UNIVERSITÀ DEGLI STUDI DELL'INSUBRIA

DIPARTIMENTO DI SCIENZA E ALTA TECNOLOGIA

Sede di Como

APPROCCIO MULTI-LIVELLO ALLO STUDIO DI ESPOSIZIONE A NANOPARTICELLE

-

MULTI-LEVEL APPROACH TO THE STUDY OF EXPOSURE TO NANOPARTICLES

A dissertation submitted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy in Environmental Sciences

by

Dott. Andrea Spinazzè

Supervisor: Prof. Domenico M. Cavallo

16 Dicembre 2014

CORSO DI DOTTORATO IN SCIENZE AMBIENTALI

- XXVII CICLO -

ANNO ACCADEMICO 2013/2014

"[…] Credo di poter affermare che nella ricerca scientifica, né il grado di intelligenza né la capacità di eseguire e portare a termine con esattezza il compito intrapreso, siano i fattori essenziali per la riuscita e la soddisfazione personale. Nell'una e nell'altra contano maggiormente la totale dedizione e il chiudere gli occhi davanti alle difficoltà: in tal modo possiamo affrontare problemi che altri, più critici e acuti, non affronterebbero"

Da *Elogio dell'imperfezione*, R.L. Montalcini

"[…] Migliaia, milioni di individui lavorano, producono e risparmiano nonostante tutto quello che noi possiamo inventare per molestarli, incepparli, scoraggiarli. È la vocazione naturale che li spinge; non soltanto la sete di denaro. Il gusto, l'orgoglio di vedere la propria azienda prosperare, acquistare credito, ispirare fiducia a clientele sempre più vaste, ampliare gli impianti, abbellire le sedi, costituisce una molla di progresso altrettanto potente che il guadagno. Se così non fosse, non si spiegherebbe come ci siano imprenditori che nella propria azienda prodigano tutte le loro energie ed investono tutti i loro capitali per ritrarre spesso utili di gran lunga più modesti di quelli che potrebbero sicuramente e comodamente ottenere con altri impieghi."

Da *Lezioni di economia agli Italiani*, L. Einaudi

Dedicated to Silvia,

Alice and my Family

CONTENTS

ABBREVIATIONS

EHS:	Environment, health and safety
LDSA:	Lung Deposited Surface Area [$\mu m^2/cm^3$]
ME:	Microenvironment
Mean-d:	Mean diameter [nm]
MNM:	Manufactured Nanomaterial
NP:	Nanoparticles (Engineered Nanoparticles)
PM:	Particulate Matter
PMC:	Particle Mass Concentration [$\mu g/m^3$]
PNC:	Particle Number Concentration [pt/cm^3]
pt/cm^3:	Number of particle/cm^3
QUFP:	Quasi-Ultrafine Particles ($PM_{0.25}$)
TWA:	Time-weighted average
UFP:	Ultrafine Particles ($PM_{0.1}$)

SUMMARY

Background and aims

Exposure to nanoaerosols (particles with diameter below 100 nm) is an important topic in epidemiological and toxicological studies and is deemed to be a major risk affecting human health, both for general population (exposed to “ultrafine particles” - UFP) and for workers (involved in the production or application of manufactured nanomaterials -MNM- and nanoparticles - NP). In fact, high concentrations of airborne nano-sized particles are associated with increased pulmonary and cardiovascular mortality. Further, recent studies assesse that UFP and NP can reach the deeper region of the respiratory system and overcome the alveolar barrier and enter the bloodstream, contributing to increased risk of cancer, thrombosis, and cardiovascular diseases. An increasing number of studies are indicating that the health risk deriving from exposure to airborne nanoaerosols is not adequately addressed by conventional exposure evaluation methods and strategies capable of measuring exposure against these attributes. In fact, in recent years, society has become increasingly sensitive to individual risks, and thus data on the exposure needs to be personalized.

Therefore, airborne particle studies were performed in the recent years to identify the main UFP and NP sources and to characterize population exposure. In this regard, personal monitoring is considered as the only way to obtain accurate exposure data, which are critical to further reduce exposure misclassification in epidemiological studies. The drawback of such method, however, is the high cost of implementation and the associated small number of observations that tends to produce sample biases. For this reason, personal monitoring is often used as a complement in exposure models to assess air pollution exposures in health studies. These models use personal or household exposure monitoring, and appear well-suited to overcome the problem of achieving population representative samples while understanding the role of exposure variation at the individual level.

The monitoring and characterization techniques discussed in this thesis aims to evaluate nanoaerosols exposure in terms of mass, surface-area and/or number concentration. These methods were developed and used to exposure characterization both in environmental and occupational settings. The goal of this PhD project is firstly to perform an exposure assessment of UFP using innovative techniques and strategies. Secondly, it will be shown that microenvironmental models are effective tool, capable to model exposure to UFP in populations and sub populations. Finally, newly developed strategies and techniques are applied in occupational settings, in order to perform an occupational exposure assessment for workers involved in the production or application of MNM and NP.

Materials and methods

Exposure to airborne pollutants can be determined using indirect exposure models or through a direct approach (i.e. air quality measurements). In this study, UFP concentrations in different urban microenvironments (ME) were firstly measured by personal monitoring in repeated sampling campaigns, along fixed routes in two Italian cities, in order to measure personal exposure in transport MEs. Measurements followed a multi-parametric and multi-metric approach, including on-line monitoring of UFP Particle Number Concentration (PNC), mean diameter (mean d) and lung-deposited surface area (LDSA).

Secondly, average daily UFP exposure of adult Milan subpopulations (defined on the basis of gender and then for age, employment or educational status), in different exposure scenarios (typical working day in summer and winter) were simulated using a microenvironmental stochastic simulation model. The basic concept of this kind of model is that time-weighted average exposure is defined as the sum of partial microenvironmental exposures, which are determined by the product of UFP concentration and time spent in each microenvironment.

Furthermore, this thesis describes the development of an instrumental approach for measurement of NP exposures in occupational settings, which takes into account the major potential route of exposure and factors that may influence biological activity and potential toxicity of nanomaterials and incorporates a risk management approach: different methods were used to measure and assess occupational exposures to engineered nanoparticles (NP) with a multi-parametric approach: the first method involved off-line gravimetric analysis of filter samples collected with Low Pressure Impactor. The second method used different, hand-held, direct-reading instruments to obtain a time series of particle number concentrations (PNC), mean diameter and surface-area concentration for NP.

Results and conclusions

This thesis provides important insights into UFP exposure in urban environments, that should be considered in developing additional and larger studies on population's exposure. First, continuous real-time monitoring provided the information necessary to define the influence of local sources or changes in local circumstances on UFP concentrations. In addition, continuous monitoring allowed for the evaluation of short-term particle concentrations and demonstrated temporal and spatial variability for the studied urban microenvironments.

Further, the simulation model, used to estimate the average daily UFP exposure of adult subpopulations in a major Italian urban area and in different exposure scenarios, have defined that demographic and socio-demographic factors (e.g., gender, age, profession, instruction level), as well as environmental patterns, have to be considered as major determinants of pollutant exposure in urban environments. Furthermore, this research detected UFP levels and average particle sizes and their seasonal variability, as well as comprehensive information on average particle number and mass concentrations, sizes and surface area in various microenvironments within urban areas, which is fundamental to evaluate the variability of human exposure in urban environments and to support the relevance of traffic-related exposure for health.

Thus, findings derived from this study may represent an important tool in the definition of health and social implication of UFP exposure for general population and to provide complete and accurate exposure assessment data for risk assessors, including exposure metrics, mostly relevant as health effects indicators.

Finally, regarding occupational settings, this study defined an experimental protocol, which was intended to be useful in determining potential exposure to engineered nanomaterials and nanoparticles in the workplace with complementary approaches. These information may also be used to determine whether engineering controls are effective in preventing release of the engineered nanomaterials to the workplace atmosphere.

1. FOREWORD

1.1. PROBLEM STATEMENT

Exposure may be defined as the concentration of a particular agent that reaches a target organism, system, or population in a specific frequency for a defined duration. Exposure can also be defined as the contact of a target and a chemical, physical, or biological agent in an environmental carrier medium. More expansively, exposure denotes the contact between an agent and a target, which takes place at a contact boundary or surface over an exposure period. Exposure to ultrafine particles (UFP) and engineered nanoparticles (NP) is an important topic in epidemiological and toxicological studies and is deemed to be a major risk affecting human health. Therefore, airborne particle studies were performed in the recent years to identify the main UFP sources and to characterize population exposure. Exposure can be measured or modeled, either directly (e.g., personal measurements) or indirectly (e.g., microenvironment approach). Aerosol exposure has historically been characterized by the mass concentration of airborne material, usually associated with specific size ranges, corresponding to different deposition regions within the respiratory system. However there are indications that mass concentration alone may not provide a suitable indication of the health risks associated with nano-sized aerosols. For example, studies indicate that biological response depend on the surface-area of particle deposited in the lungs. It has also been suggested that due to their small diameter, nanoaerosols are capable of penetrating epithelial cells, entering the bloodstream from the lungs. Health effect associated with such particle activity would be closely associated with particle size and also possibly to particle number. Particle in the nanometer size range have a high percentage of surface atoms, and are known to have unique physic-chemical properties. For all these reasons, it is expected that particles within this size range to demonstrate biological behavior closely associated with particle number, diameter, surface-area and surface activity. Further, traditionally exposure of the population to air pollution has been calculated by ignoring spatial and temporal dimensions of exposure. More complex models, taking into account population movement and/or changing air quality, are challenging with respect to data requirements and are therefore rare. Obtaining personal air pollution exposure measurements has been hampered by the cost and complexity of the analyzing equipment.

In summary, an increasing number of studies have indicated that airborne nanoaerosols may present an inhalation health risk that is not adequately addressed by conventional exposure evaluation methods. Before appropriate standards are developed, advances are needed in identifying nanoaerosols attributes critical to environmental and occupational health. Further, implementation of instruments and strategies capable of measuring exposure against these attributes, will permit to estimate exposure to nanoaerosols more accurately, in order to reduce exposure misclassification in epidemiological studies.

1.2. RESEARCH OBJECTIVES

The overall objective of this thesis is the exposure characterization to airborne nanoaerosols, firstly in urban environments and then in occupational settings, through the development of strategies capable of measuring exposure with a multi-level and multi-metric approach.

At the beginning of the study, the hypothesis was that visiting certain microenvironments (MEs) is one of the most important determinants of personal exposure to ultrafine particles (UFP) and that moving between microenvironments significantly differentiates exposure. To test this hypothesis two methodologies were used:

- Particle Number Concentration monitoring through portable monitors for UFP are being performed in specific urban microenvironments in a major Italian urban area, and in conditions characterized by high relative concentration levels and by possible risk to human health. The ultimate goal is to provide useful information for the interpretation of possible sources of UFP in urban microenvironments.

- UFP exposure of adult population in a in a major Italian urban area were simulated using a microenvironmental stochastic simulation model. Simulations predicted the mean daily exposure distributions for different subpopulations, defined on the basis of gender, and then for age, occupational status or educational level. This approach was used to compare exposure distributions of different sub-populations and to explore distributions and determinants of exposure.

From this first part of the study, it was demonstrated that appreciable differences among urban microenvironments and monitoring periods were observed: concentration patterns and variations appear related to typical sources of urban pollutants (e.g. traffic), as well as proximity to sources, time of day and seasonal patterns. Results from the exposure simulations for different subpopulations showed variations in UFP exposure related to the mode of transport and to seasonal patterns. Thus, demographic and socio-demographic factors, as well as environmental patterns, may be considered major determinants of pollutant exposure in urban environments.

In the second part of the study, newly developed techniques were used in order to asses temporal and spatial variability in UFP properties (concentration, mean diameter, surface area) in urban microenvironments of a medium-sized Italian urban area. In summary, this part of the study provides important insights into UFP exposure in urban environments, that should be considered in developing additional and larger studies on population exposure: for example, UFP concentration has been shown to be higher in specific transport ME and for particular modes of transport, where average particle size is smaller and surface area concentration is higher, supporting the relevance of traffic-related exposure for health. Further, UFP concentrations resulted to be specific for the time (period of the day, week and season) and area of sampling. Thus, once validated, findings derived from this study may represent an important tool in the definition of health and social implication of UFP exposure

(for example, exposure can be reduced avoiding traffic-related ME) and to provide complete and accurate exposure assessment data for risk assessors, including exposure metrics mostly relevant as health effects indicators (exposure monitoring stations should be placed in different urban areas with different road and traffic characteristics).

Finally, the study focused on occupational exposure to engineered nanoparticles (NP): a specific-developed strategy, which introduced innovative methodologies, was applied for the multi-metric characterization of occupational exposure in an industrial setting (case study covers a company involved in the use of nanostructured titanium dioxide). The developed strategy was used to define the potential exposure to engineered nanomaterials and nanoparticles in the workplace with complementary approaches.

The development and adoption of appropriate measurement approaches represent an essential step toward developing and implementing future exposure measurement standards for nanoaerosols. The overall aim of this thesis is to provide the means to undertake relevant exposure measurements were current methods and standards appear inadequate. This work aims to extend the knowledge on how environmental and occupational exposure to nanoaerosols should most appropriately be measured.

1.3. OUTLINE OF THE THESIS

This first chapter gives a description of the problem and states the research aims.

Chapter 2 provides information on the current knowledge about UFP and NP and details on exposure assessment both in environmental and occupational settings with direct measurement and modeling techniques.

Chapter 3 describes the results of a first monitoring campaign, which focuses on air pollution in specific urban microenvironments. For this study, monitoring of size-fractionated Particulate Matter (PM) Particle Number Concentration (PNC) and CO, was performed in a variety of urban microenvironments. The ultimate goal is to provide useful information for the interpretation of possible sources of UFP in urban transport microenvironments

Chapter 4 explores the impact of time-activity patterns on personal exposure: UFP exposure of adult Milan population were simulated using a microenvironmental stochastic simulation model. Simulations predict the mean daily exposure distributions for different subpopulations, defined on the basis of gender, and then for age, occupational status or educational level. This approach was used to compare exposure distributions of different subpopulations and different exposure scenarios (typical working day in summer and winter), and to explore distributions and determinants of exposure.

Chapter 5 focuses on UFP exposure in a medium-sized Italian urban area: exposure concentrations were measured with different metrics: UFP concentrations in different urban microenvironments (ME) were measured by personal monitoring in repeated sampling campaigns, along a fixed route.. Measurements included on-line monitoring of UFP Particle Number Concentration (PNC), mean diameter (mean d) and lung-deposited surface-area (LDSA). Besides the PNC, particle mass concentration (PMC) profiles for quasi-ultrafine particles (QUFP; $PM_{0.25}$) were estimated. The originality of the present approach lies in the continuous, contemporaneous and time-resolved multi-metric personal monitoring of UFP, which permits measurements of urban microenvironmental UFP concentrations with high temporal resolution.

Chapter 6 describes the development of an instrumental approach for assessment of NP exposures in occupational settings, which takes into account the major potential route of exposure and factors that may influence biological activity and potential toxicity of nanomaterials and incorporates a risk management approach. Different methods were used to measure and assess occupational exposures to NP with a multi-metric approach. This protocol has been used to evaluate potential exposure to engineered nanoparticles in an occupational setting: a summary of those findings is presented as case-study in chapter 7.

The last chapter, chapter 8, discusses the contributions of this dissertation to the state-of-the-science. The most important findings and knowledge gaps are discussed as well. The chapter concludes with recommendations for future studies.

2. INTRODUCTION

Aerosol exposure has historically been characterized by the mass concentration of airborne material, usually associated with specific size ranges, corresponding to different deposition regions within the respiratory system. However, there are indications that mass concentration alone may not provide a suitable indication of the health risks associated with some aerosols. A number of toxicology studies have indicated that on a mass for mass basis, some very small respirable particles may be more toxic than larger respirable particles with similar composition [1-5]. There are enough evidences to outline a particle size-related health risk following inhalation exposure to some aerosols that is not appropriately reflected by mass concentration alone. In recognition of the potential importance of particle size, the term *ultrafine aerosol* has gradually been adopted, and loosely refers to particles "smaller than 100 nm in diameter". The term is now used widely to refer to incidental aerosols where there are potential particle size-dependent health effects. Concern has also been expressed over the potential health impact of purposely generated particles with nanometer diameters or nanoscale structure [7]. In this context, the terms *engineered nanoparticle* and *engineered nanoaerosols* have also been used loosely to describe particle and aerosols with engineered nanometer-structured materials.

The use of an unambiguous terminology may lead to a misunderstanding: for clarity, in this work, the term *Ultrafine Particles (UFP)* is used to describe all aerosols particles with diameters smaller than 100 nm that present a potential inhalation health hazard ($PM_{0.1}$). The term *Nanoparticles (NP)* specifically refers to purposely engineered (or specifically produced) particles with diameters smaller than 100 nm that present a potential inhalation health hazard. NP have very different chemical and physical features from other environmental particulates that make them hazardous to human health such as dimension, mass, chemical composition, surface area, concentration, aggregation and agglomeration status, water solubility, surface chemistry, morphological structure. Nevertheless, to date there is not an unequivocal opinion on the specific correlations between manufactured nanomaterials (MNM) and toxic effects. Finally, the term *Nanoaerosols* refers to an aerosol comprised of discrete or grouped NP and/or UFP.

With only limited toxicity data and negligible exposure data, it is currently unclear how exposure to UFP and NP should be most appropriately monitored and regulated. There is a strong toxicity-based evidence that aerosol surface-area is an appropriate exposure metric for low solubility particles [2, 8-10]. However there are also indications that in some instances particle number within specific particle size ranges may be important [11-12].At the present time, there is insufficient information to determine which physical exposure metrics - size selective number, surface area, mass concentration - are most relevant, or which are the most appropriate exposure characterization techniques to use. A first step to providing the necessary information is to establish the means by which exposure can be measured against different metrics. In the short-term this will provide a means to evaluate exposures where there is concern over the inadequacy of mass-based methods and it will also provide a basis

for developing a deeper understanding of associations between aerosol exposure and health effect using a range of exposure metrics

2.1. NANO AEROSOLS AND POTENTIAL HEALTH EFFECT

A number of study have indicated that the toxicity of insoluble materials increases with decreasing particle size, on a mass for mass basis. The precise mechanisms by which these materials exhibit higher level of toxicity at smaller sizes have yet to be elucidated, although there are many hypotheses. A number of studies indicate that biological response depend on the surface-area of particle deposited in the lungs [8, 9, 13-15]. It has also been suggested that due to their small diameter, nanoparticles are capable of penetrating epithelial cells, entering the bloodstream from the lungs [16-22].

Health effect associated with such particle activity would be closely associated with particle size and also possibly to particle number. Particle in the nanometer size range have a high percentage of surface atoms, and are known to have unique physic-chemical properties. Thus one would expect particles within this size range to demonstrate biological behavior closely associated with particle number, diameter, surface-area and surface activity.

Concerning nanoaerosols' lung deposition (defines as mean probability for an inhaled particle with specific diameter to deposit somewhere in respiratory system), ICRP model [22] outlined that total deposition reaches a minimum within the lung for airborne particles around 300 nm aerodynamic diameter. At this size, particle are too large for diffusion to be effective and too small for impaction or interception to be effective. Below this minimum in deposition probably, predicted deposition increases as diffusional forces increase with decreasing particle diameter. Nanoparticles above 10 nm are deposited primarily in the alveolar region, while particles less than 10 nm have significant deposition in the head airways and to a lesser extent in the tracheo-bronchiolar region, due to their very high diffusional mobility. A substantial fraction of the inhaled nanoaerosols will deposit within the respiratory tract. For particle larger than 5 nm, deposition is predominantly in the alveolar region of the lungs. However, there is a significant predicted deposition probability for nanoparticles in the extra-thoracic and tracheo-bronchiolar regions, particularly for particle smaller than 5 nm. Particle deposition in these regions may be important in the development of airways diseases such as chronic obstructive airways disease (COPD) or asthma. In addition, once deposited in the respiratory tract, particles may be translocated elsewhere in the body. Experimental data confirm the trend of increasing deposition with breathing pattern and level of exertion [23, 24]. Thus, the deposition fraction of inhaled nanoaerosols is greater in the alveolar and tracheo-bronchial regions of human lungs, compared to the larger-diameter inhaled particles. Once deposited, nanoaerosols may also remain in the lungs longer than larger particles, due to decreased clearance and increased retention of nanoaerosols. Some type of nanoparticles (e.g. titanium dioxide, carbon) have been shown to penetrate the epithelial cell barrier more readily and enter the lung interstitium or the blood circulation in rats or hamsters [20, 25]. Once in the blood, UFP and NP may translocate to other organs in the body [21]. The fate of inhaled nanoaerosols may also depend on chemical composition [16, 21].

2.2. EXPOSURE TO ULTRAFINE PARTICLES IN URBAN ENVIRONMENTS

Sources

The ubiquitous way of UFP formation is from the gas phase [26, 27]. This process is characterized by an initial nucleation step, that is the formation of very small particle *nuclei* from the molecular phase. These *nuclei* subsequently grow by coagulation and/or surface growth mechanism. The aerosol is formed from precursor materials which are either vaporized from a liquid or solid reservoir or are existent as mixed reactive gases. The formation of condensable vapor is achieved by cooling and/or chemical reaction of the precursor gases resulting in super-saturations high enough for homogeneous nucleation occur. At high nucleation rates where a high density of nuclei is being formed, particle growth will be predominantly controlled by coagulation. At low nucleation rates leading to small concentrations of newly formed particles, direct heterogeneous condensation of the aerosol vapor on existing particle surfaces controls the dynamics of the aerosol size distribution. Condensational growth can result in quite large particles with diameters outside the nanometer size region. When particles are already present in the air, heterogeneous condensation and nucleation compete for the available condensable vapor. Therefore UFP in urban environments are emitted from primary emissions from combustion sources in transportation, industries and power generation, and by secondary formation by atmospheric photochemical reactions and conversion processes [28, 29]. UFP have a transient nature with short life times and rapidly grow through atmospheric processes of coagulation and/or condensation to larger complex aggregates [30].

