ice margin transect. Environ Sci Technol 2001;35:736–741.
78. Biester H, Kilian R, Franzen C, Woda C, Mangini A, Schöler H.F. Elevated mercury accumulation in a peat bog of the Magellanic Moorlands, Chile (538S)—An anthropogenic signal from the Southern Hemisphere. Earth Planet Sci Lett 2002;201:609–620.
79. Lamborg C.H, Fitzgerald W.F, Damman A.W.H,. Benoit J.M, Balcom P.H,

Engstrom D.R. Modern and historic atmospheric mercury fluxes in both hemispheres: global and regional mercury cycling implications. Global Biogeochem Cycles 2002;16:104.

80. Lindberg S, Bullock R, Ebinghaus R, et al. A synthesis of progress and uncertainties in attributing the sources of mercury in deposition. Acta Biomate 2007;36(1):19-32.
81. US-EPA (U.S. Environmental Protection Agency). Mercury Report to Congress, Vol VI: Characterization of Human Health and Wildlife Risks from anthropogenic Mercury Emissions in the United States. EPA-452/R-97-001f. Washington. DC. U.S. Environmental Protection Agency, 1997.
82. Schroeder W.H, Munthe J. Atmospheric Mercury – An Overview. Atmos Environ 1998;29:809-822.
83. Sprovieri F, Pirrone N, Gärdfeldt K, Sommar J. Mercury speciation in the marine boundary layer along a 6000 km cruise path around the Mediterranean Sea. Atmos Environ 2003; 37:63-71.
84. Pirrone N, Ferrara R, Hedgecock I.M, et al. Dynamic Processes of Mercury Over the Mediterranean Region: results from the Mediterranean Atmospheric Mercury Cycle System (MAMCS) project. Atmos Environ 2003;37(1):21-39.
85. Bornmann G, Henke G, Alfes H, Mollmann H. Intestinal absorption of metallic mercury (in German) Arch Toxicol 1970;26:203-209.
86. Hursh J.B. Partition coefficients of mercury (203-Hg vapor between air and biological fluids. J Appl Toxicol 1985;5:327-332.
87. Berlin M, Friberg L, Nordberg G.F, Vouk V.B. editors, Mercury In: Handbook on the toxicology of Metals, pp. 387-445, 2nd ed. New York: Elsevier; 1986.
88. WHO. Environmental health criteria 101: Methylmercury. World Health Organisation Geneva, International Programme on Chemical Safety, 1990.
89. WHO. Environmental health criteria 118: Inorganic mercury. World Health Organisation Geneva, International Programme on Chemical Safety 1991.
90. WHO. Joint FAO/WHO expert committee on food additives. Fifty-third meeting, 1999 Jun 1-10, Rome, Italy. Summary and conclusions. Available at. www.who.int/pcs/jecfa/summary53revised.pdf.
91. U.S. EPA (1997) Mercury Report to Congress. U.S. Environmental Protection Agency,
92. Research Triangle Park, NC.
93. Clarkson T.W. The toxicology of mercury. Crit Rev Clin Lab Sci 1997;34:369-403.
94. Aberg B, Ekman L, Falk R, Greitz U, Persson G, Snihs J.O. Metabolism of methylmercury (203 Hg) compounds in man. Arch Environ Health 1969;19:478-484.
95. Finlayson-Pitts B.J, Pitts J.N. Jr. Chemistry of the upper and lower atmosphere. San Diego CA (USA), Academic Press, 2000.
96. Pankow J.F, Luo W, Bender D.A, Isabelle L.M, Hollingsworth J.S, Chen C, Asher W.E, Zogorski J.S. Concentrations and co-occurrence correlations of 88 volatile organic compounds (VOCs) in the ambient air of 13 semi-rural to urban locations in the United States. Atmos. Environ. 2003; 37; 5023-5046.
97. Ciccioli P, Cecinato A, Brancaleoni E, Frattoni M. Identification and quantitative evaluation of C4-C14 volatile organic compounds in some urban, suburban and forest sites in Italy. Fresenius Envir. Bull. 1992; 1; 73-78.
98. Ciccioli P, Brancaleoni E, Cecinato A, Sparapani R. Identification and determination of biogenic and anthropogenic volatile organic compounds in forest areas of Northern and Southern Europe and a remote site of the Himalaya region by high-resolution gas chromatography - mass spectrometry. J. Chromatog. 1993; 643; 55-69.
99. Campbell N.J, McCulloch A. Climate change implications of manufacturing refrigerants: a Calculation of 'production' energy contents of some common refrigerants. Process Safety and Environ. Protect. 1998; 76; 239-244.