Potential exposure routes

Measured UFP concentrations within a variety of environments were affected by a significant variability among some indoor and outdoor microenvironments: previous studies have shown high spatial and temporal variability of UFP number concentrations in urban microenvironments [31, 32] and have documented the dependence of UFP levels on traffic volume, the built environment and meteorological characteristics [33 - 37]. The highest concentrations of UFP are found in the vicinity of the primary sources, for example, near busy roads where particle number concentrations are typically between 10^4 and 10^6 particles/cm^3 depending on driving speed, fleet composition and meteorology [38], while UFP concentrations decrease rapidly with distance from the emission sources [39,40]. Thus, the distance to the source of emissions is a major determinant of UFP levels in urban areas moreover, the distribution of UFP is often affected by physical constraints such as trees or buildings in the vicinity [41]. Therefore, the highest urban UFP concentrations are generally expected to occur while moving along busy streets or in their immediate environments, while the lower concentrations are usually detected in indoor environment and in urban green areas. With regard to population exposure to UFP, previous studies estimate indoor home exposure providing about 46% of total daily exposure, indoor office exposure about 30%, and transport environments about 24% (almost insensitive to transportation mode) [42]. Thus, focusing on outdoor environments, individuals may gain a significant contribution to their daily exposure when commuting in traffic: in fact, the levels of most air pollutants are particularly high along

busy roads, and their concentrations peak are typically registered during commute hours [35, 43]. Thus, commuting is considered as one of the high-exposure periods among various daily activities, especially in high vehicle-density metropolitan areas, even though individuals usually travel for no more than 6-8% time of the day [44].

2.3. OCCUPATIONAL EXPOSURE TO ENGINEERED NANOPARTICLES

Sources

The "voluntary" production of nanomaterials (MNM) for nanotechnologies can be realized through two different chemico-physical approaches: the "bottom up" and "top down" methods: the former is the result of nanotechnology research and consists in obtaining materials in the desired configuration by assembling atoms following pre-defined schemes; the latter is widely used in the electronic industry for materials and components miniaturization. As far as the size of material under investigation is concerned, both approaches converge on the field of NP. The "bottom-up" approach refers to the chemical and physical processes whereas the "top-down" approach usually involves mechanical processes. Nanoparticles (NP) do not always represent the final product of the technological cycle. NP in the workplace environment are often byproducts of nucleation and condensation processes of some aerosol precursors such as gases, liquids and solids. The following are processes that most develop or involve thermal energy: metals refinery and manufacturing, high-temperature spray application, welding, grinding and carving of metals or alloys; the "undesired" products are metal and/or metal oxide NP exhibiting a large surface area and, usually, a low solubility.

The greatest prevalence of nanoaerosols within workplace is associated with particle formation through nucleation and condensation. Hot processes such as metal refining and processing, thermal metal spraying, welding, gouging and metal grinding all lead to generation of metal and/or metal oxide particles with small particle sizes, high specific surface-areas and, in many cases, low solubility. Combustion also leads to the generation of NP throughout vapor reactions and nucleation/condensation. Particle size is dependent on the generating conditions, although primary particles will generally have a modal diameter between 10 nm and 50 nm. These coagulate together rapidly where high particle concentrations are initially generated, forming agglomerates that may lie outside the nanometer size region. However, it is likely that open agglomerates of these primary particles will demonstrate many similarities to nanoparticles in how they behave following deposition in the respiratory tract. Particles generated from point sources (such as welding) are more likely to undergo rapid coagulation, while disperse sources will lead to rapid quenching of the coagulation process in many cases, resulting in higher fraction of generated particles lying in the nanometer size range. A further group of processes designed to generate aerosols having a high specific surface-area include for example the formation of carbon-black, graphene, nanoscale TiO_2, fumed alumina and fumed silica. Although the products resulting from these processes are powders with particle agglomerate larger than 100 nm, specific surface-areas may be in excess of 300 m^2/g.

In addition to established technologies and processes associated with nanoparticles, the emerging field of nanotechnology is leading to the introduction of new processes and materials associated with unique NP. Nanotechnology relies on the unique physical and chemical properties of materials and devices with nanometer-scale structure, and is frequently associated with the generation and use of nano-colloids and NP. Potential applications of the new technology are widespread (ranging from electronics to medical applications and cosmetics). Commercial and industrial interests in the technology are intense, with a wide range of industries introducing nanotechnology to a new generation of commercial and consumer products. Many of the raw particulate materials associated with nanotechnology are insoluble particulates in the nanometer size region, with unusual morphologies and active surfaces. If materials consisting of or containing nanoparticles are being handled, consideration needs to be given to the likelihood of nanoaerosols being released into the air as the materials are handled and used. Powders of nanoparticles will release agglomerates and possibly discrete particles when handled. The rate of nanoaerosols release will depend on several factors (e.g. the degree of agitation during handling) and the nature of the material. Although very little is known about the release of nanoaerosols from powders, it is likely that the physical and chemical nature of the material will play a significant role in determining how easily a powder is aerosolized. NP embedded in a solid matrix are unlikely to be released during handling, although it is possible that if the matrix is subject to high mechanical and thermal energies nanoaerosols may be released. Liquid suspensions of nanoparticles will not lead to inhalation exposure directly. However, fine sprays from the suspension will lead to airborne nanoaerosols. Particles may in principle also be re-suspended from dry deposits, although it is questionable whether disturbances and air movements leading to re-suspension will be sufficient to release large quantities of discrete airborne nanoparticles. Similarly, re-suspension from deposits of nanoparticle powders may lead to nanoaerosols exposure in some case.

Potential exposure routes

Potential routes of occupational exposure to NP include inhalation, dermal contact, olfactory and ingestion. The most common route of exposure to airborne particles in the workplace is inhalation [45]. The deposition of discrete nanoparticles in the respiratory tract is determined by the particle's diameter (size-dependent). Agglomerates of nanoparticles will deposit according to the diameter of the agglomerate and not to that of each nanoparticle. As describe above, ICRP defined that inhaled particles substantially deposit primarily in the alveolar, but also in the tracheobronchial and extra-thoracic regions [46, 47]. As stated by the International Standard ISO/TR 27268 [48], provided that the biological response is associated with the surface area of deposited aerosols, the response of a given amount of material to a fractal-like agglomerate/aggregate is assumed to be similar to that of an equivalent amount of discrete particles. Besides these considerations, if the biological interactions following deposition are dependent upon the diameter of particles, the response of discrete NPs deposited in the respiratory tract is very likely to be different from that of an equivalent amount of agglomerated/aggregated particles which do not split up under deposition. On the basis of such premises, it is worth noting that the definition of reference regulatory standards

needs to take into account some fundamental aspects; in particular, the Occupational Exposure Limit Values need to take into consideration both discrete NP and NP agglomerates/aggregates if analogies in effects of human health from exposure are identified (with respect to a potential independence of the health impact assessment from particle size); otherwise, differentiated hygienic limits must be established.

In occupational settings, dermal NM exposure may occur during production, usage or contact with contaminated surfaces. It is still under debate whether and to what extent NPs are able to penetrate the intact skin and cause harmful effects. Most of experiments have been conducted with single types of NM such as TiO_2 and ZnO on intact skin. Also, evidence indicates that nano-Ag may pass through damaged skin [49] and nano-Au may penetrate mouse skin [50]. Potential effects on flexed and damaged human skin need further exploration [51-53]. The same goes for the role of solvents in skin penetration of NPs.

2.4. EXPOSURE MEASUREMENT

Although further research is needed on the physical attributes of nanoaerosols which are most closely associated with potential health risk, it is apparent that measuring exposures against mass alone is not sufficient. Of the three primary physical exposure metrics (mass, surface-area and number), there is strong evidence to suggest that nanoaerosols should be monitored with respect to surface-area. But, while a strong case may be made for using aerosol surface-area as an exposure metric, it is also necessary to consider characterizing exposure against aerosol mass and number concentration, until further information is available. In addition, some study have shown there may be critical particle sizes influencing the fate and toxicity of respirable particles in the lungs [54, 55]. For each of these exposure metrics, but particularly in the case of mass concentration, size-selective sampling will need to be employed to ensure only particle within the relevant size range are sampled [56]. However, currently, no standard sampling methods are available for the assessment of exposure to airborne nanoaerosols. Every attempt to estimate exposure to UFP and NP requires the use of multiple sampling and assessment techniques. Monitoring and characterization methods [48, 57] allow exposure assessments for NPs and nanoaerosols in terms of mass, concentration and surface area and are the basis for the development of new standards for the exposure characterization. Estimates and characterization exposure to nanoaerosols are deeply limited by the lack of efficient instrumentation for personal sampling and, therefore, the combined use of devices for in-situ assessments and offline sampling analysis represents, today, the best tool for the assessment of personal exposure; nevertheless, most of instrumentations available, are expected to be adapted in terms of compactness, portability and costs for routinary applications in the workplace.

Mass determination and chemical characterization can’t provide information on particle concentration, dimension, agglomeration status and surface but it can act as a surrogate measure if data on size distribution or specific surface area are available [58]. The use of conventional impact techniques for determining NMs exposure is limited as the limit impact size range is from 200 and 300 nm, anyway. With low pressure impactors, it is possible to measure particles of up to 10 nm as static samplers; though their dimensions and complexity do not allow a personal use. However, a personal cascade impactor is available with a lower

aerosol cut point of 250 nm [59], allowing an approximation of nanometer particle mass concentration in the worker's breathing zone. All this samplers enable the sampling of materials deposited onto membranes and already divided into particle size fractions, thus they enable off-line investigations on UFP and NP through chemical analysis and electron and scanning probe microcopies. The gravimetric measure, although deriving from the traditional monitoring approach, is very little sensitive to MNM made of NP.

The use of Condensation Particle Counter (CPC) is relatively easy and can be extended without great difficulty for particles of up to 3 nm. These instruments are widely used to measure ultrafine particles in the urban atmosphere [27, 60-62]. As these devices are not size selective (except for an initial cut-off selection), it is difficult to distinguish the different sources of UFP generated by processes from those present in the background. Nevertheless, the adoption of this measurement process carried out in the vicinity of potential sources has been put forward for the raw identification of nanoaerosols emitted by sources in the workplace [63]. Such devices can be typically used in a static way only. Instruments providing information on particle total number and size are commercially available today: the Scanning Mobility Particle Sizers (SMPS) and the Fast Mobility Particle Sizer (FMPS) can measure the size distribution of particles with a range from 3 to 800 nm [64]. These techniques allow the determination of nano-range particles but they are not able to distinguish single NPs from those formed by agglomerates of smaller particles.

The need to measure the surface area of NPs as it is shown to be more correlated to the potential biological effects: at present, the instrument which allows the measurements of aerosol surface- area is the epiphaniometer, nevertheless, more recent devices (e.g., diffusion chargers), might find a wider use, as they use the same principles of the previous instrumentation, although they operate by generation and adhesion of positive unipolar ions to the aerosol particles surface [65]. Further, a recent version of this instrument provided data which were well correlated to the surface area of particles deposited in the human respiratory tract [66].

Exposure Modeling

Exposure to UFP for general population can be measured or modeled, either directly (e.g. personal measurements) or indirectly (e.g. microenvironment approach) [67-74]. Assessing individual and population exposure to UFP in urban environments is challenging, as UFP concentrations and particle sizes vary within short distances from the source. As a result, fixed site monitors generally underestimate exposures, especially for certain subpopulations [75]. Exposure modeling is recognized as a valuable and cost-effective tool for assessing potential population exposures to air pollution and represent an element of exposure assessment, which evaluates, qualitatively and quantitatively, the degree of intake or uptake that is likely to occur. Exposure models allow estimation of pollutant exposure for groups of people and time periods for which personal monitoring has not been conducted; models can be also used to combine information from different sources to produce estimates for population exposures that would be very expensive or impossible to perform [76, 77]. Models can be used to predict population exposures for existing, past or scenario situations and for

subpopulations for whom no measurement data are available [69], by simulations based on the distributions of input parameters. However, the full description of personal exposure to an air pollutant requires knowledge of the magnitude of pollutant concentration in the exposure environments, duration and time pattern of exposure [69] and, possibly a number of other environmental parameters. A number of modeling approaches exist to estimate air pollution exposure: some long-term exposure assessment studies have applied the concept of microenvironments to take into account in-transit exposure (so-called compartment models) [78]. This approach uses the average concentrations within different environments (derived from personal or fixed station measurements), and multiplies them by the time spent in such microenvironments. Uncertainties remain for pollutants with high spatial and temporal variability within microenvironments, such as for example UFP concentrations, thus creating inter-subject variability [79, 80]. More dynamic models account for people's specific location throughout the day along with time-activity information. Exposures are estimated by overlaying air pollution models with information from census data, time-activity and/or geo-coded origin-destination information from surveys [79, 81-83].

Limitations of such exposure simulations include the imprecision of spatial data. Other models are based on a synthetic population and are generated stochastically as in the case of activity-based models. Simulated exposure estimates might be further impaired by the limited spatial resolution of the air pollutant models used which do not accurately represent the high spatial variability of pollutant concentrations, especially in urban environments [84, 85]. Developing models of high resolution requires expertise, adequate data and can be costly. However, better quantification of exposure of general population is a fundamental issue in investigations of long-term health effects.

2.5. RISK ASSESSMENT AND RISK MANAGEMENT: STATE OF THE ART

Manufactured Nanomaterials (MNM) pose specific challenges and risks to the environment, health and safety (EHS), in a broader sense, due to the great diversity of nanomaterials types, lacking of standard testing, mathematical modeling and tools/processes for the detection, the tracking and the characterization in situ or in vivo of these MNM.

Generally, a critical element of risk management programs is the capability to anticipate new and emerging risks (hazard determination) and whether they are linked to changes in the manufacturing process, equipment, or the introduction of new materials. Risk management programs for MNM should be seen as an integral part of an overall occupational safety and health (OSH) and life-cycle assessment (LCA) programs for any company producing or using MNM or nano-enabled products. This will require a critical assessment of the potential risks to workers, consumers, general population, environmental matrixes and organisms through the systematic collection of information starting from the first step of the production of raw nanomaterials to the post-production life cycles of MNM, from their synthesis as new substances, through the entry into occupational settings, to their destination in consumer and technological products, as well as atmospheric and water emissions, from their entry in the environment as 'used products', through the full range of waste treatment processes to their final fates, so that risk management indications can be driven from a realistic all-

comprehensive scenario. This assessment should be an ongoing cyclic process that provides feedback on potential sources of human exposure and environmental contamination solutions taken to correct those problems. In short, there is the need of overcoming relevant gaps of knowledge regarding environmental aspect in risk management of MNM and exposure assessment to nanoparticles (NP).

Despite recent research efforts, nanotechnology progresses are growing faster than scientific knowledge of the health and safety aspects of MNM. There are still knowledge gaps regarding the implications of nanomaterials on workers' health and safety and regarding risk assessment methods. Thus, it is important that priorities in risk assessment (including exposure assessment) and risk management processes are given not only to MNM with known effects on health and safety, but also to the large part of MNM for which there is missing, incomplete or uncertain information regarding their hazards and exposures, in which case a precautionary approach to prevent exposures to MNM in the workplace should be generally applied. According to Commission Guidelines [86], the use of the precautionary principle requires: (I) identification of the degree of scientific uncertainty following scientific evaluation; (II) assessment of the potential consequences of inaction and (III) a transparent review of available management options. Any measure taken on the basis of the precautionary principle must be proportionate, non-discriminatory and consistent, and include socioeconomic impact and cost-benefit analyses. Precautionary principle measures must be temporary, pending a review of scientific developments. In this regard, changes in REACH Annex II [87], as well as the guidance from the European Chemicals Agency (ECHA) on Safety Data Sheets [88], which gives further advice on how to address characteristics of nanomaterials, are expected to improve the quality of the given information. Guidance from the OECD [89] provides also support for assessing potential sources of emissions of airborne MNM (nanoparticles) from various types of work practices and processes. It should be also considered that the pursuit of wealth in a legal sense mainly accounts for economic human rights and legislators have to justify any interference with these in the course of preventing hazards. The process of balancing the conflicting interests is governed by the principle of proportionality and in the case of uncertainty, the economic human rights have an important weight to be balanced with safety interests [90]. For this reason, it is equally crucial to gain information on the toxicological and eco-toxicological impacts of MNM (to implement new regulations on the basis of scientific knowledge), but also on impacts of every possible regulatory indication on risk mitigation strategies to be adopted in occupational and environmental settings.

As regards the workplace, it's mandatory to ensure the health and safety of workers in every aspect related to their work, by conducting a regular risk assessment process, as specified in the 'Framework' Directive 89/391/EEC [91], including also possible risks from MNM. In addition, Directive 98/24/EC on chemical agents at work [92] imposes more restrictive indications on the management of risks from substances at work, as the hierarchy of prevention strategies that strengthens elimination or substitution as priority measures. This also applies to MNM, which fall within the general definition of 'substances'. In parallel, when a MNM or the corresponding macro-scale equivalent is carcinogenic or mutagenic,

Directive 2004/37/EC on carcinogens and mutagens at work [93] must also be fulfilled. In any case, national legislation may have stricter provisions and should be consulted. Moreover, being MNM considered "substances", the REACH regulation [94] and the CLP regulation [95] are relevant anyway.

Because of the above-mentioned gap of knowledge on the toxicity and eco-toxicity of MNM, traditional approaches for EHS risk assessment of dangerous substances cannot always be applied. Thus, according to the precautionary principle, it is necessary to minimize exposures at workplace (where high exposure levels can occur), but also control industrial emissions of MNM in air, water and soils for the protection of the general population. This can be also achieved by reducing the environmental concentrations of MNM exposure durations and the number of people exposed, through suitable prevention and protection measures.

In order to overcome the already mentioned limitations and to facilitate workplace risk assessment and management of nanomaterials, a number of helpful and informative guides and tools have been developed. The complexity and detail needed is dependent on the hazardous substance involved and the activity being undertaken. Standards and Guidelines developed in the past decade by government agencies, academia, and occupational health organizations are summarized in *table 1*. The European Commission issued some communication documents [96, 97] illustrating a plan of action to implement a safe, integrated and responsible approach to nanotechnologies, in order to properly develop, modify and implement legislation through the improvement of scientific knowledge about risk assessment and management. These communications are also relevant for the protection of the general population and consumers, as well as the environment. The European Agency for Safety and Health at Work developed more specific recommendations on how to manage risks of nanomaterials in occupational settings [98]. Moreover, the Sixth and Seventh Framework Program of the European Commission supported many research projects, aiming at developing risk assessment and management procedures for secure industrial production of MNM. From the conclusions of the NANOSAFE2 project some practical recommendations can be derived. There are also national guidelines that the Project will take into account [99]. Other projects funded by the EC (i.e., FP7 NMP – NANOREG and MARINA) are ongoing and will provide in the next few years updated indications for risk assessment and management of MNM to support regulation.

The validation of the effectiveness of the exposure controls and measurement methods for MNM remains a key research need. In the absence of regulatory occupational exposure limits (OELs) for most MNM, a strategy is needed to assess the hazard and determine the appropriate levels of exposure control to protect workers' health.

It is globally recognized that a general hierarchical approach in risk management must be implemented to eliminate the hazard when possible (i.e., substitute with a less hazardous material) or, if not feasible, control the hazard at (or as close to) the source as possible. Risk management programs should include detailed guidelines on engineering controls, education and training of workers (e.g., good work practices), selection and use of personal protective

equipment (e.g., clothing, gloves, respirators). As regards the general environment, the air, water, waste, industrial emissions and consumer safety protection have been object of interest by the European Commission [97]. The scientific opinion from EFSA [100] confirmed that the risk assessment paradigm used for the evaluation of standard food products is also appropriate for nanomaterials applications in the food and feed chain, as well as the need for a case by case approach. A similar approach was adopted also for medicines [101]. The Consortium will explore the practical feasibility, at the level of industrial sectors, to adopt this approach developed for the food industry to all the MNM users.

The environmental impact of many MNM and their potential backlash are yet to be fully understood. The potential environmental impact from nanotechnologies is huge, from the introduction in the environmental matrixes throughout their lifecycle, all stages of which may carry different potential impacts. Thus, there is much debate about the requirement for specific regulation of nanotechnologies within the environment. Several regulatory bodies are active in the investigation of MNM under existing environmental scenarios. A summary of the outputs from these bodies is provided below:

- In 2011, the European Commission (DG Environment) commissioned a "Review of Environmental Legislation for the Regulatory Control of Nanomaterials". The report concludes that, in general, all the reviewed existing legislation (*Table* 2) could be considered to be also applied to MNM. However, several pieces of legislation were found to have some limitations in the coverage of MNM environmental impact, resulting from a lack of knowledge and technical capabilities (monitoring and detection techniques), as well as from the inapplicability of existing legal mechanisms (i.e. concentration thresholds to control the presence of pollutants).
- U.S. Environmental Protection Agency (EPA). Nanotechnology is identified by the EPA as a topic that cuts across various environmental laws, regulations, or programs [102].
- Environment Canada, Health Canada & CEPA. Nanomaterials are being regulated in Canada under existing legislation including the Canadian Environmental Protection Act, 1999, the Pest Control Products Act, the Fertilizers Act, the Feeds Act and the Food and Drugs Act. It is recognized that, due to the unique properties associated with nanomaterials, the science surrounding the risk assessment of these substances needs to be further developed.

In short, there is the need of overcoming relevant gaps of knowledge regarding environmental aspect in risk management of MNM. Some of the most relevant are reported hereafter:

- The water legislation shows a lack of technical capacity in the detection and monitoring of MNM in aqueous environments and a lack of reliable eco-toxicological data for risk assessment.
- The applicability of a threshold-based approach to controlling pollutants in a context where the potential adverse effects associated with MNM are not solely dependent on exposure in terms of mass concentration is somehow questionable.

- Concerns regarding the coverage of MNM under waste legislation reflect uncertainties surrounding the classification of specific MNM as hazardous substances under the CLP Regulation. A number of limitations were addressed about the waste legislation in regards to the applicability of threshold-based limit values to MNM (i.e., Lits of Waste, Sewage Sludge Directive, Landfill Directive and RoHS Directive).
- A high proportion of the legislation is dependent upon the CLP Regulation for the identification of hazardous substances. In the absence of available nano-specific data, MNM will principally be categorized according to the bulk form and in some cases hazardous properties may not be recognized.
- Implementation of legislation suffers from the lack of data regarding the intrinsic properties of specific nanomaterials and their behavior in environmental compartments, in particular when considering their large variety stemming also from differences in size distribution and particle coating.

For all these reasons, some European projects (*Table 3*) and scientific publications (*Table 4*) aim at producing risk assessment tools and strategies for risk control by combining the mitigation of human exposures and environmental contaminations. Some other initiatives from standardization bodies will be ready in the next few years (*Table 5*).

2.6. CONCLUSION

An increasing number of studies are indicating that airborne nanoaerosols (ultrafine particle and engineered nanoparticles) may present an inhalation health risk that is not adequately addressed by conventional exposure evaluation methods. Before appropriate standards are developed, advances are needed in identifying nanoaerosols attributes critical to environmental and occupational health, as well as the implementation of instruments and strategies capable of measuring exposure against these attributes.

The monitoring and characterization techniques discussed in this thesis aims to evaluate nanoaerosols exposure in terms of mass, surface-area and/or number concentration. These methods were developed and used to exposure characterization both in environmental and occupational settings.

2.7. TABLES

Table 1. Standards and guidelines relevant for Nanosafety and for the present project

Type	Field	Year	Institution/N°	Title
IS	O	2008	ISO/TS 12885	Nanotechnologies - Health and safety practices in occupational settings relevant to nanotechnologies
IS	O	2011	ISO/TR 13121	Nanotechnologies -- Nanomaterial risk evaluation
IS	O	2012	ISO/TS 12901-1	Nanotechnologies - Occupational risk management applied to engineered nanomaterials - Part 1: Principles and approaches
IS	O	2014	ISO/TS 12901-2	Nanotechnologies - Occupational risk management applied to engineered nanomaterials - Part 2: Use of the control banding approach
IS	C	2013	CEN ISO/TS 13830	Nanotechnologies - Guidance on voluntary labelling for consumer products containing manufactured nano-objects
IS	-	2009	CEN ISO/TS 27687	Nanotechnologies - Terminology and definitions for nano-objects - Nanoparticle, nanofibre and nanoplatelets
EG	O	2013	EU-OSHA	E-fact 72: Tools for the management of nanomaterials in the workplace and prevention measures.
EG	C	2009	SCENIHR	Risk Assessment of Products of nanotechnologies
IG	C	2009	EFSA	Guidance on the risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain
NG France	O	2010	Agencie Nationale de Sécurité Sanitare	Development of a specific control banding tool for nanomaterials
NG Nederl.	O	2012	IVAM/version 4.2	Working safely with engineered nanomaterials and nanoproducts - A guide for employers and employees
NG U.K.	O	2008	BSI/PD 6699-2	Nanotechnologies—Part 2: guide to safe handling and disposal of manufactured nanomaterials.
NG U.S.A.	E	2007	U.S. EPA	Nanotechnology white paper (EPA/100/ B-07/001)
				Concept Paper for the Nanoscale Materials Stewardship Program under TSCA
NG U.K.	E	2006	U.K. Defra	UK Voluntary Reporting Scheme for engineered nanoscale materials.
				Consultation on a Proposed Voluntary Reporting Scheme for Engineered Nanoscale Materials.
NG Canada	E	2007	Environment Canada Health Canada	Proposed Regulatory Framework For Nanomaterials Under The Canadian Environmental Protection Act, 1999
				Requirements for nanomaterials under the New Substances Notification Regulations

IS = International Standard; EG = European Guideline; IG = International Guideline; NG = National Guideline; W = Workplace; C = Consumers; E = Environment

Table 2. Summary of EU environmental legislation also applicable to MNM in waste, water and other environmental matrixes

Ref.	Title
2009/98/EC	Waste Framework directive
2000/532/EC	The list of waste decision
199/31/EC	The Landfill Directive
2003/33/EC	Waste acceptance criteria in landfills in Decision
86/278/EEC	Sewage sludge directive
2002/95/EC; 2002/96/EC	WEEE and RoHS directives
2000/53/EC	Directive on end-of-life vehicles
94/62/EC	Directive on packaging waste
2000/60/EC	Water Framework Directive
2008/105/EC	Directive on protection of groundwater against pollution and deterioration
91/271/EEC	Urban waste Directive
98/83/EC	Drinking water directive
2006/11/EC	Directive on pollution caused by certain dangerous substances discharged into the aquatic environment
96/82/EC	Directive on the control of major-accident hazards involving dangerous substances
Reg. EC 66/2010	Regulation on the EU Ecolabel

Table 3(a). EU-projects relevant for producing recommendations on nanosafety regulation

Acronym	Year	Project n°	Title
NANOREG	2013-	FP7 310584	A common European approach to the regulatory testing of Manufactured Nanomaterials
NANOSAFE	2003-4		Risk Assessment in Production and use of Nanoparticles with Development of Preventive Measures and Practice Codes
NANOSAFE2	2005-9		Safe production and use of nanomaterials
NANO SUPPORT		DG ENV-JRC	Scientific technical support on assessment of nanomaterials in REACH registration dossiers and adequacy of available information
EU-KNIGHTS		FP7 608926	
NANOPOLYTOX		FP7 247899	Toxicological impact of nanomaterials derived from processing, weathering and recycling from polymer nanocomposites used in various industrial applications
NANOVALID	2011-16		Nanoparticle Fate Assessment and Toxicity in the Environment
NANOFATE	2010-14	NMP4-SL-2010-247739	Nanoparticle Fate Assessment and Toxicity in the Environment
MARINA	2011-		Managing risks of nanomaterials
ENNSATOX	2009-12	FP7 NMP4-SL-2009-229244	Nanoparticles in the aquatic environment
NANOGENOTOX	2010-13		Facilitating the safety evaluation of manufactured nanomaterials by characterising their potential genotoxic hazard.
ENPRA	2009-12		Risk assessment of engineered nanoparticles
Nanolyse			Validation of methods for the detection and quantification of engineered nanoparticles in food
ENRHES	2008-09		Engineered Nanoparticles: Review of Health and Environmental Safety
NHECD	2008-12	FP7 218639	Creation of a critical and commented database on the health, safety and environmental impact of nanoparticles
NanoEIS	2012-15	FP7 319054	Nanotechnology Education for Industry and Society
NanoImpactNet	2008-	FP7 NMP4-CA-2008-218539	European Network on the Health and Environmental Impact of Nanomaterials
NANOfutures	2010-		European initiative for sustainable development by Nanotechnologies
SIINN	2011-14	FP7 265799	Safe Implementation of Innovative Nanoscience and Nanotechnology
NANOdefine	2013-17	FP7 265799	
NanEx	2009-10	FP7 NMP-2009-CSA-3	Development of Exposure Scenarios for Manufactured Nanomaterials

Table 3(b). EU-projects relevant for producing recommendations on nanosafety regulation

Acronym	Year	Project n°	Title
NanoPuzzles	2013-16	P7 NMP-2012-SMALL-6-309837	Modelling properties, interactions, toxicity and environmental behaviour of engineered nanoparticles
NanoStair	2012-14	FP7 NMP4-SA-2012-319092	Establishing a process and a platform to support standardization for nanotechnologies implementing the STAIR approach
NanoSustain		FP7 NMP4-SL-2009-247989	Development of sustainable solutions for Nanotechnology-based products based on hazard characterization and LCA
Scaffold	2012-16	FP7 NMP4-SL-2012-280535	Innovative strategies, methods and tools for occupational risk management of manufactured nanomaterials (MNM) in the construction industry
Nephh		FP7 CP-FP 228536-2	Nanomaterials Related Environmental Pollution and Health Hazards Throughout their Life Cycle
LICARA	2012-14	FP7 315494	Life cycle approach and human risk impact assessment, product stewardship and stakeholder risk/benefit communication of nanomaterials

Table 4. Scientific publications and reports on risk management of MNM

Author	Year	Citation	Title
GAO	2014	GAO-14-181SP	Nanomanufacturing. Emergence and Implications for U.S. Competitiveness, the Environment, and Human Health
OECD	2009	ENV/JM/MONO(2009)18	Report of an OECD Workshop on Exposure Assessment and Exposure Mitigation: Manufactured Nanomaterials
Read et al	2014	In book: Handbook of Nanosafety - Measurement, Exposure & Toxicology, Chapter 2; pp.17-58	Nanotechnology and Exposure Scenarios
Berges et al	2014	In book: Handbook of Nanosafety - Measurement, Exposure & Toxicology, Chapter 8; pp. 279-326	Risk Assessment and Risk Management
Aitken et al	2009	IOM Report TM/09/01	EMERGNANO: A review of completed and near completed environment, health and safety research on nanomaterials and nanotechnology
Maynard et al	2005	J. Nanopart. Res. 7: 587–614.	Airborne nanostructured particles and occupational health
Oberdörster et al	2005	Environ. Health. Perspect. 113: 823–39	Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles.
Stone et al	2010	Project Final Report	Engineered nanoparticles: Review of health and environmental safety (ENRHES)
Roco et al	2011	ISBN: 978-94-007-1167-9 (Print) 978-94-007-1168-6 (Online)	Nanotechnology research directions for societal needs in 2020
Nel et al	2010	WTEC panel report (http://www.wtec.org/nano2)	Nanotechnology environmental, health, and safety issues

Table 5. *Other on-going activities about nanosafety standard and regulation implementation*

Organization	Type	Number	Title
CEN TC 352	SO		Nanotechnology
ISO/TC 229	SO	ISO/AWI TR 18637	General framework for the development of occupational exposure limits for nano-objects and their aggregates and agglomerates
ISO/TC 229	SO	ISO/AWI TR 18196	Nanotechnologies -- Measurement method matrix for manufactured nano-objects

SO = Standardization Organizations

2.8. REFERENCES

1. Oberdörster, G.; Celein, R.M.; Ferin, J.; Weiss, B. Association of particulate air pollution and acute mortality: involvement of ultrafine particles? Inhal. Toxicol. 1995, 7(1): 111-124.

2. Oberdürster, G. Toxicology of ultrafine particles: in vivo studies. Philosophical Transactions of the Royal Society of London. Series A: Mathematical, Physical and Engineering Sciences, 2000, 358(1775): 2719-2740.

3. Donaldson, K.; Stone, V.; Gilmour, P.S.; Brown, D.M.; MacNee, W. Ultrafine particles: mechanisms of lung injury. Philosophical Transactions of the Royal Society of London. Series A: Mathematical, Physical and Engineering Sciences 2000, 358(1775): 2741-2749.

4. Tran, C.L.; Buchanan, D.; Cullen, R.T.; Searl, A.; Jones, A.D.; Donaldson, K. Inhalation of poorly soluble particles. II. Influence of particle surface area on inflammation and clearance. Inhal. Toxicol. 2000, 12(12): 1113-1126.

5. MacNee, W.; Donaldson, K. Mechanism of lung injury caused by PM_{10} and ultrafine particles with special reference to COPD. Eur. Respir. J. 2003, 21(40): 47s-51s.

7. Maynard, A.D.; Kuempel, E.D. Airborne nanostructured particles and occupational health. J. Nanopart. Res. 2005, 7(6): 587-614.

8. Brown, D.M.; Wilson, M.R.; MacNee, W.; Stone, V.; Donaldson, K. Size-dependent proinflammatory effects of ultrafine polystyrene particles: a role for surface area and oxidative stress in the enhanced activity of ultrafines. Toxicol. Appl. Pharmacol. 2001, 175(3): 191-199.

9. Tran, C.L.; Buchanan, D.; Cullen, R.T.; Searl, A.; Jones, A.D.; Donaldson, K. Inhalation of poorly soluble particles. II. Influence of particle surface area on inflammation and clearance. Inhal. Toxicol. 2000, 12(12): 1113-1126.

10. Lison, D.; Lardot, C.; Huaux, F.; Zanetti, G.; Fubini, B. Influence of particle surface area on the toxicity of insoluble manganese dioxide dusts. Arch. of Toxicol. 1997, 71(12): 725-729.

11. Beck-Speier, I.; Dayal N.; Karg E. et al. Agglomerates of ultrafine particles of elemental carbon and TiO_2 induce generation of lipid mediators in alveolar macrophages. Environ. Health Perspect. 2001, 109: 613–618.

12. Peters, A.; Wichmann H.; Tuch, T.; Heinrich, J.; Heyder J. Respiratory effects are associated with the number of ultrafine particles. Am. J. Respir. Crit. Care Med. 1997, 155: 1376–1383.

13. Donaldson, K.; Beswick, P.H.; Gilmour, P.S.; Free radical activity associated with the surface of particles: a unifying factor in determining biological activity? Toxicol. Lett. 1996, 88(1): 293-298.

14. Hamoir, J.; Nemmar, A.; Halloy, D.; Wirth, D.; Vincke, G.; Vanderplasschen, A.; Gustin, P. Effect of polystyrene particles on lung microvascular permeability in isolated perfused rabbit lungs: role of size and surface properties. Toxicol. App. Pharmacol., 2003, 190(3): 278-285.

15. Oberdörster, G.; Ferin, J.; Lehnert, B. E. Correlation between particle size, in vivo particle persistence, and lung injury. Environ. Health Perspect. 1994, 102(5): 173.

16. Kreyling, W.G.; Semmler, M.; Erbe, F.; Mayer, P.; Takenaka, S.; Schulz, H.; et al. Translocation of ultrafine insoluble iridium particles from lung epithelium to extrapulmonary organs is size dependent but very low. J. Toxicol. Environ. Health, 2002, 65(20): 1513-1530.

17. Oberdörster, G.; Oberdörster, E.; Oberdörster, J. Nanotoxicology: An emerging discipline evolving from studies of ultrafine particles. Environ. Health Perspect. 2005, 113(7): 823–839

18. Schlesinger, R.B. Deposition and clearance of inhaled particle. In Concepts in Inhalation Toxicology, 2nd ed., R.O. McClella and R.F. Henderson (eds.), pp. 191–224. London: CRC Press, 1995.

19. Takenaka, S.; Karg, E.; Roth, C.; Schulz, H.; Ziesenis, A.; Heinzmann, U.; et al. Pulmonary and systemic distribution of inhaled ultrafine silver particles in rats. Environ. health Perspect. 2001, 109(4), 547.

20. Nemmar, A.; Hoet, P.M.; Vanquickenborne, B.; Dinsdale, D.; Thomeer, M.; Hoylaerts, M. F.; et al. Passage of inhaled particles into the blood circulation in humans. Circulation, 2002, 105(4): 411-414.

21. Oberdörster, G.; Sharp, Z.; Atudorei, V.; Elder, A.; Gelein, R.; Lunts, A.; et al. Extrapulmonary translocation of ultrafine carbon particles following whole-body inhalation exposure of rats. J. Toxicol. Environ. Health. A. 2002, 65(20): 1531-1543.

22. ICRP, International Commission on Radiological Protection, Publication 66: Human respiratory tract model for radiological protection. Pergamon, Elsevier Science Ltd, Oxford, 1994.

23. Jaques, P. A.; Kim, C. S. Measurement of total lung deposition of inhaled ultrafine particles in healthy men and women. Inhal. Toxicol. 2000, 12(8): 715-731.

24. Daigle, C.C.; Chalupa, D.C.; Gibb, F.R.; Morrow, P.E.; Oberdörster, G.; Utell, M.J.; Frampton, M.W. Ultrafine particle deposition in humans during rest and exercise. Inhal. Toxicol., 2003, 15(6): 539-552.

25. Ferin, J.G.D.P.; Oberdörster, G.; Penney, D.P. Pulmonary retention of ultrafine and fine particles in rats. Am. J. Respir. Cell Mol. Biol. 1992, 6(5): 535-542.

26. Friedlander, S. K. Smoke, dust, and haze. Fundamental of aerosol dynamics., 2nd ed. Oxford University Press, New York, 2000.

27. Hind, W.C. Aerosol Technology: Properties, Behavior, and Measurement of airborne particles. Second edition, 2nd ed. Wiley-Interscience, New York, 1999.

28. Westerdahl, D., Fruin, S., Sax, T., Fine, P.M. and Sioutas, C. Mobile Platform Measurements of Ultrafine Particles and Associated Pollutant Concentrations on Freeways and Residential Streets in Los Angeles. Atmos. Environ. 2005, 39: 3597–3610.

29. Seinfeld, J.H.; Pandis, S.P. Atmospheric Chemistry and Physics: From Air Pollution to Climate Change, 2nd Edition, Wiley, Hoboken, New Jersey, 2006.

30. Pope III, C.A.; Dockery, D.W. Health Effects of Fine Particulate Air Pollution: Lines That Connect. J. Air Waste. Manage. Assoc. 2006, 56: 709–742.

31. Moore, K.; Krudysz, M.; Pakbin, P.; Hudda, N.; Sioutas, C. Intra-community Variability in Total Particle Number Concentrations in the San Pedro Harbor Area (Los Angeles, California). Aerosol Sci. Technol. 2009, 43: 587–603.

32. Hudda, N.; Cheung, K.; Moore, K.F.; Sioutas, C. Inter-community Variability in Total Particle Number Concentrations in the Eastern Los Angeles air Basin. Atmos. Chem. Phys. 2010, 10: 11385–11399.

33. Boogaard, H.; Montagne, D.R.; Brandenburg, A.P.; Meliefste, K.; Hoek, G.; Comparison of short-term exposure to particle number, PM10 and soot concentrations on three (sub) urban locations. Sci. Total. Environ. 2010, 408: 4403-4411.

34. Hoek, G.; Beelen, R.; Kos, G.; Dijkema, M.; van der Zee, S.C.; Fischer, P.H.; Brunekreef, B. Land use regression model for ultrafine particles in Amsterdam. Environ. Sci. Technol. 2011, 45: 622-628.

35. Morawska, L.; Ristovski, Z.; Jayaratne, E.R.; Keogh, D.U.; Ling, X.; Ambient nano and ultrafine particles from motor vehicle emissions: characteristics, ambient processing and implications on human exposure. Atmos. Environ. 2008, 42: 8113-8138.

36. Ragettli, M.S.; Corradi, E.; Braun-Fahrlander, C.; Schindler, C.; de Nazelle, A.; Jerrett, M.; Ducret-Stich, R.E.; Künzli, N.; Phuleria, H.C. Commuter exposure to ultrafine particles in different urban locations, transportation modes and routes. Atmos. Environ. 2013, 77: 376-384.

37. Rivera, M.; Basagana, X.; Aguilera, I.; Agis, D.; Bouso, L.; Foraster, M.; et al. Spatial distribution of ultrafine particles in urban settings: a land use regression model. Atmos. Environ. 2012, 54: 657-666.

38. Nikolova, I.; Janssen, S.; Vrancken, K.; Vos, P.; Mishra, V.; Berghmans, P. Size Resolved Ultrafine Particles Emission Model - A Continuous Size Distribution Approach. Sci. Total Environ. 2011, 409: 3492–3499.

39. Zhu, Y.; Fung, D.C.; Kennedy, N.; Hinds, W.C.; Eiguren-Fernandez A. Measurements of Ultrafine Particles and Other Vehicular Pollutants inside a Mobile Exposure System on Los Angeles freeways. J. Air Waste Manage. Assoc. 2008, 58: 424–434

40. Hagler, G.; Thoma, E.D.; Baldauf, R.W. High resolution Mobile Monitoring of Carbon Monoxide and Ultrafine Particle Concentrations in a Near-road Environment. J. Air Waste Manage Assoc. 2010, 60: 328–336.

41. Fuller, C.H.; Brugge, D.; Williams, P.L.; Mittleman, M.A.; Durant, J.L.; Spengler, J.D. Estimation of ultrafine particle concentrations at near-highway residences using data from local and central monitors. Atmos. Environ. 2012, 57: 257-265.

42. Lonati, G.; Ozgen, S.; Luraghi, I.; Giugliano, M. Particle number concentration at urban microenvironments. Chem. Eng. Trans. 2010, 22: 137–142.

43. Moreno, T.; Querol, X.; Alastuey, A.; Viana, M.; Gibbons, W. Profiling transient daytime peaks in urban air pollutants: city centre traffic hotspot versus urban background concentrations. J. Environ. Monit. 2009;11:1535–42.

44. Kaur, S.; Nieuwenhuijsen, M.J.; Colvile, R.N. Fine particulate matter and carbon monoxide exposure concentrations in urban street transport microenvironments (Review). Atmos. Environ. 2007, 41(23): 4781–810.

45. INAIL, Department of Occupational Medicine (formerly ISPESL): “White book”: Exposure to engineered nanomaterials and occupational health and safety effects. Ed. 2010. ISBN 978-88-7484-192-9.

46. ISO/NP TS. International Standardization Organisation. Guidelines for occupational risk management applied to engineered nanomaterials based on a “control banding approach”. ISO/NP TS 12901-2/2008.

47. Yeh, H.C.; Cuddihy, R.G.; Phalen, R.F.; Chang, I.Y. Comparison of calculated respiratory-tract deposition of particles based on the proposed ICRP model and the new ICRP 66 model. Aerosol Sci. Technol. 1996, 25: 134-140.

48. ISO/TR. International Standardization Organisation. Workplace atmospheres - Ultrafine, nanoparticle and nano-structured aerosols - Inhalation exposure characterization and assessment. Technical Report ISO/TR 27628/2007.

49. Larese Filon, F.; D'Agostin, F.; Crosera, M.; Adami, G.; Renzi, N.; Bovenzi, M.; Maina, G. Human skin penetration of silver nanoparticles through intact and damaged skin. Toxicol. 2009, 255: 33-37.

50. Sonavane, G.; Tomoda, K.; Sano, A.; Ohshima, H.; Terada, H.; Makino, K. In vitro permeation of gold nanoparticles through rat skin and rat intestine: Effect of particle size. Colloids Surf. B Biointerfaces 2008: 65, 1-10.

51. Tinkle, S.S.; Antonini, J.M.; Rich, B.A.; Robert, J.R.; Salmen, R.; De Pree, K.; Adkins, E.J. Skin as a route of exposure and sensitization in chronic beryllium disease. Environ. Health. Perspect. 2003, 111: 1202-1208.

52. Nohynek, G.J.; Lademann, J.; Ribaud, C.; Roberts, M.S. Grey goo on the skin? Nanotechnology, cosmetic and sunscreen safety. Crit. Rev. Toxicol. 2007, 37: 251-277.

53. Crosera, M.; Bovenzi, M.; Maina, G.; Adami, G.; Zanette, C.; Florio, C.; Larese, F.F. Nanoparticle dermal absorption and toxicity: a review of the literature. Int. Arch. Occup. Environ. Health. 2009, 82(9): 1043-1055.

54. Li, N.; Sioutas, C.; Cho, A.; Schmitz, D.; Misra, C.; Sempf, J.; et al. Ultrafine particulate pollutants induce oxidative stress and mitochondrial damage. Environ. Health. Persp. 2003, 111(4): 455.

55. Hofmann, W.; Sturm, R.; Winkler-Heil, R.; Pawlak, E. Stochastic model of ultrafine particle deposition and clearance in the human respiratory tract. Radiat. Prot. Dosim. 2003, 105(1-4): 77-79.

56. ISO. International Standardization Organisation. Air Quality – Particle size fraction definitions for health-related sampling, ISO 7708:1995, 1995

57. ISO. International Standardization Organisation. Health and Safety Practices in Occupational. Settings Relevant to Nanotechnologies. Technical Report ISO 12885/2008.

58. Möhlmann, C. German activity on the ultrafine particles in the workplaces. Proceedings of the First International Symposium on Occupational Health Implications of Nanomaterials-Nanomaterials: a risk to health at work? 12-14 Oct 2004, Buxton, UK. pp 128-130.

59. Misra, C.; Singh, M.; Shen, S.; Sioutas, C.; Hall, P.M. Development and evaluation of a personal cascade impactor sampler (PCIS). J. Aerosol Sci. 2002, 33(7): 1027-1048.