100. Highwood E.J, Shine K.P, Hurley M.D, Wallington T.J. Estimation of direct radiative forcing due to non-methane hydrocarbons. Atmos. Environ. 1999; 33; 759-767.
101. Rodriguez J.M. Probing stratospheric ozone. Science 1993; 261; 1128-1129.
102. Rowland F.S. Atmospheric chemistry: causes and effects. J. Mar. Technol. Soc. 1991; 25; 12-18.
103. Derwent R.G, Jenkin M.E, Saunders S.M. Photochemical ozone creation potentials for a large number of reactive hydrocarbons under European conditions. Atmos. Environ. 1996; 30; 181-199.
104. Croute F, Poinsot .J, Gaubin Y, Beau B, Simon V, Murat J.C, Soleilhavoup J.P. Volatile organic compounds cytotoxicity and expression of HSP72, HSP90 and GRP78 stress proteins in cultured human cells. Biochim. Biophys. Acta (BBA)/ Molec. Cell Res. 2002; 1591; 147-155.
105. European Parliament and Council. Directive 96/62/EC of 27 September 1996 on Ambient Air Quality Assessment and Management. Strasbourg, 1996.
106. European Parliament and Council. Directive 2000/69/EC of 16 November 2000 relating to Limit Values for Benzene and Carbon Monoxide in Ambient air. Strasbourg, 2000.
107. European Parliament and Council. Directive 2001/80/EC of 23 October 2001 on Limitation of Emissions of Certain Pollutants into the Air from Large Combustion Plants. Strasbourg, 2001.
108. European Parliament and Council. Directive 2001/81/EC of 23 October 2001 on National Emission Ceilings for Certain Atmospheric Pollutants. Strasbourg, 2001.
109. European Parliament and Council. Directive 2002/3/EC of 12 February 2002 Relating to Ozone in Ambient Air. Strasbourg, 2002.
110. European Parliament and Council. Directive 2002/45/EC of 25 June 2002 amending the Council Directive 76/769/ EEC relating to Restrictions on the Marketing and Use of Certain Dangerous Substances and Preparations (short-chain chlorinated paraffins). Strasbourg, 2002.
111. European Parliament and Council (2003). Directive 2003/87/EC of the of 13 October 2003 establishing a Scheme for Greenhouse Gas Emission Allowance Trading within the Community and amending Council Directive 96/61/EC. Strasbourg, 2003.
112. European Parliament and Council. Commission Decision 2004/297/EC of 19 March 2004 concerning guidance for implementation of Directive 2002/3/ EC of the European Parliament and the Council relating to Ozone in Ambient Air. Strasbourg, 2004.
113. European Parliament and Council. Directive 2004/101/EC of 27 October 2004 amending Directive 2003/87/EC establishing a Scheme for Greenhouse Gas Emission Allowance Trading within the Community, in Respect of the Kyoto Protocol's Project Mechanisms. Strasbourg, 2004.
114. Italian Ministry of the Environment. Ministry Decree of 25 November 1994 relative to Updating of the technical rules concerning the concentration limits and the precautionary and warning levels regarding the atmospheric pollutants in the urban areas and indications for the measure of some pollutants referred in the Ministerial released on April 15th. Gazzetta Ufficiale Italiana della Repubblica Italiana, Suppl. No. 290, 13 December 1994. Rome, 1994.
115. Alves C, Pio C, Duarte A. Composition of extractable organic matter of air particles from rural and urban Portuguese areas. Atmos. Environ. 2001; 35; 5485-5496.
116. Simoneit B.T.R. Organic matter in the troposphere – III – Characterization and sources of petroleum and pyrogenic residues in aerosols over the western United States. Atmos. Environ.1984; 18; 51-67.
117. Simoneit B.R.T, Mazurek M.A. Organic matter of the troposphere-II. Natural background of biogenic lipid matter in aerosols over the rural Western United

States. Atmos. Environ. 1982; 16; 2139-2159.