60. Kim, S.; Shen, S.; Sioutas, C.; Zhu, Y.; Hinds, W. Size distribution and diurnal and seasonal trends of ultrafine particles in source and receptor sites of the Los Angeles Basin. J. Air Waste Manage. Assoc. 2002; 52: 297-307

61. Aalto, P.; Hämeri, K.; Paatero, P.; Kulmala, M.; Bellander ,T.; Berglind, N.; et al. Aerosol particle number concentration measurements in five European cities using TSI-3022 condensation particle counter over a three-year period during health effects of air pollution on susceptible subpopulations J. Air Waste. Manage. Assoc. 2002, 55: 1064–1076.

62. Marconi, A.; Cattani, G.; Cusano, M.; Ferdinandi, M.; Inglessis, M.; Viviano, G.; Settimo, G.; Two years of fine and ultrafine particles measurements in Rome, Italy. J. Toxicol. Environ. Health, Part A. 2007, 70: 213-221.

63. Brouwer, D.H.; Gijsbers, J.H.; Lurvink, M.W. Personal exposure to ultrafine particles in the workplace: exploring sampling techniques and strategies. Ann. Occup. Hyg. 2004, 48(5): 439-453.

64. Flagan, R.C. Electrical techniques. In: Aerosol measurement: principles, techniques and applications, Baron PA and Willeke K. John Wiley &Sons, New York, 537-568, 2001.

65. Keller, A.; Fierz, M.; Siegmann, K.; Siegmann, H.C.; Fillippov, A. Surface science with nanosized particles in a carrier gas. J. Vacuum Sci. Technol. 2001,19(1): 1-8.

66. Wilson, W.E. Use of the electrical aerosol detector as an indicator for the total particle surface area deposited in the lung. Proceedings of the 2004 Air and Waste Management Association Conference.

67. Duan, N. Models for human exposure to air pollution. Environ. Int. 1982, 8: 305–309.

68. Ott, W.R. Total human exposure: an emerging science focuses on humans as receptors of environmental pollution. Environ. Sci. Technol. 1985, 19(10): 880–886.

69. Ryan, P.B. An overview of human exposure modeling. J. Expos. Anal. Environ. Epidemiol. 1991, 1(4): 453–474.

70. Duan, N. Stochastic microenvironmental models for air pollution exposure. J. Expos. Anal. Environ. Epidemiol. 1991, 1(2): 235–257.

71. Ott, W.R. Exposure estimates based on computer generated activity patterns. J. Toxicol. Clin. Toxicol. 1984, 21: 97–128.

72. Ott, W.; Thomas, J.; Mage, D.; Wallace, L. Validation of the simulation of human activity and pollutant exposure (SHAPE) model using paired days from the Denver, Colorado carbon monoxide field study. Atmos Environ 1988, 22: 2101–2113.

73. Lioy, P.J. Assessing total human exposure to contaminants - a multidisciplinary approach. Environ. Sci. Technol. 1990, 7: 938–945.

74. Duan, N.; Mage, D.T. Combination of direct and indirect approaches for exposure assessment. J Expos. Anal. Environ. Epidemiol. 1997, 7(4): 439–470

75. Knibbs, L.D.; Cole-Hunter, T.; Morawska, L. A review of commuter exposure to ultrafine particles and its health effects. Atm. Environ. 2011, 45: 2611e2622.

76. Hanninen, O.; Kruize, H.; Lebret, E.; Janutnen, M. EXPOLIS simulation model: $PM_{2.5}$ application and comparison with measurements in Helsinki. J. Expos. Anal. Environ. Epidemiol. 2003, 13: 74 – 85

77. Letz, R.; Ryan, B.P.; Spengler, J.D.; Estimated distributions of personal exposure to respirable particles. Environ. Monit. Assess. 1984, 4: 351– 359

78. Ott, W. Concepts of Human Exposure to Air Pollution. Environ. Int. 1982, 7: 179-196

79. Gulliver, J.; Briggs, D.J. Time-space modeling of journey-time exposure to traffic-related air pollution using GIS. Environ. Res. 2005, 97: 10–25.

80. Steinle, S.; Reis, S.; Sabel, C.E. Quantifying human exposure to air pollution—Moving from static monitoring to spatio-temporally resolved personal exposure assessment. Sci. Total Environ. 2013, 443: 184–193.

81. Setton, E.M.; Keller, C.P.; Cloutier-Fisher, D.; Hystad, P.W. Spatial variations in estimated chronic exposure to traffic-related air pollution in working populations: A simulation. Int. J. Health. Geogr. 2008, 7, doi:10.1186/1476-072X-7-39.

82. Mölter, A.; Lindley, S.; de Vocht, F.; Agius, R.; Kerry, G.; Johnson, K.; Ashmore, M.; Terry, A.; Dimitroulopoulou, S.; Simpson, A. Performance of a microenviromental model for estimating personal NO_2 exposure in children. Atmos. Environ. 2012, 51: 225–233.

83. Marshall, J.D.; Granvold, P.W.; Hoats, A.S.; McKone, T.E.; Deakin, E.; Nazaroff, W. Inhalation intake of ambient air pollution in California's South Coast Air Basin. Atmos. Environ. 2006, 40: 4381–4392.

84. Beckx, C.; Panis, L.I.; van de Vel, K.; Arentze, T.; Lefebvre, W.; Janssens, D.; Wets, G. The contribution of activity-based transport models to air quality modelling: A validation of the ALBATROSS-AURORA model chain. Sci. Total Environ. 2009, 407: 3814–3822.

85. Setton, E.; Marshall, J.D.; Brauer, M.; Lundquist, K.R.; Hystad, P.; Keller, P.; Cloutier-Fisher, D. The impact of daily mobility on exposure to traffic-related air pollution and health effect estimates. J. Expo. Sci. Environ. Epidemiol. 2011, 21: 42–48.

86. Commission of the European Communities COM (2000) 1: Communication from the Commission on the precautionary principle

87. Commission Regulation (EU) No 453/2010 of 20 May 2010 amending Regulation (EC) No 1907/2006 of the European Parliament and of the Council on REACH, OJ L 133, 31 May 2010.

88. European Chemicals Agency (ECHA), Guidance on the Compliance of Safety Data Sheets, December 2011.

89. OECD Emission assessment for the identification of sources and release of airborne manufactured nanomaterials in the workplace: compilation of existing guidance, ENV/JM/MONO, number 11, 2009

90. Heselhaus 2010 - Nanomaterials and the Precautionary Principle in the EU. J. Consum. Policy. 2010, 33:91–108

91. Council Directive of 12 June 1989 on the introduction of measures to encourage improvements in the safety and health of workers (89/391 EEC), OJL 183, 29 June 1989.

92. Council Directive 98/24/EC of 7 April 1998 on the protection of the health and safety of workers from the risks related to chemical agents at work.

93. European Parliament and Council Directive 2004/37/EC on the protection of workers from the risks related to exposure to carcinogens or mutagens at work.

94. Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 (REACh Regulation), OJ L 396, 30 December 2006.

95. Regulation (EC) No 1272/2008 of the European Parliament and of the Council (CLP Regulation), OJ L 353, 31 December 2008.

96. Communication from the Commission to the European Parliament, the Council and the European Economic and Social Committee. Regulatory aspects of nanomaterials [Sec(2008) 2036]

97. Communication from the Commission to the European Parliament, the Council and the European Economic and Social Committee - Second Regulatory Review on Nanomaterials, Brussels, 3.10.2012

98. EU-OSHA - E-fact 72: Tools for the management of nanomaterials in the workplace and prevention measures. 2013

99. Working safely with engineered nanomaterials and nanoproducts. IVAM, version 4.2 - August 2012.

100. Guidance on the risk assessment of the application of Nanoscience and nanotechnologies in the food and feed chain - EFSA Journal 2011;9(5):2140. doi:10.2903/j.efsa.2011.2140

101. Ehmann, et al. 2013 - Next-generation nanomedicines and nanosimilars: EU regulators' initiatives relating to the development and evaluation of nanomedicines. Nanomedicine 8(5): 849-856

102. (http://www.epa.gov/pesticides/regulating/nanotechnology.html)

3. TEMPORAL VARIATION OF ULTRAFINE PARTICLES IN SELECTED URBAN MICROENVIRONMENTS

This chapter focuses on air pollution in specific urban microenvironments and conditions characterized by high relative concentration levels and by possible risk to human health. For this reason, monitoring of Particle Number Concentration (PNC) with a wide, size-resolved particle-size range, and CO (an indicator of combustion sources; e.g. traffic), was performed in a variety of microenvironments. The ultimate goal of this part of the study is to provide useful information for the interpretation of possible sources of high levels of in urban transport microenvironments.

Concentrations of ultrafine particles (UFP), size-fractionated particulate matter (PM), and carbon monoxide (CO) was measured in the central area of Milan over three week-long periods, one each during summer, autumn, and winter, with three monitoring sessions per day. Experimental data were collected continuously during each monitoring period along an established urban pathway, moving afoot or by different private and public means of transport, to assess the relevance of time and spatial factors affecting atmospheric concentrations of UFP, PM, and CO. Measurements were divided on the basis of different microenvironments (MEs), seasons, days of the week, and periods of the day; data analysis shows statistically significant differences across MEs and monitoring periods. The highest measured median concentrations and data variability were observed for busy streets, walking or moving by motorized vehicle (CO, UFP) and in metro trains (PM); the lowest concentrations were observed in park areas and in indoor environments. The highest concentrations were measured during working-day morning monitoring sessions. Regarding seasonal variation, UFP, PM and CO showed different patterns: the highest median concentrations were observed in summer for CO, and in autumn and winter for the UFP and PM. Appreciable differences among all MEs and monitoring periods were observed: concentration patterns and variations appear related to typical sources of urban pollutants (traffic), proximity to sources, and time of day.

3.1. INTRODUCTION

Milan is the second-largest city in Italy, with more than one million inhabitants. As with many large cities, it suffers from high levels of air pollution. Its urban area is characterized by a high density of residential and commercial buildings and a very high volume of vehicular traffic, while many factories are located at the city's outskirts. Moreover, Milan is situated in the Po Valley, which experiences adverse atmospheric dispersion conditions due to its topography. Milan, especially in winter, exhibits prolonged periods of high pollution, during which air quality limits are exceeded and exceptionally high particulate matter (PM) mass concentrations are frequently measured [1].

Human exposure in transport microenvironments (MEs) is of particular interest because many residents and commuters spend a substantial proportion of their outdoor time in urban transport microenvironments. In addition, a recent review points out that time spent in transit

can contribute substantially to total daily exposure [2]. Fixed monitoring stations measuring outdoor air quality are routinely used to assess general exposure levels to pollutants such as PM and carbon monoxide (CO). However, studies show that this approach significantly underestimates the exposure of particular population subgroups, pointing to the need for direct personal exposure measurements [3, 4]. Further, there are indications that, as an exposure metric, particle number concentration may be more important than mass concentrations when considering impact on health [5 - 6]. Currently, concentrations of particles with aerodynamic diameter > 10 um (PM_{10}) and > 2.5 ($PM_{2.5}$) are regulated.

The originality of the present study lies in its integrated measurement of PNC for both UFP and size-resolved PM (and CO, as a co-pollutant), with a study design aimed at analyzing space and time variations (different seasons, three daily monitoring periods, many urban indoor and outdoor MEs). Research is not directly comparable to this study design; as they assessed the variability of a single, specific fraction of particles, such as UFP [7] or $PM_{2,5}$ [8]. Studies focused on the analysis of the variations of exposure to UFP (PNC), in association with different fractions of particulate matter (PM_1, $PM_{2.5}$, PM_5, PM_{10}) [9-12], but these latter are expressed as mass concentrations. When attention is focused only on the PNC, typically, only a single fraction of particle is considered [7]. Further, when the research is focused on size-fractioned PM, only a few fractions are considered [13, 14] and usually UFP are not monitored. The number concentration metric was selected because of its better accuracy compared to continuously measured mass concentrations, if these latter are not properly corrected using simultaneous gravimetric data [15]. For this reason, a continuous, individual and time-resolved monitoring of PNC (with a wide, size-resolved dimensional particle-size range) and CO (an indicator of combustion sources; e.g. traffic), was performed in a variety of microenvironments; the choice of PNC as measurement metric was made because of its better accuracy in the measurement of spatial and temporal variations of concentration levels, compared to photometric mass concentration. The ultimate goal is to provide useful information for the interpretation of possible sources of high levels of in urban transport microenvironments.

Particulate Matter

High concentrations of airborne particles are associated with increased pulmonary and cardiovascular mortality [16, 17], mostly for fine particle (i.e., particles < 2.5 micron in aerodynamic diameter; $PM_{2.5}$) [18]. PM of particular concern for adverse impacts on human health ranges in size from the largest PM10 (aerodynamic diameter < 10µm) and $PM_{2.5}$ (diameter < 2.5µm), to ultrafine particles (UFP), (aerodynamic diameter < 0.1µm). Particle size defines the capacity for penetration in the respiratory system. 'Inhalable fraction' includes all particles that can be inhaled from the nose and mouth. The UFP and the finer PM fractions ($PM_{2,5}$) can reach the deeper region of the respiratory system [19] and recent studies assess the possibility that nanoparticles may overcome the alveolar barrier and enter the bloodstream, contributing to increased risk of cancer, thrombosis, and cardiovascular diseases [20, 21]. Further, there are indications of a specific toxicological role for UFP in respiratory diseases [6]. For example, UFP may induce inflammatory and pro-thrombotic responses,

promoting atherosclerosis, thrombogenesis, and other cardiovascular events [22]. Human data suggest that inhaled UFP influence lung physiology and may affect the autonomic nervous system or act directly on cells in various organs, inducing mutations [23-27].

These findings have, in the last few years, led to international guidelines and standards, and exposure assessment studies focused on specific, health-relevant fractions of PM. The impact on human health from exposure to PM and UFP is strongly associated with particle chemical composition, and in particular with the presence of trace metal (Pb, Cd, Cr, As, Zn, Hg, among others) and of organic compounds classified as carcinogenic (e.g., Polycyclic aromatic hydrocarbons, dioxins). Typical sources of these pollutants include combustion processes (e.g., road traffic) and industrial processes, as well as natural sources (e.g., wind erosion, sea-spray, spores, and pollen). Road transport is recognized as a principal source of PM, and combustion of fuel is the main source of precursor gases in the urban environment [28]. Motor vehicle emissions are the major contributors out of all other combustion and non-combustion sources in urban areas. With decreasing particle size the contribution of road transport to the total emissions increases. Contributions from other combustion sources tend to decrease with decreasing particle size [29].

PM concentrations in Milan have been widely investigated, and seasonal trends, chemical compositions, and sources have been described [1, 7, 30-35]. Chemical speciation analyses enabled the identification and quantification of PM_{10} and $PM_{2.5}$ sources: secondary aerosol contribution was found to be most prevalent and this was ascribed to nitrates and secondary organic compounds originating primarily from NOx, Volatile Organic Compounds (VOCs) emissions, which are typically traffic-related pollutants, as well as from NH_3, typically emitted in farming activities [32]. Road traffic turns out to be the most important source of PM_{10} pollution in Milan, accounting for approximately 60% of the PM_{10} mass. $PM_{2.5}$ appears strictly related to anthropogenic activities such as combustion processes, industrial processes, and traffic emissions; while the coarse $PM_{(10-2.5)}$ fraction is dominated by soil-related elements such as Al, Si, Ca, Ti, and Fe.

Despite background UFP concentrations ascribed to natural processes, processes such as fossil fuel combustion was shown to be the dominant source in urban areas [1, 36, 37], with heavy-duty diesel powered vehicles making a disproportionately large contribution to UFP concentrations [38].

Previous studies estimate a daily average exposure to UFP (20-1000 nm) of 1.6×10^4 particles/cm^3 for people commuting in Milan, with indoor home exposure providing about 46% of total daily exposure, indoor office exposure about 30%, and transport environments about 24% (almost insensitive to transportation mode) [30]. Moreover, the average particle number concentration measured in the 20–1000 nm size range was usually on the order of 1.0×10^4 up to 4.0×10^4 particles/cm^3 in different indoor MEs and on the order of 3×10^4 particles/cm^3 (with a maximum up to about 10^5 particles/cm^3) in different transport MEs; thus, the transport ME represents a significant component of exposure to UFP in urban areas [7, 30].

Carbon Monoxide

Some studies show that exposure to CO in urban populations can be variable and dependent on several factors [39]. This can be relevant in particular MEs, such as congested roads or tunnels, where the presence of road traffic at moderate speeds can contribute to high concentrations of CO. Other important determinants of exposure to CO may be distance from emission sources, season (higher values of CO are found in winter), or even day of the week (which can affect traffic fluxes). Moreover, a study in England finds a moderately strong correlation between personal exposure to UFP and CO [8]. Further, there is a notable difference between ambient levels and personal exposure to CO, with the latter much higher particularly when walking near roads or in traffic. These differences should not be neglected when determining impact on health [40]. There is growing evidence that exposure to low concentrations (e.g., from less than 10 ppm) of CO can affect organ systems. However, the effects on health of prolonged low-level exposure to CO are unclear. It is suggested that chronic exposure to CO may produce mild neurological effects, although there are as yet no conclusive studies showing such a correlation [41]. Experimental studies show that those most affected by increases of ambient CO concentrations are those already prone to heart disease, and that low levels of CO adversely affect patients with heart disease when exercising [42]. There is also consistent epidemiological evidence that exposure to CO increases risk of cardiovascular mortality and cardiovascular disease hospitalization, even at ambient levels [43 - 46]. In Italy, the change in mortality for all natural causes associated with a 1 mg/m^3 increase in CO was found to be 0.93% [47].

3.2. METHODS

As discussed above, urban areas are characterized by a variety of MEs, in which airborne particle levels and CO concentrations may strongly vary, yielding different contributions to personal daily total exposures. The aim of this study is to measure concentrations of size-fractionated PM, UFP, and CO, as a function of environmental settings (investigating selected MEs in the Milan urban area) and time (examining different periods of the year and days of the week).

Study Design

Experimental data were collected within the central area of Milan over three one-week periods in three different seasons (summer, autumn, winter). This study followed the same procedure used in a previous study investing exposure to airborne UFP in the same area [7]. Monitoring was performed only in the absence of rainfall.

The monitoring strategy involved data collection while walking along busy streets (code ‘W1’ and ‘W2’), travelling by bus (code ‘B’), car (code ‘C’), and metro (code ‘UG’). Concentrations inside a car (2001 FIAT Punto) were measured while driving a vehicle fuelled by petrol along a very busy street, turning off the mechanical ventilation system and with windows closed. The car was equipped with a cabin pollen filter, and a three-way catalytic converter.

Measurements were collected along the route represented in Figure 1, according to a sequential protocol that started and finished at the Department of Clinical Sciences and Community of the University of Milan, also used as an office sampling area (code 'IN'). The protocol also includes investigation of a urban park (code 'P') and a large, multi-road intersection (code 'CR'). All environments in which the operator was exposed to the study pollutants in other unspecified situations (e.g., waiting in the metro station) are classified as 'transition environments' (code 'T').

The monitoring protocol is designed to collect data for at least 15 minutes in each selected ME. Data were collected at three times: morning ('AM': 08:00–10:30 am), lunchtime ('EA': 12:00–14:30), and afternoon ('LA': 16:00–18:30). A time-activity diary was completed in order to accurately separate the concentration data as a function of the different monitored environments; data were collected with a frequency of measurement of 30 seconds (averaging time). The instruments were placed into a backpack and carried by one investigator. Since the sampling inlets were not placed in strict correspondence with the breathing zone (the hemisphere of 30 cm radius extending in front of the face) [48], the study results refer to the so-called 'individual' measurement (in proximity of subjects - within 3 m), as discussed in Cattaneo et al [49] The 'individual measurements' approach allows measurement of concentrations without losing accuracy with respect to breathing zone (personal) measurements, except for coarse particles [49].

Size-resolved PM

Numeric concentrations of airborne PM were measured using an optical particle counter (OPC) and a condensation particle counter (CPC). Both OPCs and CPCs are able to provide real-time measurement of particles, although each type of instrument has its own sensitivity to specific particle characteristics. Immediately before the study, these instruments were calibrated by factory-supplied services.

OPCs allow measurement of particle number concentration (PNC) separated as a function of their size, by detecting the light scattered by individual particles. The size range normally measured by OPCs is 0.3 to 20 µm, while the maximum number concentration is dependent on particle size. The OPC used in this study (mod. Handheld 3016, Lighthouse Worldwide Solutions, Fremont, CA; Counting Efficiency: 50% @ 0.3 µm; 100% for particles > 0.45 µm) uses an active sampling mode (flow rate = 2.83 L/min). It is based on the principle of light scattering of a linear radiation produced by a diode laser focused on the air flow to measure PNC. Each signal is counted and classified into 6 different dimensional fractions (0.3-0.5; 0.5-1.0; 1.0-2.5; 2.5-5.0; 5.0-10.0; > 10.0 µm). The timing of signal processing is extremely fast and allows measurements with high monitoring frequency (30 second-weighted average concentration).

The CPC quantifies the PNC, using isopropanol to artificially enlarge particles through condensation of vapors on the particle surface. Unlike OPCs, however, this instrument does not allow differentiation of particles into different dimensional classes; so it is not possible to obtain information about the size distribution of PM.

The CPC used in this study (P-Trak Ultrafine Particle Counter model 8525; TSI Inc., Shoreview, MN, USA; Concentration Range 0 to 5 x 105 particles/cm^3) measures particles ranging from 0.02 to > 1 μm in size (so for this study the 'UFP' fraction includes particle with dimension >100 nm, although their number concentration is negligible with respect to those in the 0-100 nm interval). This instrument is not equipped with a flow control system. The P-Trak operated with batteries at an inlet flow rate of 700 cm^3/min; 100 cm^3/min of the total sampled volume was automatically analyzed. Usually, portable and easy–to–use devices are characterized by worse metrological performance than counters well–suited for basic aerosol research in terms of measurement accuracy, minimum detectable particle diameter, and maximum measurable concentrations [50]. The portable rapid-response instruments used in this study have shown adequate capacity to measure short-term variation of PNC in urban environments [7].

Carbon Monoxide

CO concentrations were measured via a passive analyzer (CO Measurer mod. T15v; Langan Products, Inc. San Francisco, CA, USA - Nominal concentration range: 0-200 ppm; Resolution: 0,05 ppm) consisting of an electrochemical cell that allows continuous monitoring over several days. Measured concentrations and temperature were recorded via an internal data logger with an acquisition time of 30 seconds. The instruments were calibrated in a glove bag before every monitoring campaign with two different certified–concentration standard gases (< 0.5 ppm and 10.3 ppm). Data were downloaded using the factory–supplied software and corrected as a function of temperature using an experimental quadratic equation.

Data Analysis

For this study, the data were gathered in 4 dimensional classes: UFP (0.02-1 μm), 2 different classes of fine particles (FP': 0.3-1 μm; FP: 1-2.5 μm) and coarse particles (CP: > 2.5 μm); this classification was implemented on the basis of the significant correlation defined between the various dimensional classes determined by the OPC. The collected data were examined and handled to provide for the exclusion of zero values and missing data (casewise deletion), and synchronized in function of their acquisition interval following good practice in statistics [51-53]. Statistical analysis of collected data was performed via PASW Statistics 18.0; analysis consists of non–parametric tests to analyze data distribution (Kolmogorov–Smirnoff test - KS) and to assess the differences among groups (Kruskal–Wallis one–way ANOVA - KW). To refine data analysis, we performed a non–parametric significance test (Mann - Whitney U-test - MW) to point out statistically significant differences among groups of variables and to determine whether the observations from both groups are independent of each other. Finally, another non–parametric test (tau test) was used to evaluate the association between measured pollutants in each ME.

3.3. RESULTS

In total, N = 17562 data observations were collected using the three instruments, of which, respectively, N = 16264 were valid for CPC, N = 16983 for OPC, and N = 13341 for the CO

analyzer (a more detailed summary is shown in *table 1*); some samples were lost due to data–logging errors or alcohol wick depletion for CPC.

Measurements were analyzed on the basis of the considered variables (MEs, seasons, days of the week, and period of the day); statistically significant differences were found ($p_{KW} < 0.05$) for every variable and group of variables ($p_{MW} < 0.05$). Thus, data analysis shows that concentrations was specific to each sampled ME and time period.

Microenvironmental variations

The highest median concentration and the widest data variability (*table 2*) were observed for CO and UFP while walking along busy streets ('W1' and 'W2'), and moving by motorized vehicles, like buses (B) and cars (C). The highest concentrations of FP', FP, and CP were measured in metro trains. In particular, the FP fraction shows the highest median value and widest difference among the considered MEs (always with $p_{MW} < 0.05$). On the contrary, the lowest median concentrations were typically observed in green areas (P) and in the indoor environment (IN), both for particles and CO ($p_{MW} < 0.05$ compared to all the other MEs). The variability and mean concentrations obtained in the other MEs generally fall between these values and are comparable to each other. Measured PNC within a variety of indoor and outdoor MEs were affected by significant variability among some indoor MEs and relative homogeneity in outdoor MEs, as outlined in Levy et. al. [13]. It has already been shown that the mode of transport has a very large influence on personal exposure to UFP [54, 55], and CO [56]. Further, route choice and behavioral factors are likely to also be important determinants of exposure [54, 57, 58]. As expected, road traffic appears to be the most relevant determinant of PM (especially the finer fractions) and CO, as the highest concentrations were detected in traffic–related environments ('B', 'C', 'W1', 'W2'). As outlined in a recent review article, vehicle emissions are the dominant source of UFP [38]. Further, whereas some studies report higher levels of PM in metro systems compared to other modes of transport and street canyons or ambient air [31, 59] other studies report lower levels in metro systems [60]. The main explanation given for the high levels in metro systems in most cases is the wear of steel from friction between wheel and rail, wear of brakes, and vaporization of metals due to sparking involving the third rail. Further, other sources were identified including brake shoes, passenger activities, the train piston effect (airflow at the front of the platform), and floor cleaning. Tunnels, ventilation, and traffic of trains were suggested to be other important determinants of high levels of exposure to PM. Regarding coarse PM, the role of re-suspension of particles from the floor and of personal clouds should not be neglected, owing to the high density of occupants in some transport modes [61]. This hypothesis seems to be confirmed by the high concentrations measured not only in UG, but also in the T and B MEs. Thus, as outlined by several studies [62-63] measured concentrations in transport MEs are clearly dependent on mode of transport, time of day, and proximity to sources, as well as source characteristics.

Seasonal variation

Analysis of seasonal variation of concentrations reveals several trends among PM size fractions (*table 3*). The highest median concentrations and variability of measurement were observed during winter and autumn for UFP and PM, while the lowest values were measured in summer. With respect to CO, the highest concentration and variability of measurements were observed during summer, while lower values were measured in autumn. Published studies for Milan show a strong seasonal effect in PM concentration values, mainly due to differences in average dispersion conditions of the atmosphere in summer and winter [32, 34]. PM mass concentrations show strong seasonal variability, which is more evident for the finer particle-sizes, with higher values in winter. This seasonal modulation is, essentially, more linked to differences in average thermodynamic and meteorological conditions of the atmosphere than to variations of the type or number of emitting sources. However, the observed seasonal behaviour of particulate concentrations may also be ascribed to the presence of additional emission sources (i.e., domestic heating) during the cold season, which contributes to primary PM as well as to secondary aerosol production because of the large emission of gaseous precursors. This seasonal trend is confirmed in a study conducted in residential premises near Milan, with findings of higher CP concentrations in summer than in winter [64]

Daily variation

Concentrations measured during working days (*table 4*) show higher mean values and wider data distributions in comparison with those collected on non–working days for UFP, PM and CO. The highest particle concentrations were observed during the monitoring sessions on Tuesdays (UFP and PM), while the highest median concentration of CO was reached on Mondays (this latter result, however, may be due to the small number of data collected during the Monday session - *table 1* - which could have resulted in a bias for median CO levels). The lowest median values were measured, as expected, on Saturdays or Sundays both for particles and for CO. Differences between non–working days and working days were always statistically significant ($p_{MW} < 0.05$), while differences among working days showed higher values of significance ($0.096 < p_{MW} < 0.968$).

The lower concentration measured during non-working days may be interpreted in light of the decrease in traffic volumes (especially heavy-vehicles) and reduced contributions from industrial emissions on those days. In the case of coarse particles, a reduced contribution from tire abrasion products and soil dust re-suspension from streets can explain the low concentration measured on non-working days, as previously observed for PM mass concentrations [32].

Variation among periods of the day

Analysis of concentrations collected during three different periods of the day (*table 5*) reveals statistically significant differences ($p_{MW} < 0.05$) between the morning sessions (AM) and the remainder of the day for UFP, PM, and CO. Data variability and mean concentrations

obtained during the afternoon sessions (Early Afternoon - EA, and Late Afternoon - LA) were generally lower (and comparable to each other - $p_{MW} > 0.05$). It should be remembered that PM mass concentrations show great daily variability related to meteorological variables and thermodynamic conditions of the atmosphere and, in particular, to the height of the mixing layer [32]. However, the AM monitoring sessions were performed during rush hours characterized by heavy traffic and commuting, in contrast with the afternoon sessions, in which commuting flows and traffic were less intensive. Thus, daily variations in concentrations are likely influenced by differences in traffic volumes and corresponding emissions: this is in agreement with the behaviour shown by CO and UFP (lower median concentrations in EA); while for size–resolved particles, this trend is only partially observed. This finding is reported in several studies that identify traffic–related parameters as significant determinants of exposure to PM and CO [62, 63, 66, 67].

Relationships among air pollutants

Finally, a non–parametric hypothesis test (Tau test) is used to measure the association between the measured pollutants in each ME; the results are generally always statistically significant ($p < 0.01$) and are shown in *table 6*. The strongest correlations are determined for CP vs. FP and for FP' vs. FP, while the correlations among other dimensional fractions and between particles and CO are weaker, due probably to the different physical states.

UFP may be not strongly correlated with the different PM size fractions, as they are the result of nucleation of low vapor pressure compounds, while fine particles and CP are typically formed through other processes (e.g., coagulation–accumulation, atmospheric chemical reactions, mechanical–abrasion processes, re-suspension from the ground). On the contrary, FP, FP', and CP are better correlated, as they are partially composed of particles with the same origin, such as nitrates and crustal elements [68]. UFP may be not strongly correlated with the different PM size fractions, as they are the result of nucleation of low vapor pressure compounds, while fine particles and CP are typically formed through other processes (e.g., coagulation-accumulation, atmospheric chemical reactions, mechanical–abrasion processes, re-suspension from the ground). On the contrary, FP, FP', and CP are better correlated, as they are partially composed of particles with the same origin, such as nitrates and crustal elements [68].

It is possible to observe that some MEs show similar behaviour: the MEs typically associated to urban traffic (W1, W2, B, C) show similar correlations and the strongest correlations between UFP and CO, pointing out these two pollutant are mainly produced from urban traffic [29]. UG shows the highest correlation recorded between the FP and CP, highlighting the presence of a localized source of coarse particles, as already discussed earlier. Finally, the indoor ME (IN), shows levels of correlation between the pollutants to be substantially different and lower from those observed at the other microenvironments, while P and CR show correlation values very similar to each other, and intermediate with respect to the correlations determined for the other MEs, probably due to the presence of non-localized sources for these MEs.

3.4. DISCUSSION

The highest concentrations CO and UFP occurs while moving along busy streets or in their immediate environs, either afoot (‘W1’) or by motorized vehicle (‘B’ and ‘C’), as shown in recent studies [7, 56]. Different considerations are required for PM: highest concentrations to FP’, FP, and CP is detected during time spent in the metro (‘UG’). For all pollutants considered, the lowest concentrations are detected in an office environment (‘IN’) and in a green area (‘P’). The concentrations measured in vehicles with instruments and in conditions similar to those adopted in this study (driving along a very busy street with closed windows) are comparable to the ones reported in similar international researches and consistent with literature [11, 13] implying that individual exposures can depend on ME’s concentration condition.

Considering the variation of concentration as function of time, an appreciable and statistically significant difference between working and non–working days is observed for CO, UFP, and PM: all the highest concentrations are observed during working days, while lower values are measured on Saturdays and Sundays. Moreover, analysis of concentrations collected during different periods of the day reveals a general traffic–related trend, both for particles (UFP) and CO. Analysis of temporal variability of collected measures within the week reveals relevant differences between weekdays and the rest of the week. Sunday is characterized by lower concentration values, while UFP levels measured on Saturday lie in an intermediate position between Sunday and the working days. A similar behaviour have been observed for data variability: the most wide-spread distributions have been found during weekdays. The concentrations observed in different days of the week confirms a relationship with traffic trends, similarly to what described in other international research studies [10, 11, 36, 69, 70], where the diurnal particle number concentration profiles show that the bulk of the particulate matter measured on weekdays comes from anthropogenic sources that are dominant on weekdays (probably traffic as opposed to domestic heating). The impact of traffic intensity on measured concentrations is also highlighted looking at the distributions of UFP and PM concentrations according to three different diurnal time periods are displayed. Indeed, more wide-spread distributions and higher particle number concentrations were measured during rush hours (08:00-10:30), when an increase of particle emissions is caused by the elevated number of circulating vehicles. Similarly to other European cities [69, 71, 72], peaks of concentrations were found in the morning, between 7:00 and 11:00 a.m., while in the evening measured UFP levels are slightly higher than during the central hours of daytime. Similar weekday diurnal profiles of PNC and CO, with a major morning peak and in some instances a late afternoon peak, have been identified by others [10, 11, 36, 73, 74]. Finally, regarding seasonal variation, the highest concentrations are observed in winter and autumn for UFP and PM, and in summer for CO: seasonal pattern affects all sites, with highest concentrations in the winter months and lowest concentrations in the summer. Atmospheric mixing processes are generally poorer in the winter months leading to less adequate dispersion and higher concentrations.

Limitations and Strengths

Although this study demonstrated temporal and spatial variability for the studied microenvironments, the study design and methods imply some limitations in the generalizability of these findings: measurements were taken within the Milan area, and the microenvironments evaluated in our study may not correspond directly to similar microenvironments elsewhere. Interpretation of particle count concentration data is limited by the fact that the PNC for UFP include particles > 100 nm (from 20 to 1000 nm). Finally, although the categories of microenvironments were chosen to reflect common urban activities and general trends, the specific locations were selected according to a systematic and technical protocol, but they may not be representative of the average concentrations in that microenvironment across Milan or in other cities. Nevertheless, the patterns in PNC and CO are demonstrative of relationships that would remain consistent in a broader investigation.

3.5. CONCLUSIONS

Continuous real-time monitoring can provide the information necessary to define the influence of a local source or changes in local circumstances on particulate matter counts or mass concentrations. In addition, continuous monitoring permit to evaluate short-term particle concentrations [75]. This study has favored the accuracy in individual measurement strategy (using portable instruments), rather than the instrumental accuracy. Future studies should focus on the assessment of personal exposure to nanoparticles and/or UFP (mass, number and surface area concentrations). Moreover, the use of micro-environmental models should be encouraged for estimating the total daily exposure of general populations.

3.6. ACKNOWLEDGEMENTS

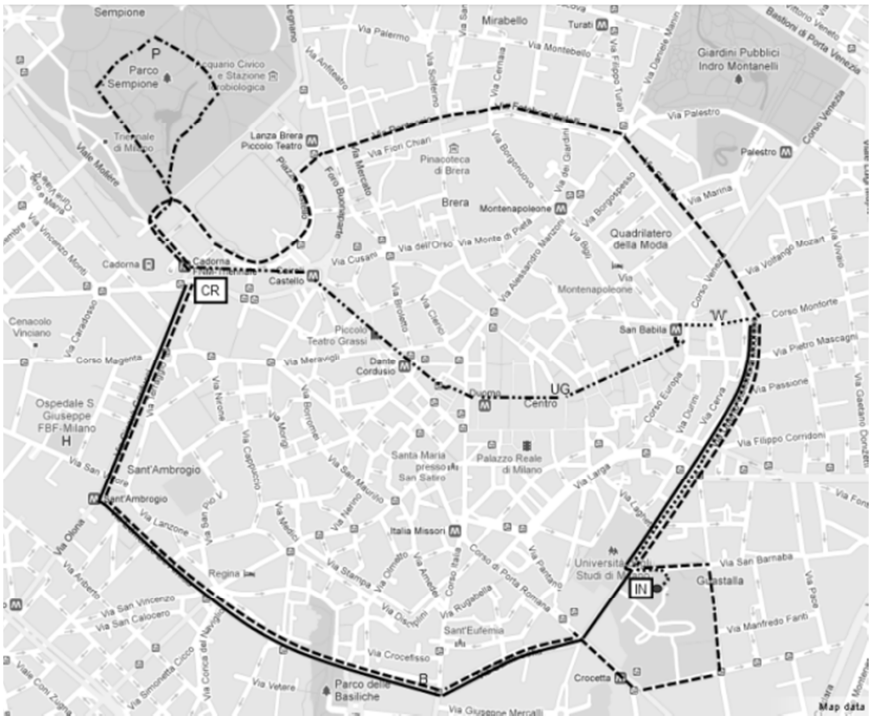
A special acknowledgment to dr. Stefano Taronna and dr. Greta Peverelli for their fundamental contribution in the environmental sampling.

This chapter is based on:

- Spinazzè, A. Cattaneo, G. Garramone, D. M. Cavallo
  Temporal variation of size-fractionated particulate matter and carbon monoxide in selected microenvironments of the Milan urban area.
  J. Occup. Environ. Hyg. 2013;10:11,652-662
- Spinazzè, A. Cattaneo, D.M. Cavallo (2013)
  Individual exposure to size-fractionated particulate matter and carbon monoxide in selected micro-environments of the urban area of Milan
  (Abstract): Environ. Health. Perspect. DOI: http://dx.doi.org/10.1289/ehp.ehbasel13
- Spinazzè A., Cattaneo A., Taronna S., Cavallo, D.M. (2013)
  Esposizione individuale a particolato atmosferico, particelle ultrafini e monossido di carbonio in microambienti urbani
  Atti del Atti del 30° Congresso Nazionale AIDII" (ISBN 978-88-86293-22-8)

3.7. FIGURES

Figure 1. Milan's urban area monitoring route (adapted from Google Maps, Google Maps © 2012 Google); Legend: IN = indoor (office); CR = Multi-road intersection; W1 & W2 (dotted line) = walk in busy streets; B = bus (continuous line); UG = metro (dash-double dot line); P = urban green area (dash-dot line); C = car (dashed line)



3.8. TABLES

Table 1. Summary of the valid observations collected using the three instruments, in each microenvironment and in each time bin.

	MICROENVIRONMENT								
	T	W1	UG	P	CR	B	W2	C	IN
UFP	3091	1957	722	1891	1215	2172	1127	2664	1425
CO	2623	1569	575	1517	978	1731	943	2160	1245
Size-resolved PM	3187	2000	725	1904	1244	2281	1194	2880	1568
	WEEKDAY								
	Mon	Tue	Wed	Thu	Fri	Sat	Sun		
UFP	1366	2148	2367	2490	2409	2328	3156		
CO	801	1818	2512	1686	1691	1584	3249		
Size-resolved PM	1680	2292	2512	2437	2461	2352	3249		
	MONITORING SESSION								
	08:00-10:30			12:00 - 14:30			16:00-18:00		
UFP	5566			5291			5407		
CO	4656			4373			4312		
Size-resolved PM	5949			5400			5634		
	SEASON								
	July			Oct-Nov			Feb-Mar		
UFP	5260			6273			4731		
CO	6054			2481			4806		
Size-resolved PM	5543			6697			4743		

Notes: IN = indoor (office); CR = Multi-road intersection; W1 & W2 (dotted line) = walk in busy streets; B =bus (continuous line); UG = metro (dash-double dot line); P = urban green area (dash-dot line); C = car (dashed line); T = 'transition' environments. UFP: valid observations collected with TSI P-TRAK CPC; CO: valid observation collected with Langan CO analyser; Size-resolved PM: valid observations collected with Lighthouse 3016 OPC. UFP, CO and Size-resolved PM data were measured with a monitoring frequency of 30 seconds (averaging time)

Table 2. Concentration of CO [ppm], UFP, FP', FP, CP [pt/cm^3] in different urban transport MEs (SD = standard deviation, IQR = interquartile range).

		MICROENVIRONMENT								
		T	W1	UG	P	CR	B	W2	C	IN
CO	*Mean*	1.49	1.76	1.46	1.03	1.30	2.03	2.09	2.67	1.09
	SD	1.14	0.97	0.54	0.53	0.62	1.01	1.83	1.30	0.71
	Median	1.26	1.59	1.34	0.96	1.18	1.88	1.80	2.45	1.06
	IQR	0.78	1.14	0.60	0.56	0.68	1.34	1.41	1.46	0.45
UFP	*Mean*	32746	48450	25817	18840	32693	51805	56353	64223	13643
	SD	30590	43132	12330	17861	23974	35036	44047	48543	15271
	Median	24693	33030	22665	14560	25536	43606	43693	53985	10536
	IQR	22587	39420	12140	9957	25766	45605	55036	61748	6718
FP’	*Mean*	201.6	208.4	236.3	202.1	204.8	178.5	194.2	188.1	101.6
	SD	203.4	209.5	166.8	215.8	213.8	167.5	197.1	191.7	124.1
	Median	125.7	128.7	191.9	125.5	127.1	116.4	118.9	113.5	61.2
	IQR	207.1	221.8	179.3	223.9	225.3	187.6	197.6	193.4	73.8
FP	*Mean*	3.32	1.34	13.89	1.25	1.33	2.62	1.50	1.49	0.83
	SD	8.60	1.23	15.29	1.53	1.39	2.20	1.87	1.05	1.91
	Median	1.22	0.93	7.74	0.68	0.84	2.09	1.01	1.20	0.56
	IQR	1.97	1.45	10.03	1.44	1.51	2.22	1.56	1.35	0.78
CP	*Mean*	1.42	0.55	5.02	0.50	0.44	1.37	0.77	0.67	0.50
	SD	4.02	0.52	6.41	0.61	0.29	1.22	3.94	0.41	0.94
	Median	0.52	0.44	2.50	0.37	0.38	0.98	0.50	0.57	0.28
	IQR	0.66	0.37	3.37	0.36	0.34	1.26	0.50	0.50	0.33

Notes: Particles size ranges: UFP: 0.02-1 µm, FP’: 0.3-1 µm; FP: 1-2.5 µm; CP: > 2.5 µm. Microenvironments: IN = indoor; CR = Multi-road intersection; W1 & W2 = walk in busy streets; B = bus; UG = metro; P = urban green area; C = car; T = ‘transition’ environments . Variables: UFP: valid observations collected with TSI P-TRAK CPC; CO: valid observation collected with Langan CO analyzer; Size-resolved PM: valid observations collected with Lighthouse 3016 OPC.

Table 3. Concentration of CO [ppm], UFP, FP', FP, CP [pt/cm^3] in different seasons (SD = standard deviation, IQR = interquartile range).

		SEASON		
		Summer	Autumn	Winter
CO [ppm]	*Mean*	1.88	1.50	1.63
	SD	1.22	1.01	1.21
	Median	1.55	1.29	1.37
	IQR	1.43	1.44	0.94
UFP [pt/cm^3]	*Mean*	28969	50020	40252
	SD	30344	41603	38319
	Median	19420	35690	27173
	IQR	23432	47743	36790
FP' [pt/cm^3]	*Mean*	93.2	219.8	257.7
	SD	66.2	186.6	253.3
	Median	81.5	162.4	153.5
	IQR	67.2	166.1	350.2
FP [pt/cm^3]	*Mean*	3.08	2.15	1.95
	SD	9.47	1.94	2.30
	Median	0.67	1.81	1.28
	IQR	0.74	1.56	2.12
CP [pt/cm^3]	*Mean*	1.48	0.90	0.69
	SD	4.46	0.98	0.72
	Median	0.43	0.59	0.50
	IQR	0.47	0.64	0.67

Notes: Particles size ranges: UFP: 0.02-1 µm, FP': 0.3-1 µm; FP: 1-2.5 µm; CP: > 2.5 µm.

Table 4. Concentration of CO [ppm], UFP, FP', FP, CP [pt/cm^3] on different days of the week (SD = standard deviation, IQR = interquartile range).

		WEEKDAY						
		Mon	Tue	Wed	Thu	Fri	Sat	Sun
CO	*Mean*	2.64	1.57	1.85	1.89	2.10	1.41	1.35
	SD	1.21	0.87	1.59	1.07	1.41	0.76	0.80
	Median	2.33	1.18	1.51	1.61	1.80	1.22	1.23
	IQR	1.43	1.13	1.41	1.31	1.47	0.75	0.84
UFP	*Mean*	42523	54703	45276	44606	45125	27014	28886
	SD	35940	47804	39618	35656	39958	27439	32498
	Median	28947	37902	31903	33913	32190	17576	18642
	IQR	35767	52047	43500	40957	38543	21621	21586
FP’	*Mean*	151.7	371.9	126.9	157.2	206.2	87.3	212.0
	SD	105.9	276.4	75.5	101.8	157.0	65.5	259.8
	Median	124.7	307.8	104.0	116.2	141.3	84.7	92.7
	IQR	204.1	498.4	102.8	155.4	278.5	105.2	318.6
FP	*Mean*	2.30	3.33	2.15	2.52	2.59	1.84	2.15
	SD	4.12	4.56	6.13	6.21	6.21	5.08	6.27
	Median	1.52	2.21	0.84	1.20	1.73	0.69	0.68
	IQR	1.61	2.81	0.77	1.42	1.41	1.71	2.02
CP	*Mean*	1.17	1.14	0.93	1.14	1.25	0.69	0.95
	SD	1.86	1.53	1.95	2.29	4.15	1.99	3.30
	Median	0.70	0.79	0.47	0.53	0.65	0.33	0.35
	IQR	0.72	0.71	0.45	0.64	0.54	0.31	0.51

Notes: Particles size ranges: UFP: 0.02-1 µm, FP’: 0.3-1 µm; FP: 1-2.5 µm; CP: > 2.5 µm.