118. Baltensperger U, Dommen J, Alfarra M.R, et al. Combined determination of the chemical composition and of health effects of secondary organic aerosols: The POLYSOA project. J. Aerosol Med. Pulmonary Drug Deliv. 2008; 21; 145 – 154
119. Binkovà B, Vesely D, Veselà D, Jelinek R, Sram R.J. Genotoxicity and embryotoxicity of urban air particulate matter collected during winter and summer period in two different districts of the Czech Republic. Mutat. Res. Genetic Toxicol. Environ. Mutagen. 1999; 440; 45-58.
120. Fabiani R, De Bartolomeo A, Rosignoli P, Morozzi G, Cecinato A, Balducci C. Chemical and toxicological characterization of airborne total suspended particulate and PM_{10} organic extracts. Polycyc. Aro. Comp. 2008; 28; 486-499.
121. Hannigan M.P, Cass G.R, Penman B.W, et al. Bioassay-directed chemical analysis of Los Angeles airborne particulate matter using a human cell mutagenicity assay. Environ. Sci.Technol. 1998; 32; 3502-3514.
122. Cass G.R. Organic molecular tracers for particulate air pollution sources. Trends in Analyt. Chem. 1998; 17; 356-366.
123. Kavouras I.G, Khalili N.R, Scheff P.A, Holsen T.M. PAH source fingerprints for coke ovens, diesel and gasoline engines, highway tunnels and wood combustion emissions. Atmos. Environ. 1995; 29; 533-542.
124. Kamens R.M, Guo Z, Fulcher J.N, Bell D.A. The influence of humidity, sunlight, and temperature on the daytime decay of polyaromatic hydrocarbons on atmospheric soot particles. Environ. Sci. Technol. 1988; 22; 103–108.
125. International Agency for Research on Cancer IARC. Polynuclear aromatic compounds. Part I. Chemical, environmental and experimental data. Monographs on the evaluation of carcinogenic risk of chemicals to humans, vol. 32. Lyon (F), IARC, 1983.
126. Claxton L.D, Matthews P.P, Warren S.H. The genotoxicity of ambient outdoor air, a review: Salmonella mutagenicity. Mutat. Res. 2004; 567, 347–399.
127. Turpin B. J, Saxena P, Andrews E. Measuring and simulating particulate organics in the atmosphere: problems and prospects. Atmos. Environ. 2000; 34; 2983-3013.
128. Chrysikou L.P, Samara C.A. Seasonal variation of the size distribution of urban particulate matter and associated organic pollutants in the ambient air. Atmos. Environ. 2009; 43; 4557-4569.
129. Tremblay R.T, Riemer D, Zika R.G. Organic composition of $PM_{2.5}$ and size-segregated aerosols and their sources during the 2002 Bay Regional Atmospheric Chemistry Experiment (BRACE), Florida, USA. Atmos. Environ. 2007; 41; 4323-4335.
130. European Parliament and Council. Directive 2004/107/EC of 15 December 2004 relating to Arsenic, Cadmium, Mercury, Nickel and Polycyclic Aromatic Hydrocarbons in Ambient Air. Strasbourg, 2004.
131. European Parliament and Council. Directive 2008/1/EC of 15 January 2008 concerning Integrated Pollution Prevention and Control. Strasbourg, 2008.
132. European Parliament and Council. Directive 2008/50/EC of 21 May 2008 on Ambient Air Quality and Cleaner Air for Europe. Strasbourg, 2008.
133. Italian Ministry of the Environment. Law by Decree No. 152 of 3 August 2007 on "Implementation of the 2004/107/CE Directive concerning arsenic, cadmium, mercury, nickel and polycyclic aromatic hydrocarbons in ambient air". Gazzetta Ufficiale della Repubblica Italiana No. 213, 13 September 2007, Suppl. No. 194. Rome, 2007.
134. NNI (National Nanotechnology Initiative). 2004. What Is Nanotechnology? Available: http://www.nano.gov/html/facts/whatIsNano.html.