Table 5. Concentration of CO [ppm], UFP, FP', FP, CP [pt/cm^3] in different periods of the day (SD = standard deviation, IQR = interquartile range).

		MONITORING SESSION		
		08:00-10:30	12:00-14:30	16:00-18:30
CO	*Mean*	1.88	1.52	1.76
	SD	1.41	0.99	1.07
	Median	1.54	1.28	1.47
	IQR	1.35	1.01	1.24
UFP	*Mean*	51069	32735	36828
	SD	42967	31937	36566
	Median	38193	21993	24416
	IQR	50940	26206	30110
FP’	*Mean*	192.8	197.0	177.4
	SD	199.9	206.1	175.2
	Median	117.1	125.2	110.3
	IQR	172.6	205.9	214.4
FP	*Mean*	2.46	2.39	2.34
	SD	5.37	6.12	5.60
	Median	1.28	1.14	1.11
	IQR	1.85	1.56	1.85
CP	*Mean*	1.10	0.99	0.99
	SD	2.40	2.71	2.90
	Median	0.56	0.48	0.47
	IQR	0.66	0.57	0.59

Notes: Particles size ranges: UFP: 0.02-1 µm, FP’: 0.3-1 µm; FP: 1-2.5 µm; CP: > 2.5 µm.

Table 6. Tau b (Kendall) index for non–parametric correlation tau test

	ME	UFP	FP’	FP	CP
	T	0.205**	0.154**	0.204**	0.259**
	W1	0.207**	0.247**	0.209**	0.222**
	UG	0.254**	0.225**	0.080**	0.051
	P	0.120**	0.130**	0.155**	0.222**
CO	CR	0.128**	0.134**	0.166**	0.234**
	B	0.356**	0.195**	0.255**	0.269**
	W2	0.257**	0.166**	0.195**	0.245**
	C	0.247**	0.171**	0.196**	0.178**
	IN	-0.083**	0.162**	0.238**	0.303**
	T		0.286**	0.268**	0.241**
	W1		0.273**	0.312**	0.324**
	UG		0.192**	-0.044	0.054*
	P		0.284**	0.351**	0.250**
UFP	CR		0.223**	0.319**	0.301**
	B		0.288**	0.350**	0.324**
	W2		0.294**	0.351**	0.351**
	C		0.283**	0.322**	0.207**
	IN		0.201**	0.212**	0.080**
	T			0.605**	0.441**
	W1			0.692**	0.534**
	UG			0.303**	0.267**
	P			0.716**	0.520**
FP’	CR			0.715**	0.573**
	B			0.412**	0.286**
	W2			0.623**	0.466**
	C			0.731**	0.564**
	IN			0.620**	0.292
	T				0.698**
	W1				0.658**
	UG				0.852**
	P				0.627**
FP	CR				0.664**
	B				0.747**
	W2				0.681**
	C				0.628**
	IN				0.544**

Notes:

Particles size ranges: Particles size ranges: UFP: 0.02-1 µm, FP’: 0.3-1 µm; FP: 1-2.5 µm; CP: > 2.5 µm. Microenvironments: IN = indoor (office); CR = Multi-road intersection; W1 & W2 = walk in busy streets; B = bus; UG = metro; P = urban green area; C = car; T = transition environment.

3.9. REFERENCES

1. Marcazzan, G.M.; Valli, G.; Vecchi, R. Factors influencing mass concentration and chemical composition of fine aerosols during a PM high pollution episode. Sci. Total. Environ. 2002, 298:65–79.

2. Knibbs, L.D.; Cole–Hunter, T.; Morawska, L. A review of commuter exposure to ultrafine particles and its health effects. Atmos. Environ. 2011, 45: 2611–2622.

3. Adams, H.S.; Nieuwenhuijsen, M.J.; Colvile, R.N.; McMullen M.A.S.; Khandelwal P. Fine particle ($PM_{2.5}$) personal exposure levels in transport microenvironments, London, UK. Sci. Total. Environ. 2001, 279: 29–44.

4. Gulliver J.; Briggs, D.J. Personal exposure to particulate air pollution in transport microenvironments. Atmos. Environ. 2004, 38: 1–8.

5. Beck–Speier, I.; Dayal, N.; Karg, E.; Maier, K.L.; Roth, C.; Ziesenis, A.; Heyder, J. Agglomerates of ultrafine particles of elemental carbon and TiO_2 induce generation of lipid mediators in alveolar macrophages. Environ. Health. Perspect. 2001, 109: 613–618.

6. Peters, A.; Wichmann, H.; Tuch, T.; Heinrich, J.; Heyder, J. Respiratory effects are associated with the number of ultrafine particles. Am. J. Respir. Crit. Care Med 1997, 155: 1376–1383.

7. Cattaneo, A.; Garramone, G.; Taronna, M.; Peruzzo, C.; Cavallo, D.M. Personal exposure to airborne ultrafine particles in the urban area of Milan. J. Phys. Conf. Ser. 2009, 151 (1): 012039

8. Kaur S.; Nieuwenhuijsen, M.; Colvile, R. Personal exposure of street canyon intersection users to $PM_{2.5}$, ultrafine particle counts and carbon monoxide in Central London, UK. Atmos. Environ. 2005, 39: 3629–3641.

9. Borsós T.; Řimnáčová, D.; Ždímal, V.; Smolík, J.; Wagner, Z.; Weidinger, T.; et al. Comparison of particulate number concentrations in three Central European capital cities. Sci. Tot. Env. 2012, 433: 418–426.

10. Harrison R.M.; Jones, A.M. Multisite study of Particle Number Concentrations in urban air. Environ. Sci. Technol. 2005, 39: 6063-6070.

11. Hussein T.; Puustinen, A.; Aalto, P.P.; Makela, J.M.; Hameri, K.; Kulmala, M. Urban aerosol number size distributions. Atmos. Chem. Phys. 2004, 4: 391-411.

12. Wu D.L.; Lin M.; Chan C.Y.W.Z.; Li, J.; Tao, Y.P.; et al. Influences of Commuting Mode, Air Conditioning Mode and Meteorological Parameters on Fine Particle ($PM_{2.5}$) Exposure Levels in Traffic Microenvironments. Aerosol and Air Qual. Res. 2013, 13: 709–720.

13. Levy J.I.; Houseman, E.A.; Ryan, L.; Richardson, D.; Spengler, J.D. Particle concentrations in urban microenvironments. Environ. Health Persp. 2001, 108: 1051 – 1057.

14. Weijers, E.P.; Khlystov, A.Y.; Kos, G.P.A.; Erisman, J.W. Variability of particulate matter concentrations along roads and motorways determined by a moving measurement unit. Atmos. Environ. 2004, 38: 2993-3002.

15. Binnig J.; Meyer, J.; Kasper G. Calibration of an optical particle counter to provide $PM_{2.5}$ mass for well-defined particle materials. J. Aerosol Sci. 2007, 38: 325–332.

16. Dockery, D.W.; Pope, C.A.; Xu, X.; Spengler, J.D.; Ware, M.E. et al. An association between air pollution and mortality in six US cities. N. Engl. J. Med. 1993, 329: 1753–1759.

17. Pope, C. A.; Thun, M.J.; Namboodiri, M.M.; Dockery, D.W.; Evans, J.S.; Speizer, F.E.; Heath, C.W. Particulate air pollution as a predictor of mortality in a prospective study of US adults. Am. J. Respir. Crit. Care Med. 1995, 151: 669–74.

18. Schwartz, J.; Norris, G.; Larson, T.; Sheppard, L; Claiborne, C.; Koenig, J. Episodes of high coarse particle concentrations are not associated with increased mortality. Environ. Health Perspect. 1999, 107(5): 339.

19. American Thoracic Society, Committee of the Environmental and Occupational Health: Health effects of outdoor pollution. Am. J. Respir. Crit. Care Med. 1996, 153: 3–50.

20. Oberdörster, G.; Oberdörster, E.; Oberdörster, J. Nanotoxicology: An Emerging Discipline Evolving from Studies of Ultrafine Particles. Environ. Health. Perspect. 2005, 113(7): 823–839.

21. Schlesinger R.B. Deposition and Clearance of Inhaled Particle. In Concepts In Inhalation Toxicology, second edition. R.O. McClella and R.F. Henderson (ed) pp. 191-224. London, CRC Press, 1995.

22. Schulz, H.; Harder, V.; Ibald-Mulli, A.; Khandoga, A.; Koenig, W.; Krombach, F.; et al. Cardiovascular effects of fine and ultrafine particles. J. Aerosol Med. 2005, 18: 1–24.

23. Daigle C.C.; Chalupa, D.C.; Gibb, F.R.; Morrow, P.E.; Oberdörster, G.; Utell, M.J. Frampton, M.W. Ultrafine particle deposition in humans during rest and exercise. Inhal. Toxicol. 2003, 15: 539–552.

24. Frampton, M.W. Does inhalation of ultrafine particles cause pulmonary vascular effects in humans? Inhal. Toxicol. 2007, 19(1): 75–79.

25. Phalen, R.F.; Oldham, M.J. Aerosol dosimetry considerations. Clin. Occup. Environ. Med. 2006, 5: 773–784.

26. Pietropaoli, A.P.; Frampton, M.W.; Hyde, R.W.; Morrow, P.E.; Oberdörster, G.; Cox, C.; et al. Pulmonary function, diffusing capacity, and inflammation in healthy and asthmatic subjects exposed to ultrafine particles. Inhal. Toxicol. 2004, 16(s1): 59-72.

27. Samet, J.M.; Demarini, D.M.; Malling, H.V. Do airborne particles induce heritable mutations? Science 2004, 304 (5673): 971-972

28. Kittelson, D.B. Engines and nanoparticles: A review. J. Aerosol Sci. 2008, 29: 575–88.

29. Morawska, L.; Zhang, J. Combustion sources of particles. 1. Health relevance and source signatures. Chemosphere 2002, 49: 1045–1058

30. Lonati, G.; Ozgen, S.; Luraghi, I.; Giugliano M. Particle number concentration at urban microenvironments. Chem. Eng. Trans. 2010, 22: 137–142.

31. Lonati, G.; Ozgen, S.; Ripamonti, G.; Cernuschi, S.; Giugliano M. Pedestrian exposure to size–resolved particles in Milan. J. Air Waste Manage Assoc. 2011, 61: 1273–1280.

32. Marcazzan, G.M.; Ceriani, M.; Valli, G.; Vecchi, R. Source apportionment of PM_{10} and $PM_{2.5}$ in Milan (Italy) using receptor modeling. Sci. Total Environ. 2003, 317: 137–147.

33. Giugliano, M.; Lonati, G.; Butelli, P.; Romele, L.; Tardivo, R.; Grosso M. Fine particulate ($PM_{2.5}$–PM_1) at urban sites with different traffic exposure. Atmos. Environ. 2005, 39: 2421–2431.

34. Vecchi, R.; Marcazzan, G.; Valli, G.; Ceriani, M.; Antoniazzi, C. The role of atmospheric dispersion in the seasonal variation of PM_1 and $PM_{2.5}$ concentration and composition in the urban area of Milan (Italy). Atmos. Environ. 2004, 38: 4437–4446.

35. 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. J. Aerosol Sci. 2008, 39: 827–49.

36. Cyrys, J.; Stolzel, M.; Heinrich, J.; Kreyling, W.G.; Menzel, N.; Wittmaack, K.; et al. Elemental composition and sources of fine and ultrafine ambient particles in Erfurt, Germany. Sci. Tot. Environ. 2003, 305: 143–156.

37. Shi, J.P.; Khan, A.A.; Harrison, R.M.; Measurements of ultrafine particle concentration and size distribution in the urban atmosphere. Sci. Tot. Environ. 1999, 235: 51–64.

38. Morawska, L.; Ristovski, Z.; Jayaratne, E.R.; Keogh, D.U.; Ling X. Ambient nano and ultrafine particles from motor vehicle emissions: Characteristics, ambient processing and implications on human exposure. Atmos. Environ. 2008, 42: 8113–38.

39. Flachsbart, P.G. Human exposure to carbon monoxide from mobile sources. Chemosphere 1999, 1:301–29.

40. Morris, R.D. Low–level carbon monoxide and human health. In: Carbon monoxide toxicity. D.G. Penney (ed) pp. 381–391. London: CRC Press, 2000.

41. Townsend, C.L.; Maynard R.L. Effects on health of prolonged exposure to low concentrations of carbon monoxide. Occup. Environ. Med. 2002, 59: 708–11.

42. Allred, E.N.; Bleecker, E.R.; Chaitman, B.R.; Dahms, T.E.; Gottlieb, S.O.; Hackney, J.D.; et al. Short–term effects of carbon monoxide exposure on the exercise performance of subjects with coronary artery disease. N. Engl. J. Med. 1989, 321: 1426-1432.

43. Burnett R.T.; Dales, R.E.; Brook, J.R.; Raizenne, M.E.; Krewski, D. Association between ambient carbon monoxide levels and hospitalizations for congestive heart failure in the elderly in 10 Canadian cities. Epidemiology 1997, 8: 162–67.

44. Chen T.M.; Gokhale, J.; Shofer, S.; Kuschner, W.G. Outdoor air pollution: Nitrogen dioxide, sulfur dioxide, and carbon monoxide health effects. Am. J. Med. Sci. 2007, 333(4): 249–256.

45. Reboul, C.; Thireau, J.; Meyer, G.; André, L.; Obert, P.; Cazorla, O.; Richard, S. Carbon monoxide exposure in the urban environment: An insidious foe for the heart? Respir. Physiol. Neurobiol. 2012, 184(2): 204-212.

46. Yang, W.; Jennison, B.L.; Omaye, S.T.; Cardiovascular disease hospitalization and ambient levels of carbon monoxide. J. Toxicol. Environ. Health. A. 1998, 55: 185–196.

47. Biggeri, A.; Baccini, M.; Bellini, P.; Terracini, B. Meta–analysis of the Italian studies on short–term effects of air pollution (MISA) 1990–1999. Int. J. Occup. Environ. Health. 2005, 11: 107–22.

48. Rodes, C.E.; Thornburg, J.W. Breathing zone exposure assessment. In: Aerosols handbook: Measurement, dosimetry, and health effects. L.S. Ruzer, N.H. Harley (ed) pp. 61–74. Boca Raton: CRC Press, 2005.

49. Cattaneo, A.; Taronna, M.; Garramone, G.; Peruzzo, C.; Schlitt, C.; Consonni, D.; Cavallo, D.M. Comparison between personal and individual exposure to urban air pollutants. Aerosol Sci. Technol. 2010, 44: 370–379.

50. Buonanno, G.; Fuoco, F.C.; Stabile, L. Influential parameters on particle exposure of pedestrians in urban microenvironments. Atmos. Environ. 2011, 45: 1434–1443.

51. Robakiewicz, P.; Ryder, E.F. Statistic: Detecting differences Among groups. In Current protocols in protein Science (commonly used techniques). Supplement 21: A.3 G.1-A.3G.22 New York: John and Wiley & Sons, Inc., 2000.

52. Norman, G.; Streiner, D.L. Biostatistica: Quello che avreste voluto sapere. Milano: Casa Editrice Ambrosiana – Zanichelli, 2000

53. Miller, J.N.; Miller, J.C. Statistics and Chemiometrics for Analytical Chemistry. Fifth Edition. Harlow. Pearson Education Ltd, 2005.

54. Kaur, S.; Nieuwenhuijsen, M.J.; Colvile, R.N. Fine particulate matter and carbon monoxide exposure concentrations in urban street transport microenvironments. Atmos Environ 2007, 41: 4781–4810.

55. Kaur, S.; Nieuwenhuijsen, M.J. Determinants of personal exposure to $PM_{2.5}$, ultrafine particle counts, and CO in a transport microenvironment. Environ. Sci. Technol. 2009, 43:4737–4743.

56. Duci, A.; Chaloulakou, A.; Spyrellis, N. Exposure to carbon monoxide in the Athens urban area during commuting. Sci. Total Environ 2003, 309: 47–58.

57. Hertel, O.; Hvidberg, M.; Ketzel, M.; Storm, L.; Stausgaard, L. A proper choice of route significantly reduces air pollution exposure – a study on bicycle and bus trips on urban streets. Sci. Tot. Env. 2008, 389: 58–70.

58. McCreanor, J.; Cullinan, P.; Nieuwenhuijsen, M.J.; Stewart–Evans, J.; Malliarou, E.; Jarup, L.; et al. Respiratory effects of exposure to diesel traffic in persons with asthma. New. Engl. J. Med. 2007, 357: 2348–2358.

59. Colombi, C.; Angiu, S.; Gianelle, V.; Lazzarini, M. Particulate matter concentrations, physical characteristics and elemental composition in the Milan underground transport system. Atmos. Environ. 2013, 70: 166–178.

60. Nieuwenhuijsen, M.J.; Gomez–Perales, J.E.; Colvile, R.N. Review: Levels of particulate air pollution, its elemental composition, determinants and health effects in metro systems. Atmos. Environ 2007, 41: 7995–8006.

61. Thatcher, T.L.; Layton, D.W. Deposition, resuspension, and penetration of particles within a residence. Atmos. Environ. 1995, 29: 1487–1497.

62. Dor, F.; Le Moullec, Y.; Festy, B. Exposure of city residents to carbon monoxide and monocyclic aromatic hydrocarbons during commuting trips to Paris metropolitan area. J. Air Waste Manage. Assoc. 1995, 45: 103–110

63. Rank, J.; Folke, J.; Jesperen P. Differences in cyclists and car drivers exposure to air pollution from traffic in the city of Copenhagen. Sci Total. Environ. 2001, 279: 131–136.

64. Cattaneo, A.; Peruzzo, C.; Garramone, G.; Urso, P.; Ruggeri, R.; Carrer, P.; Cavallo, D.M. Airborne particulate matter and gaseous air pollutants in residential structures in Lodi province, Italy. Indoor Air 2011, 21: 489–500.

65. Kaur, S.; Clark, R.; Walsh, P.; Arnold, S.J.; Colvile, R.N.; Nieuwenhuijsen, M.J. Exposure visualisation of ultrafine particle counts in a transport microenvironment. Atmos. Environ. 2006, 40(2): 386.

66. Koushki, P.A., K.H. Al–Dhowalia, S.A. Niazi: Vehicle occupant exposure to carbon monoxide. J. Air. Waste Manage. Assoc. 1992, 42(12): 1603.

67. Zagury, E., Le Moullec, Y.; Momas, I. Exposure of Paris taxi drivers to automobile air pollutants within their vehicles. Occ. Env. Med. 2000, 57: 406.

68. Kleeman M.J., Cass, G.R. Source contributions to the size and composition distribution of urban particulate air pollution. Atmos. Environ. 2008, 32: 2803–2816.

69. Aalto, P.; Hämeri, K.; Paatero, P.; Kulmala, M.; Bellander, T.; Berglind, N.; et al. Aerosol particle number concentration measurements in five european cities using TSI-3022 condensation particle counter over a three-year period during health effects of air pollution on susceptible subpopulations J. Air Waste Manage. Assoc. 2005, 55: 1064–1076.

70. Wåhlin, P.; Palmgren, F.; Van Dingenen, R. Experimental studies of ultrafine particles in streets and the relationship to traffic. Atmos. Environ. 2001, 35 (S1) S63-S69.

71. Diapouli, E; Chaloulakou, A.; Spyrellis, N. Levels of ultrafine particles in different microenvironments - Implications to children exposure. Sci. Total Environ. 2007, 388: 128-136.

72. Marconi, A.; Cattani, G.; Cusano, M.; Ferdinandi, M.; Inglessis, M.; Viviano, G.; et al. Two-years of fine and ultrafine particles measurements in Rome, Italy J. Toxicol. Env. Heal. 2007, A70 213-221.

73. Molnar, P.; Janhall, S.; Hallquist, M. Roadside measurements of fine and ultrafine particles at a major road north of Gothenberg. Atmos. Environ. 2002, 36: 4115-4123.

74. Gomiscek, B.; Hauck, H.; Stopper, S.; Preining, O.; Spatial and temporal variations of PM_1, $PM_{2.5}$, PM_{10} and particle number concentration during the AUPHEP-project. Atmos. Environ. 2004, 38: 3917-3934.

75. Brauer, M.; Hirtle, R.D.; Hall, A.C.; Yip, T.R. Monitoring personal fine particle exposure with a particle counter. J. Expo. Anal. Environ. Epidemiol. 1999, 9: 228–236.

4. POPULATION EXPOSURE TO ULTRAFINE PARTICLES

Average daily ultrafine particles (UFP) exposure of adult Milan subpopulations (defined on the basis of gender, and then for age, employment or educational status),in different exposure scenarios (typical working day in summer and winter) were simulated using a microenvironmental stochastic simulation model. The basic concept of this kind of model is that time-weighted average exposure is defined as the sum of partial microenvironmental exposures, which are determined by the product of UFP concentration and time spent in each microenvironment. In this work, environmental concentrations were derived from previous experimental studies that were based on microenvironmental measurements in the city of Milan by means of personal or individual monitoring, while time-activity patterns were derived from the EXPOLIS study. A significant difference was observed between the exposures experienced in winter (W: 28415 pt/cm^3) and summer (S: 19558 pt/cm^3). Furthermore, simulations showed a moderate difference between the total exposures experienced by women (S: 19363 pt/cm^3; W: 27623 pt/cm^3) and men (S: 18806 pt/cm^3; W: 27897 pt/cm^3). In addition, differences were found as a function of (I) age, (II) employment status and (III) educational level; accordingly, the highest total exposures resulted for (I) 55–59 years old people, (II) housewives and students and (III) people with higher educational level (more than 10 years of scholarity). Finally, significant differences were found between microenvironment-specific exposures.

4.1. INTRODUCTION

Exposure may be defined as the concentration of a particular agent that reaches a target organism, system, or population in a specific frequency for a defined duration [1]. Exposure can also be defined as the contact of a target and a chemical, physical, or biological agent in an environmental carrier medium [2 - 4]. More expansively, exposure denotes the contact between an agent and a target, which takes place at a contact boundary or surface over an exposure period. Exposure to ultrafine particles (UFP, <100 nm) is an important topic in epidemiological and toxicological studies and is deemed to be a major risk affecting human health. Therefore, airborne particle studies were performed in the recent years to identify the main UFP sources and to characterize population exposure.

Exposure can be measured or modeled [5], either directly (personal measurements) or indirectly (microenvironment approach) [3 - 10]. In order to properly evaluate the UFP exposure, personal monitoring is considered as the only way to relate particle exposure levels to the activities performed and microenvironments visited. For example, a recent study carried out in central Italy during summer and winter in 2012 [11] evaluated the influence of time-activity patterns on the personal exposure of 24 Italian couples to UFP based on their time-activity patterns, through an experimental measurement of personal exposure over 48 h. Time-activity patterns, particle number concentration exposure and the related dose received by the participants (in terms of particle alveolar deposited surface area) were measured. Similarly, in another study [12] the examination of personal behavior and activity was combined with the measurement of particulate matter with high temporal resolution and over

full 24 h periods using an optical aerosol spectrometer. Personal monitoring offers the most accurate measurements of exposure to air pollutants. The drawback of such methods, however, is the high cost of implementation and the associated small number of observations that tends to produce sample biases: only specific types of subjects would carry monitors and record their daily activities for a relatively prolonged time period. For this reason, personal monitoring is often used as a complement in exposure models to assess air pollution exposures in health studies. These models use personal or household exposure monitoring, and appear well-suited to overcome the problem of achieving population representative samples while understanding the role of exposure variation at the individual level. Thus, exposure modeling is recognized as a valuable and cost-effective tool for assessing potential population exposures to air pollution and represent an element of exposure assessment, which evaluates, qualitatively and quantitatively, the degree of intake or uptake that is likely to occur. Exposure models allow estimation of pollutant exposure for groups of people and time periods for which personal monitoring has not been conducted; models can be also used to combine information from different sources to produce estimates for population exposures that would be very expensive or impossible to perform [13, 14]. For example, some studies reviewed in Jerrett et al. [15] combine personal or regional monitoring with other air pollution exposure methods (hybrid models) in order to compare or validate results from exposures assigned from modeling of ambient exposure with the use of experimental monitoring at differing scales (i.e., personal and regional monitoring). These methods appear well-suited to overcome the conundrum of achieving population representative samples while understanding the role of exposure variation at the individual level. Remote sensing and activity–space analysis will complement refinements in pre-existing methods, and permit to reduce scientific uncertainties in exposure analysis. An application of activity–space analysis may be found in a recent study [16], in which activity-pattern data were combined with microenvironmental data (human activities and particle number size distributions) using an indirect approach, in order to evaluate the doses of alveolar and tracheobronchial deposited particle number and surface area experienced by different age groups in south and north Italy. This study used the average particle number size distribution data obtained from an experimental measurement survey in major microenvironments, together with activity pattern data to estimate the tracheobronchial and alveolar dose of sub-micrometer particles for different population age groups in Italy. Furthermore, time-activity patterns were combined with microenvironmental data through a Monte Carlo simulation in order to evaluate the daily alveolar and tracheobronchial number or surface area deposited doses for different age group populations [17]. More generally, physical stochastic models describe parameters with frequency or probability distributions instead of single values. These models can be used to predict population exposures for existing, past or scenario situations and for subpopulations with no available measurement data [5], by simulations based on the distributions of input parameters. In this case, the full description of personal exposure to an air pollutant requires knowledge of the magnitude of pollutant concentration in the exposure environment, duration and time pattern of exposure [5]. As mentioned before, the microenvironment (ME) approach [18] has been commonly used to model exposures [7,14,19 - 21].

In such a case, the exposure (E) is calculated as the sum of the partial exposures across the visited MEs according to the relationship described by *Equation 1* (*where Ci is the concentration in the i^{th} microenvironment, T_i is the fractional time spent in the i^{th} microenvironment, and N is the number of microenvironments*). The exposure E is often defined as "total exposure", but this study refers to the term "time-weighted average exposure", because the simulated exposure E is the total exposure (expressed in particle/cm^3) for the considered subpopulation, expressed as the average concentrations weighted on the integration period (24 h).

$$E = \sum_i^N C_i T_i \qquad (1)$$

This paper describes the simulation of exposure to UFP and evaluates the differences of the estimates by subpopulation and season. A microenvironmental probabilistic exposure model was developed in order to simulate the exposure of different subpopulations to UFP in the city of Milan, distinguished by gender, age, employment status and educational level. Our approach includes the use of time-activity data of subpopulations within the study area and average concentrations in different environments collected by on-site experimental measurements.

The present study was carried out in the city of Milan, which is the second largest city in Italy and has a population of more than one million inhabitants. Its urban area (181 km^2) is characterized by a high density of residential and commercial buildings and very high traffic volumes, while many factories are located at the city's outskirts. As with many large cities, Milan suffers from high levels of air pollution, especially in winter, during which time air quality limits are frequently exceeded and exceptionally high particulate matter (PM) mass concentrations are frequently recorded [22]. The UFP concentrations are usually particularly high along busy roads, common in urban transport environments [23 - 25], generated in large quantities by fuel combustion processes, with vehicular traffic exhaust being the predominant source in urban environments [26]. The main objective of this study was to estimate individual UFP exposures in general subpopulations during a typical weekday for summer and winter periods within the metropolitan area of Milan (Italy). The quantification of daily exposures for the general population is important to provide better estimates in investigations of long-term health effects. Other specific aims are (I) to use the model to simulate the daily mean exposures to UFP and (II) to observe exposure distributions and differences among different subpopulations as a function of seasonal variability (summer and winter) and behavioral factors (time use).

4.2. METHODS

Exposure models based on *Equation 1* should describe the microenvironmental concentration of the considered pollutant and fully characterize the behavior (time use) of the study population. Relevant microenvironments need to be defined to perform exposure simulation; at the same time, data on time-activity patterns are needed, specified as the amount of time spent in each microenvironment. People spend their time differently, depending for example on employment status, age [14], season, and day of week [27].

Exposure models require data on human time patterns: time-activity data are required implicitly to determine the status of source use, the activity level of subjects, and other activities that may affect exposure components. Therefore, it is important to define groups of people with similar time-activity patterns. The exposure distributions for subpopulations need to be simulated separately, and eventually merged together to get an exposure distribution for the overall population.

Input Data: Time Activity Patterns

The present study focused on the city of Milan; the subpopulations were firstly defined on the basis of gender, and then for age, employment status or educational level (Table 1). Subpopulations were defined on the basis of expected general similarity of time-activity patterns within groups. This selection of subpopulations and MEs was also made in accordance with the availability of activity pattern data: the selected source was the EXPOLIS study, in which the time (mean, standard deviation) spent in 11 different MEs (*table 2*) by Milan's subpopulation (years: 1996 - 2000) was described and whose results are available online [28]. Time-activity data refer to the typical working day (excluding weekend and holiday) without seasonal distinction and allow defining the amount of time spent daily in each microenvironment, including time spent in commuting and at home, work or school locations. A summary of UFP concentrations segregated by MEs and time use for the whole study population is listed in *table 2*. The ME where people spent the majority of their time was the indoor environment, with the highest contribution to the daily exposure deriving from residential indoor environments (49-78%). Time spent outdoors and commuting was generally limited (0-17% and 0-8%, respectively). However, some transport ME may represent an important component of human exposure. Commuting time was mostly spent on active (walk/bike: 2-8 %) or motorized transport (car: 2-7 %), both of which represent MEs with high UFP mean concentrations (*table 2*).

Input data: microenvironmental concentrations

Microenvironmental concentrations (*table 2*) were derived from previous studies performed in the city of Milan. UFP concentration for "In-transit" MEs and "Work-Indoor" ME were obtained from a study performed within the central area of Milan in different seasons, in which experimental data were collected continuously during each monitoring period along an established urban pathway, moving through different MEs [29]. These data were then updated with an up-to-date measurement, performed following the same study design in 2013 for a total amount of about more than 100 hours of performed measurements, distributed in 28 days. The UFP concentrations for the remaining MEs were obtained from a study (PM-CARE project) involving 81 non-smoking senior volunteers living in the urban and suburban area of Milan. During the PM-CARE project, 162 24-hour monitoring sessions were performed in the warm and cold seasons of 2005-2006 following the same sampling protocol and study design [30]. In all these studies a time-activity diary was completed in order to accurately define the concentration data as a function of the different monitored activity and environments. Particle number concentrations (PNC) of airborne UFP were measured using a condensation particle counter (CPC) capable to provide real-time

measurement of particles. Data were collected with high sampling frequency (30 seconds); the instruments were placed in a backpack and carried by one investigator [19] or in a mobile monitoring unit (MMU) developed to sample simultaneously some urban pollutants of interest for public health purposes [30, 31]. Since the sampling inlets were not placed in strict correspondence with the breathing zone (the hemisphere of 30 cm radius extending in front of the face) [32], the results refer to the so-called "*individual exposure*" (in proximity of subjects, within 3 m). The individual exposure approach allows the determination of concentrations without losing accuracy with respect to personal measurements performed in the breathing zone, except for coarse particles [31]. The number concentration metric was selected because of its better accuracy in the continuous monitoring of spatial and temporal variations of UFP concentration, compared with continuous photometric measurement of mass concentrations (especially if these latter are not properly corrected using simultaneous gravimetric data) [24]. Before analysis, data cleaning was performed to exclude invalid values and clear up missing data.

Exposure Model

A microenvironmental probabilistic exposure model was used to simulate the daily personal exposures of urban subpopulations by combining the UFP concentration in selected MEs and the time spent by people in those MEs. In each ME a homogeneous UFP concentration is assumed. The choice for this kind of model (basic microenvironmental model, using a stochastic approach) has been defined due to some considerations. The first was the availability of information on time-activity patterns and environmental concentrations, as this kind of model needs real data in the model-building process. The second consideration was the usability of the model's output: statistical models are considered to be useful for descriptive analysis and hypothesis testing [5], and thus they are well-suited for this study. In this work, the combination of the UFP concentration in an ME and the time spent by a subpopulation in the ME was described by *equation 1* and implemented in a Microsoft Excel workbook. An Excel add-on software package was needed to supply the probabilistic functions for the stochastic functionality; a Monte Carlo simulation approach with Latin hypercube sampling (2000 iterations) was chosen for calculation. A probability distribution function is assumed for each parameter: time-activity data were fitted on beta distribution (alpha and beta parameters were calculated starting from the mean and standard deviation of time spent in each ME), while UFP concentrations were fitted on lognormal distributions (calculated again from the means and standard deviations) with Monte Carlo sampling (2000 iterations). From these simulated distributions, random values were then taken using the Latin hypercube method. The sampled parameters were combined to result in a partial exposure for each ME. By summing the partial exposures for each individual ME, the total exposure distributions ("in-transit", "indoor", "outdoor" and "total" exposure) was calculated for each considered subpopulations and both for summer and winter. Statistical analysis was performed to identify statistically significant differences ($p < 0.01$) via IBM SPSS Statistics 20.0 (IBM, Armonk, NY, USA), which consisted of factorial analysis of variance (ANOVA) with Helmert contrast and Turkey post-hoc test. All results refer to the

daily (24-hour weighted average) mean exposure, using number of particle for cubic centimeter (pt/cm^3) as the unit.

4.3. RESULTS

The seasonal trends of exposure to UFP of different subpopulations living within the city of Milan during a typical working day were estimated using the stochastic microenvironmental model described above. Based on the characteristics reported in *table 1*, a total of 26 subpopulations were identified. For each of them an exposure simulation was performed, both for summer and winter, thus generating 52 exposure profiles. A factorial ANOVA was conducted to explore the impact of season, gender and population characteristic on the simulated UFP exposure levels. Subjects were divided into groups according to their gender and consequently age, employment status or educational level, then two seasonal patterns (summer and winter) were defined for exposure simulations. On average, statistically significant differences ($p < 0.05$) were found in total UFP exposure as a function of season and subgroup characteristics (age, employment, education) but not as a function of gender ($p = 0.067$). Differences were also found among exposures simulated in the studied MEs. On average, the total daily exposure indoors was characterized by the same statistically significant differences in relation to the same variables, but with a better statistical significance between genders ($p = 0.066$). In contrast, there were no statistically significant differences in outdoor total exposures as a function of season ($p = 0.088$) and of population characteristics such as age, employment or education ($p = 0.905$).

UFP Concentrations and Exposure in Urban Microenvironments

The UFP concentrations in Milan have been widely investigated, and seasonal trends, chemical compositions and sources have been described [26, 29, 34-38]. The measured UFP concentrations of various indoor and outdoor MEs demonstrate a significant variability among indoor MEs and relative homogeneity in outdoor MEs [29]: the highest urban UFP concentrations generally occur while moving along busy streets or in their immediate environments, either on foot or by motorized vehicles and the lowest concentrations are usually detected in indoor environment and in urban green areas. This is consistent with a previous study [39], which states that personal exposure to PM levels were similar between bicycle, bus, and car, while the underground rail tube showed higher concentrations; cyclists were the group with slightly lower exposure, which was influenced by the cyclists' position on the street and the ability to avoid traffic jams. Regarding the temporal variation of environmental UFP concentrations, appreciable differences were found between working and non-working days, between different periods of the day and between seasons [26, 29].

Simulated Exposure

The results of the exposure simulations, segregated by each ME, are shown in Table 3. Results are shown as statistics calculated among all the study subpopulations within the Milan urban area. The following findings were obtained from the analysis of the exposure concentrations in different MEs: highest median exposure (19,561 pt/cm^3; 80.6% of the total

exposure) was obtained, as expected, for indoor environments (Home, Work, Other), which was one order of magnitude higher than the outdoor exposure (651 pt/cm^3; 2.6%) and well above the exposure simulated for the whole "in-transit" environments (4217 pt/cm^3; 16.3%). Despite the highest UFP personal concentrations occurred in traffic ME (Table 2), the simulated exposures were actually dominated by indoor environments as (I) the time spent in these environments is very high (Table 2) and (II) the residential environment in Italy is characterized by specific sources, among which the most important is gas cooking [14]. A previous study estimated a daily average UFP exposure of about 16×10^4 pt/cm^3 for people commuting in Milan, with indoor home exposure providing about 46% of total daily exposure, indoor office exposure about 30%, and transport environments about 24% (almost insensitive to transportation mode) [25]. The results from the present study are up to 20% higher, but in the same order of magnitude, with an average exposure (among all the profiles) of $2.4 \times 10^4 \pm 4.65 \times 10^3$ pt/cm^3, with indoor home exposure providing 61.9 ± 5.4% of total daily exposure, indoor office exposure about 11.1 ± 4.1%, and transport environments about 16.7 ± 4.4% (but sensitive to transportation mode). The in-transit MEs show a significant contribution to the total exposure, especially considering the limited amount of time spent in these MEs (8-13%). Among these, Car/Taxi (1783 pt/cm^3; 7.5%) and Walk/Bike (1230 pt/cm^3; 5%) recorded the highest simulated median exposure. On the contrary, the lowest exposure were obtained for Motorbike/Scooter and Train/Metro MEs, which are also characterized by the worst temporal representativeness of collected data (Table 2). Finally, time spent in outdoor MEs did not show a significant contribution to the total estimated exposure (651 pt/cm^3; 2.6%). The differences in the calculated exposures for each ME were statistically significant ($p < 0.01$) (Kruskal-Wallis one-way ANOVA). Thus, the exposure levels were highly dependent on the spatial behavior and the surrounding microenvironment conditions.

Seasonal patterns

Published studies for Milan showed a strong seasonal effect in particle concentration values, mainly due to the differences in average dispersion conditions of the atmosphere in summer and winter. Particle concentrations were strongly influenced by seasonal variability, which is more evident for the finer particle sizes, with higher values in winter [35 - 37]. This seasonal variation is, essentially, linked more to the differences in average thermodynamic and meteorological conditions of the atmosphere than to the variations in the type or number of emitting sources. However, the observed seasonal behavior of particulate concentrations may also be ascribed to the presence of additional emission sources (i.e., domestic heating) during the cold season, which contributes to primary as well as to secondary aerosol production because of the large emission of gaseous precursors. The simulated exposures, segregated by ME and season, are shown in Figure 1 and Figure 2. Here, a significant difference ($p < 0.01$; Mann–Whitney U Test) was observed between the exposures experienced in the two different seasons: on the whole, the highest median exposure (28415 pt/cm^3) was obtained in winter (W), as expected; this level was about 45% higher than in summer (S) (19558 pt/cm^3). Similarly, the average UFP exposure experienced by 24 Italian couples was higher in winter (women: 2.9×10^4; men: 1.3×10^4 part/cm^3) than summer

(women: 1.8×10^4; men: 9.2×10^3 part/cm^3) [11]. The whole indoor exposure showed a significant increase in median values from summer to winter (16041 pt/cm^3 and 21511 pt/cm^3, respectively), but this is accompanied by a slight decrease in the relative contribution of indoor environments to the total exposure (S: 84.4%, W: 77.5%). The exposure levels calculated for outdoor environments appear to be almost unchanged between the seasons (S: 561 pt/cm^3; W: 716 pt/cm^3) as well as their contribution to the total exposure (S: 2.8%; W: 2.6%). Contrarily, transit MEs show a strong variation between seasonal simulations: in-transit median exposure in winter (5690 pt/cm^3) was about 130% higher than in summer (2459 pt/cm^3). Moreover, the relative contribution of in-transit exposure to the total daily exposure increases from a median value of 13.2% in summer to 20.7%. The Car/Taxi and Walk/Bike MEs recorded the highest increases in partial exposure simulation. The lowest exposure was obtained for Motorbike/Scooter and Train/Metro MEs.

Subpopulation characteristics: gender

The UFP exposure simulations were made on the basis of the representative time pattern profile determined for males and females. Results separated by season and gender show a moderate difference between the total exposures of male (M) and female (F) subpopulations (Table 4). This is consistent with previous studies on exposure to sub-micrometer particles [11,17], which estimated that females receive higher daily doses than males. This difference should be not addressed to a "gender effect", but can be explained through the different lifestyle between female and male. According to the time use data, differences were found for the separate MEs: the mean total indoor exposure represents again the highest fraction of the total estimated exposure, which is stably higher for women (S: 16634 pt/cm^3; W: 22121 pt/cm^3) than for men (S: 15464 pt/cm^3; W: 20789 pt/cm^3). Mean contributions of in-transit exposure to the total exposure were higher for men (S: 2567 pt/cm^3; W: 6119 pt/cm^3) than for women (S: 2347 pt/cm^3; W: 4929 pt/cm^3), as well as the simulated outdoor exposures. After the indoor MEs, the major mean contribution to the total exposure was found in the in-transit MEs: the Car/Taxi and Walk/Bike MEs recorded the highest modeled exposures. Finally, the lowest mean exposures were obtained for Motorbike/Scooter, Train/Metro and "Other Outdoor" MEs.

Subpopulation characteristic: age

UFP exposure simulations were made for 4 different age-stratified subpopulations. The results, separated by season, are shown in Table 5. The in-transit and indoor MEs show a significant variation among subpopulations: indoor exposure ranged from 76.1% (in winter) to 85.2% (in summer) of total estimated exposure, while the relative contribution of in-transit exposure was about 12% in summer and 20% in winter, as discussed above. Simulated outdoor exposures were almost unchanged with age, representing always a small fraction of the total exposure (2.4 - 3.6 %). The highest mean indoor exposure was observed for 55-59 years old people, both in summer (16823 pt/cm^3) and in winter (22361 pt/cm^3). This could explain the fact that this subpopulation was characterized by the highest mean total exposure, too (S: 19933 pt/cm^3; W: 29064 pt/cm^3). In contrast, the highest mean in-transit exposure was observed for the youngest population (25-34 years), both in summer (2567 pt/cm^3) and in

winter (5860 pt/cm^3), as a direct consequence of the major amount of time spent in this kind of ME, in which the highest UFP concentrations were found. In fact the "age effect" may be ascribable to the different lifestyles, since other factors (characteristics of the different age groups and performed activities) were found to have negligible effect on daily doses of sub-micrometer particles [12,16,17]. Thus, the reason of the outlined differences between subgroups (and in comparison with previous studies) may be found in the different particle exposure levels experienced in different MEs

Subpopulation characteristics: employment status

UFP exposure simulations were also made for five subpopulations of different employment status. The results (Table 6) showed a significant variation among these subpopulations: the total indoor exposure represented the highest fraction of the total estimated exposure, but ranging in a wide interval (67.7 - 84.7%). The contributions of in-transit exposure to the total exposure were also found to be quite variable (10.1 - 24.5%), as well as the simulated exposure in outdoor environments, where a relevant fraction of the total exposure was also estimated for some profiles (up to 7.8%). The highest mean indoor exposures were observed, both in summer and winter, for housewives (S: 19564 pt/cm^3; W: 25377 pt/cm^3) and students (S: 17954 pt/cm^3; W: 22791 pt/cm^3). In contrast, the highest mean commuting exposures were observed, both in summer (3862 pt/cm^3) and winter (8012 pt/cm^3), for retired people, as well as for their total outdoor exposure (S: 1516 pt/cm^3; W: 2561 pt/cm^3). Thus, once again, differences between people can be explained by the time-activity pattern of the individuals, as well as the environments in which they spend their time. In fact, people can experience different exposure profiles and short-term exposures that may contribute significantly to daily average exposure: recently it has been found that the average exposure to UFP experienced by Italian homemakers were higher (roughly twice) than their spouses (full-time workers) [11].

Subpopulation characteristic: educational level

Table 7 shows the UFP exposure estimates for four different subpopulations segregated by their educational level (years of scholarity). As discussed above, all winter exposures were typically higher than summer exposures. The total indoor exposure represents the highest fraction of the total estimated exposure, ranging in a limited interval (74.7%–81.6 %). The contributions of in-transit exposure to the total exposure were also found to be quite variable (12.4 - 21.7%), as well as simulated exposure for outdoors (2.0 - 5.3%). The highest mean indoor exposures were observed, both in summer and winter, for the categories with higher educational level ("10-14 years" and "≥17 years"), which also experience the highest total exposure. Again, the highest mean in-transit exposure was found for the category "10-14 years", while for the outdoor MEs, the highest mean exposures was found for the category "0-9 years".