135. Kulmala H, Vehkamaki H, Petaja T, Dal Maso M, Lauri A, Kerminen V.-M, Birmili W, McMurry P.H. Formation and growth rates of ultrafine atmospheric particles: a review of observations. J. Aerosol Sci, 2004, 35, 143-176.
136. Fushimi A, Hasegawa S, Takahashi K, Fujitani Y, Tanabe K, Kobayashi S. Atmospheric fate of nuclei-mode particles estimated from the number concentrations and chemical composition of particles measured at roadside and background sites. Atmos Environ. 2008;42:949–59.
137. Hirano S, Nitta H, Moriguchi Y, Kobayashi S, Kondo Y, Tanabe K, et al. Nanoparticles in emissions and atmospheric environment: now and future. J Nanopart Res. 2003;5:311–21.
138. Hirano S. A current overview of health effect research on nanoparticles. Environ Health Prev Med (2009) 14:223–225.
139. Zhou J, Swietlicki E, Hansson H.C, Artaxo P. Aerosol particle size distribution and hygroscopic growth in the Amazonian rain forest. J. Aerosol Sci, 1999, 30, S163-S164.
140. Bower K.N, Beswick K.M, Burgess R.; Stromberg I.M, Gallagher M.W. Aerosol, trace gas and thermal gradient structure of urban conurbations measured by aircraft. J. Aerosol Sci, 2000, 31, 114-115.
141. Ramanathan, R. A note on the use of the analytic hierarchy process for environmental impact assessment. J. Environ. Management, 2001, 63, 27-35.
142. U.S. EPA. 2004. Air Quality Criteria for Particulate Matter. Vol 3. 600/P-95-001cF. Washington DC:U.S. Environmental Protection Agency, Office of Research and Development
143. Oberdörster G, Oberdörster E. and Oberdörster J, 2005. Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. Review. Environ. Health Perspect, 113 (7), 823 – 839.
144. McCreanor J, Cullinan P, Nieuwenhuijsen M.J, Stewart-Evans J, Malliarou E, Jarup L, Harrington R, Svartengren M. Han I.K, Ohman-Strickland P, Chung K.F, Zhang J. Respiratory effects of exposure to diesel traffic in persons with asthma. N Engl J Med; 2007;357:2348-2358.
145. Andersen Z.J, Loft S, Ketzel M, Stage M, Scheike T, Hermansen M.N, Bisgaard H. Ambient air pollution triggers wheezing symptoms in infants. Thorax; 2008; 63:710-716.
146. Rückerl R, Ibald-Mulli A, Koenig W, Schneider A, Woelke G, Cyrys J, Heinrich J, Marder V, Frampton M, Wichmann H.E, Peters A. Air pollution and markers of inflammation and coagulation in patients with coronary heart disease. Am J Respir Crit Care Med 2006; 15;173:432-441.
147. Calderón-Garcidueñas L, Solt A.C, Henríquez-Roldán C, et al. Long-term air pollution exposure is associated with neuroinflammation, an altered innate immune response, disruption of the blood-brain barrier, ultrafine particulate deposition, and accumulation of amyloid beta-42 and alpha-synuclein in children and young adults. Toxicol Pathol, 2008;36:289-310.
148. Forastiere F, Stafoggia M, Picciotto S, et al. A case-crossover analysis of out-of-hospital coronary deaths and air pollution in Rome, Italy. Am J Respir Crit Care Med;172:1549-1555.
149. Viana M,. Kuhlbusch T.A.J, Querol X, Alastuey A, Harrison R.M, Hopke P.K, et al. Source apportionment of particulate matter in Europe: A review of methods and results. Aerosol Science, 2008;39, 827 – 849.
150. Chow J.C, Watson J.G, Kuhns H, Etyemezian V. et al. Source profiles for industrial, mobile, and area sources in the Big Bend Regional Aerosol Visibility and Observational study. Chemosphere, 2004;54 (2), 185 – 208.
151. Morozzi G, Mastrandrea V, Trotta F. et al. Chemical characterization and biological properties of airborne particulate matter. Aerobiologia, 1992, 8, 451-457.
152. Fabiani R, De Bartolomeo A, Rosignoli P, Morozzi G. et al. Chemical and toxicological characterization of airborne total suspended particulate and PM10 organic extracts. Polycyclic Aromatic

Compounds, 2008, 28, 486-499
153. Peters A, Dockery D.W, et al. Particulate Air Pollution and Nonfatal Cardiac Events. Health Effects Institute (HEI) Report, 2005, Vol. 124.
154. Maynard A.D. The development of a new thermophoretic precipitator for scanning transmission electron microscope analysis of ultrafine aerosol particles. Aerosol Sci. Technol, 1995, 23, 521-533.
155. Canagaratna M.R, Jayne J.T, Jimenez J.L, et al. Chemical and microphysical characterization of ambient aerosols with the aerodyne aerosol mass spectrometer. Mass Spectrometry Reviews, 2007, 26, 185– 222.
156. Ferge T, Muhlberger F, Zimmermann R. 2005. Application of infrared laser desorption vacuum-UV single-photon ionization mass spectrometry for analysis of organic compounds from particulate matter filter samples. Anal Chem 77:4528–4538.
157. Jayne J.T, Leard D.C, Zhang X, Davidovits P, Smith K.A, Kolb C.E. Worsnop DR. 2000. Development of an aerosol mass spectrometer for size and composition analysis of submicron particles. Aerosol Sci Technol 33:49–70
158. Venkatachari P, Hopke P.K, Grover B. D. et al, 2005. Measurement of particle-bound reactive oxygen species in Rubidoux aerosols. J. Atmos. Chem. 50, 49 – 58.