4.4. DISCUSSION

Analysis of literature data on the time spent in the study MEs [28] showed that people used to spend much less time outdoors (about 1% of the day) than indoors (male: 87 ± 25%; female: 90 ± 31%) (*table 4*). The time indoors was mostly spent at home, equaling approximately two thirds of all the time spent indoors and more than 50% of the day. People spent on average about 30% of their time at workplace, mostly indoors (male: 29 ± 11%; female: 24 ± 13%). This was true for all subgroups by gender, age, educational level, employment status and season. Women had the highest average time spent indoors, and regarding employment status, self-employed workers and retired people spent the least amount of time indoors. Typically, rather long time periods (with small standard deviations) were spent on average in major MEs such as home indoors or work indoors. In contrast, only short periods (with relatively high standard deviations) are spent outdoors or in-transit. In-transit time represented about 10% of the typical working day (*table 4*). Traveling by car and walking or biking are the most popular means of transportation for the adult urban population of Milan. There are also noticeable differences in the average use of some means of transportation. On average, driving a car and walking/biking each account for approximately more than half of the total time spent in-transit. Difference in total time in traffic was found between specific subgroups; gender and employment status are very important factors. Time spent in cars has been shown to be one of the most important determinants of traffic exhaust exposure [40]. Walking or biking on city roads also often results in very close proximity to fresh traffic exhaust. Public transportation in general was more likely to be used among women. Age did not significantly contribute to the time-activity patterns in our study, while the general employment status often affected the time-activity patterns. Men generally spent more time in-transit than women. Employed participants spent more time in-transit than others. The exposure levels show a stronger correlation with time spent in each ME ($r_{spearman}$ 0.952; $p < 0.01$) rather than with the ME's UFP concentrations ($r_{spearman}$ 0.149; $p < 0.01$), thus the results from the present study showed that the variability in UFP exposure is mainly related to behavioral factors (e.g., mode of transport) and seasonal patterns, both of which have a very large influence on the human exposure to UFP [35-37]. Thus, demographic and socio-demographic factors may be considered as the major determinants of UFP exposure in urban environments. The results from this modeling study are consistent with literature [13, 14, 18]. UFP concentrations in Milan have been widely investigated, and seasonal trends, chemical compositions, and sources have been described [26, 29, 34-38].

The measured UFP concentrations within a variety of indoor and outdoor MEs demonstrate significant variability among some indoor MEs and relative homogeneity in outdoor MEs [29]: the highest urban UFP concentrations generally occur when moving along busy streets or in their immediate environments, either on foot or by motorized vehicles, and the lowest concentrations are usually detected in indoor environment and in urban green areas. Regarding the temporal variation of environmental UFP concentrations, appreciable differences were found [26, 29] between working and non-working days, at different times of the day and between seasons. Previous studies estimated a daily average exposure to UFP of about 1.6×10^4 pt/cm^3 for people commuting in Milan, with indoor home exposure

accounting for 46% of total daily exposure, indoor office exposure about 30%, and transport environments about 24% (almost insensitive to transportation mode) [25]. The results from the present study have the same order of magnitude, with a higher average estimated exposure (among all profiles) of $24 \times 10^4 \pm 4.65 \times 10^3$ pt/cm^3, with indoor home exposure giving 61.9 ± 5.4% of the total daily exposure, indoor office exposure about 11.1 ± 4.1%, and transport environments about 16.7 ± 4.4% (but sensitive to transportation mode). The results from the present study confirm that the variability in UFP exposure is also related to behavioral factors (e.g., mode of transport) and seasonal patterns, both of which have a very large influence on human exposure to UFP [41-43]. Thus, demographic and socio-demographic factors may be considered as major determinants of UFP exposure in urban environments.

Assumptions and limits

The following assumptions were included in the model:

(I) The CPC used in these studies (model P-Trak Ultrafine Particle Counter 8525; TSI Inc., Shoreview, MN, USA) can measure particles ranging from 0.02 to 1 μm in size (so UFP data include also particles with dimension >100 nm, although their number concentration is assumed to be very low with respect to those in the 0–100 nm interval) and has shown effectiveness in detecting the variations of PNC in urban environments [26]. As a general concept, for some in-transit MEs involving transient aerosol dynamics (i.e., characterized by very rapid aerosol generation and dilution processes), the measurements should be performed using aerosol measurements with frequencies high enough to track the steep changes that the aerosols undergo, thus a higher temporal resolution would be desirable for in-transit environments [44].

(II) The lognormal distribution was used as the default distribution for UFP concentrations. Despite possible deviancies from lognormality, this assumption could work fine in the current model as environmental pollutant concentrations are often found to follow lognormal distribution [8, 11, 38]. Moreover, the current model used also fitted beta distributions to describe the time fractions spent in each ME [11]. For one ME (motorbike/scooter) UFP measurement was not available, thus an indirect estimate was made, using outdoor concentrations recorded for sporadic measurement. Note that this approximation introduced only a very limited error in the total exposure estimations, because a very limited time (< 1%) were globally spent in this ME.

(III) Model validations were not possible because it was not possible to collect exposure data for the study subpopulations. Since the study examined the exposure distributions among the selected subpopulations, it is almost impossible to conduct a model validation, which should require personal measures for a large population. Thus, the performance of the model simulations strictly depends on the quality of the input data for time-activity pattern and microenvironmental measures. Therefore, it is crucial that these data appropriately reflect the specific subgroup of population under various environmental conditions. This problem has been stressed by many researchers in the field of exposure modeling and it would be very helpful if more databases on environmental concentration

data and exposure-relevant time-activity data could be published and made available [15]. Despite this, a previous research [2] showed that the time-activity data used for modeling [28] are a helpful tool for evaluating air pollution exposures in different scenarios, population groups and locations (the model predicted mean population exposure levels in four European cities with an accuracy of >20%) and for helping researchers to understand the factors that affect exposure levels. Further, the concentration input parameters used for the simulations were obtained from previous researches [26, 29], which are expected to reflect the microenvironmental concentration under certain conditions (typical weekday, in two season) for the general (non-smoking) population. This approach should provide a sufficient understanding of exposures in urban areas. Still, it is clearly evident that exposure research in the urban microenvironment has numerous inferences and there are various factors that can potentially affect personal exposure concentrations [38], especially when considering particular subpopulations.

(IV) In the absence of validation, however, it is questionable what solution would approach the real exposure situation most accurately. Model uncertainty includes uncertainties in the selection of the distributions, definition of the MEs and modeled activities, selection of averaging times and number of iterations, and generation of the random numbers, and so forth [45, 46]. In the basic equation of our model uncertainty is not included. The simplifications used in the selection of microenvironments and the selection of parametric distributions, however, introduce uncertainties to the model structure [11]. Full analysis of the model uncertainty would significantly broaden the focus and volume of this article. Further, the comparison of the modeled and measured exposures is not possible, because this study used a retrospective approach, using old data, incorporating a number of factors that cannot be captured by a single air monitoring campaign nowadays. Therefore, only measurement errors causing parameter uncertainty may be evaluated in the presented models. Thus, a nominal range sensitivity analysis was carried out accordingly. This sensitivity analysis was performed by investigating the effect of parameters on the estimated exposures [46]: the model's inputs were individually varied across their entire range of plausible values, while holding all other inputs at their base values. The sensitivity was presented as a positive or negative percentage change compared with the base values. Time spent in indoor MEs and the corresponding concentrations were found to be the most important parameter leading to possible prediction errors (± 60%). In-transit MEs (car/taxi, bike/walk) were another source of possible uncertainties, as they were affected by high variability (± 10% for each ME).

(V) A limitation of this study is the rather small sample size in the definition of time-activity patterns and the use of quite old data [18], with the intrinsic assumption that time-activity patterns were unchanged in the last 15 years. Furthermore, measurements in the urban areas were not carried out simultaneously for practical reasons, but were derived from a previous study of the research group. This may have induced systematic differences in measurements because of temporal factors. The lack of such data was one of the main reasons we conducted this retrospective exposure modeling, incorporating several factors

that were not captured by a single air monitoring campaign but strongly influence personal exposures, such as time-activity patterns, residential and workplace measurements.

(VI) This paper describes the simulation of daily mean exposure for different subpopulations in indoor and outdoor MEs. Potential bias may occur when considering the source data and in particular, the temporal representativity of the summer and winter seasons, the representativity of the considered MEs and the representativity of the presence of indoor and outdoor sources during the measurements. Although the reference studies [29, 30] have considered temporal and spatial variability for the studied MEs, the design and methods imply some limitations in the generalizability of these findings. For example, although the MEs were chosen to reflect common urban activities and general trends, the specific locations were selected according to a systematic and technical protocol. Thus, these MEs could not be representative of the average concentrations in the same kind of MEs across Milan or in other cities. The UFP residential and indoor concentrations were mainly collected within the Milan urban and suburban area, involving a quite large number of volunteers (N = 81) for a wide monitoring period (N = 162 days, >3800 h). Thus, the results from the monitoring campaigns are assumed to reflect common residential activities and general trends, but it must be considered that a potential bias may occur when ignoring specific variability factors. On the contrary, the motorbike/scooter and train/metro MEs were investigated for a limited time (few hours on the whole, see Table 2), and the corresponding measurements of UFP concentrations cannot be considered necessarily representative of the general exposure scenario occurring in these specific in-transit conditions.

4.5. CONCLUSIONS

In this work, a microenvironmental stochastic simulation model was used in order to simulate the average daily ultrafine particles exposure of adult subpopulations (defined on the basis of gender, age, employment status and educational level) in a major Italian urban area and in different exposure scenarios (typical working day in summer and winter). Although the number of profiles taken for this study is too small to yield statistical evidence, some general conclusions can be drawn and this study provide seasonal information on the average exposure to UFP in various microenvironments for a wide range of subpopulations. The estimated average daily exposure was higher in winter than in summer. The highest median exposures were obtained, as expected, for indoor environments, which were one order of magnitude higher than outdoor exposures and well above the simulated commuting exposures. The in-transit MEs contributed significantly to the total daily exposure, mostly considering the limited amount of time spent in these MEs. The Car/Taxi and Walk/Bike MEs were characterized by the highest simulated median exposures. The outdoor MEs did not show an important contribution to the total estimated exposure. Total daily exposure simulations also showed a moderate difference between genders; differences between genders were also found in some specific MEs. The mean total indoor exposure, which represented the highest fraction of the total estimated exposure, was stably higher for women than for men. In contrast, the mean contribution of commuting (in-transit) and outdoor environments to the total daily exposure was higher for men. Thus, demographic and socio-demographic factors,

as well as environmental patterns, have to be considered as major determinants of pollutant exposure in urban environments. Large-scale experiments including personal measurements might help to improve modeling approaches for a better estimation of actual exposure on a statistically sound basis.

4.6. ACKNOWLEDGMENTS

The author extends a special acknowledgment to Matteo Taronna, Gaetano Garramone, Franco Vercelli, Salvatore Pulvirenti for their contribution to the environmental samplings during PM-CARE project.

This chapter is based on:

- Spinazzè, A. Cattaneo, C. Peruzzo, D. M. Cavallo
  Modeling population exposure to ultrafine particles in a major Italian urban area
  Int. J. Environ. Res. Public Health 2014, 11(10):10641-10662.

4.7. FIGURES

Figure 1: Box plots of calculated UFP exposures (%) estimated in indoor and outdoor microenvironments for summer and winter period. (*The central box comprises values between the 25th and 75th percentiles, the whiskers show the range of values that falls within 1.5 times the interquartile range beyond the box*).

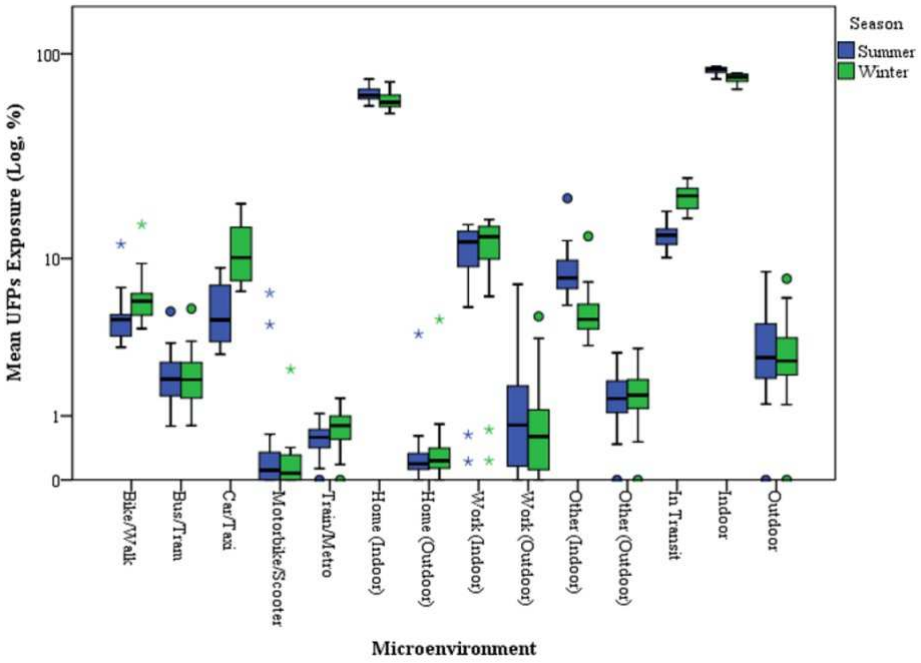
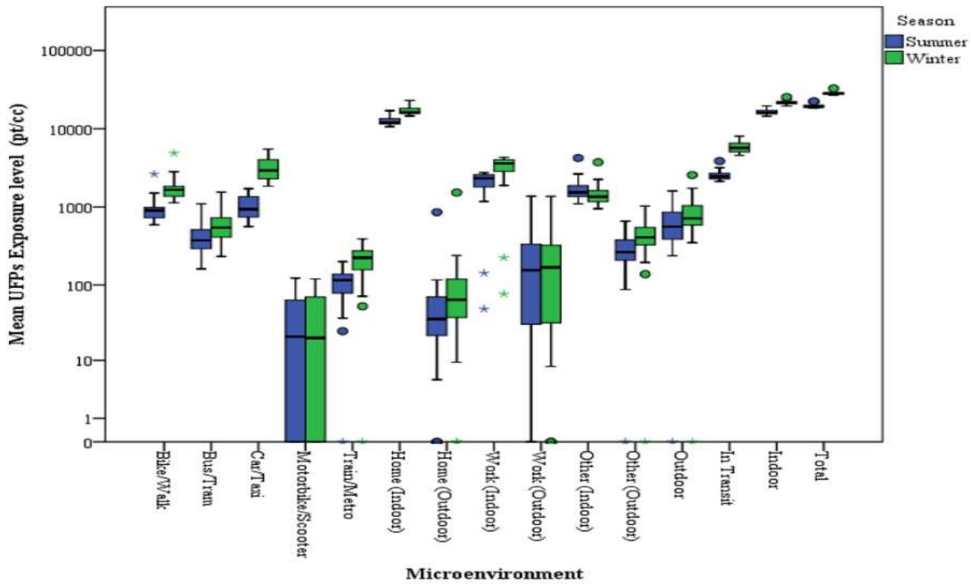


Figure 2. Box plots of calculated UFP exposures (pt/cm^3) estimated in indoor and outdoor microenvironments for summer and winter. (*The central box comprises values between the 25th and 75th percentiles, and the whiskers show the range of values that falls within 1.5 times the interquartile range beyond the box*).



4.8. TABLES

Table 1. Subpopulation defined as function of gender and, then, for Occupational Status, Educational Level or Age.

Population Characteristic	**Characteristic Subgroup**	
Gender	Male	Female
Occupational Status	Employed	
	Retired**	
	Housewife*	
	Self Employed	
	Student	
Education Level	0-9 years	
	10-13years	
	14 - 16years	
	≥ 17years	
Age	25-34 years	
	35 -44 years	
	45-54 years	
	55 – 59 years	
-	Mean subject	

**only Female subjects; **only Male subjects*

Table 2. Microenvironments used for the exposure simulation, total sampling time [hours] UFP environmental concentrations for the summer and winter periods (mean, standard deviation) [particle/cm^3] and time-activity patterns [daily % of the mean working day].

Category of ME	ME	UFP concentration [pt/cm^3] [a, b]						Time Use [c] [daily %]		
		Summer			Winter					
		Total sampling time [h]	Mean	S.D.	Total sampling time [h]	Mean	S.D.	Mean	Min	Max
"In-transit"[a]	Bike/Walk	11.2	32214	31679	9.7	60277	47588	2.9	2.0	8.0
	Bus/Tram	6.1	36798	30207	6.8	52386	23821	1.1	0.0	2.0
	Car/Taxi	10.8	27034	29966	10.2	82890	53130	3.8	2.0	7.0
	Motorbike/Scooter*	2.8	12016	7898	2.8	12016	7898	0.3	0.0	1.0
	Train/Metro	4.5	15730	9126	2.2	30643	13272	0.7	0.0	1.0
Indoor [b]	Home Indoor	1705.8	21645	21986	1736.5	29347	29369	58.0	49.0	78.0
	Work Indoor	57.3	8849	3917	46.8	13865	6364	0.5	0.0	7.0
	Other Indoor	16.2	25694	31743	24.1	22148	15309	23.2	1.0	31.0
Outdoor	Other Outdoor [a]	10.0	21008	19847	10.3	32219	24508	1.2	0.0	7.0
	Home Outdoor [b]	34.7	12722	8820	5.6	23042	15917	6.8	4.0	17.0
	Work Outdoor [b]	6.7	18716	18502	8.4	18880	11524	1.4	0.0	3.0

Sources: [a] Spinazzè et al., 2013; [b] PM-CARE Project (Schlitt et al., 2008); [c] Source: Expolis project (Expofact: http://expofacts.jrc.ec.europa.eu/ - accessed on 04/07/2014)
** indirect estimation*

Table 3. Summary of the mean simulated exposures, segregated for each ME. Results are calculated among all subpopulations within the Milan-city area (SD = standard deviation, Min = minimum, Max = maximum, p5 = 5th percentile, p95 = 95th percentile). All results are expressed in particle/cm^3 [pt/cm^3], Mean value are expressed also as percentual contribution to total daily exposure [%].

Microenvironment	*Mean*	*Mean [%]*	*SD*	*Min*	*p5*	*Median*	*p95*	*Max*
Discrete MEs								
Bike/Walk	1368	5.5	736	591	668	1230	2637	4874
Bus/Tram	504	2.1	256	161	225	434	1048	1549
Car/Taxi	2112	8.3	1350	561	650	1783	4653	5510
Motorbike/Scooter	37	0.4	42	0	0	21	114	123
Train/Metro	163	0.7	91	0	25	140	318	390
Home (Indoor)	14786	61.9	2862	10656	11294	15098	19225	22948
Home (Outdoor)	109	0.4	244	0	0	57	239	1530
Work (Indoor)	2639	11.1	1121	49	142	2600	4194	4301
Work (Outdoor)	267	1.2	345	0	0	164	1057	1373
Other (Indoor)	1629	7.1	652	951	987	1419	2640	4230
Other (Outdoor)	372	1.5	200	0	88	349	745	1031
Cumulative MEs								
Outdoor	748	3.1	458	0	237	651	1603	2561
In-Transit	4184	16.7	1790	2121	2240	4217	6987	8012
Indoor	19055	80.1	2974	14470	15176	19561	23346	25377
Total	23987	100.0	4650	18334	18479	24604	30038	32730

Table 4. Summary of the mean simulated exposures, distincted for each ME and season. Results are shown as statistics calculated for the mean male and female profile among all subpopulations within Milan-urban area

ME	Mean Subpopolation Exposure (Male)								Mean Subpopolation Exposure (Female)							
	Time use (fraction)		Summer			Winter			Time use		Summer			Winter		
	Mean	SD	Mean (%)	Mean (pt/cm^3)	SD (pt/cm^3)	Mean (%)	Mean (pt/cm^3)	SD (pt/cm^3)	Mean	SD	Mean (%)	Mean (pt/cm^3)	SD (pt/cm^3)	Mean (%)	Mean (pt/cm^3)	SD (pt/cm^3)
Discrete																
Bike/Walk	0.02	0.03	3.8	706	1329	4.8	1324	2221	0.03	0.02	5.1	993	1530	6.5	1791	2143
Bus/Tram	0.01	0.02	1.8	345	871	1.7	477	1020	0.01	0.02	2.5	478	864	2.6	713	1331
Car/Taxi	0.05	0.05	7.1	1330	2873	14.4	4027	5523	0.03	0.03	3.7	713	1339	7.7	2134	3064
Motorbike/Scooter	0.01	0.02	1.8	74	252	7.1	70	213	0.00	0.01	0.1	21	119	0.1	25	214
Train/Metro	0.01	0.02	06	112	290	0.8	221	515	0.01	0.02	0.7	140	318	1.0	265	539
Home Indoor	0.53	0.08	61.2	11502	12541	55.9	15581	15996	0.59	0.12	66.4	12866	13271	62.5	17273	17293
Home Outdoor	0.00	0.01	0.3	61	206	0.4	103	342	0.00	0.01	0.2	43	138	0.3	75	221
Work Indoor	0.29	0.11	13.5	2534	1551	14.2	3962	2504	0.24	0.13	11.2	2160	1637	12.3	3404	2666
Work Outdoor	0.02	0.06	2.3	430	1432	1.6	446	1321	0.00	0.02	0.4	76	338	0.3	79	279
Other Indoor	0.06	0.06	7.6	1428	2888	4.5	1247	1791	0.06	0.06	8.3	1608	2690	5.2	1444	1975
Other Outdoor	0.01	0.03	1.5	284	772	1.6	440	1213	0.01	0.02	1.4	265	569	1.5	420	837
Cumulative																
Outdoor	0.04	0.10	4.1	775	1667	3.5	988	1842	0.02	0.13	2.0	383	676	2.1	574	915
In Transit	0.09	0.13	13.7	2567	3311	21.9	6119	6067	0.08	0.08	12.1	2347	2238	17.8	4929	3963
Indoor	0.87	0.25	82.2	15464	12992	74.5	20789	16318	0.90	0.27	85.9	16634	13627	80.1	22121	17546
Total	1.00	0.01	100.0	18806	13395	100.0	27897	17453	1.00	0.01	100.0	19363	13820	100.0	27623	18055

Table 5. Summary of the mean simulated exposures, distincted for cumulative MEs and season. Results are shown as statistics calculated age-distincted subpopulations

Subpopulation (age)	ME (cumulative)	Summer					Winter				
		Time use (fraction)		Exposure			Time use (fraction)		Exposure		
		Mean	SD	Mean (%)	Mean (pt/cm^3)	SD (pt/cm^3)	Mean	SD	Mean (%)	Mean (pt/cm^3)	SD (pt/cm^3)
25-34 yrs	Outdoor	0.03	0.01	3.2	610	344	0.03	0.01	2.8	795	359
	In Transit	0.10	0.01	13.5	2567	148	0.10	0.01	21.1	5860	823
	Indoor	0.88	0.03	83.3	15820	61	0.88	0.03	76.1	21113	308
	Total			100.0	18997	431			100.0	27768	874
35-44 yrs	Outdoor	0.04	0.02	3.6	679	295	0.04	0.02	2.7	750	183
	In Transit	0.08	0.01	12.1	2282	229	0.08	0.01	19.4	5307	1040
	Indoor	0.89	0.02	84.3	15935	1167	0.89	0.02	77.9	21385	1481
	Total			100.0	18896	642			100.0	27442	258
45-54 yrs	Outdoor	0.03	0.01	2.4	444	175	0.03	0.01	2.5	692	247
	In Transit	0.09	0.01	12.4	2364	92	0.09	0.01	19.4	5408	642
	Indoor	0.89	0.01	85.2	16244	1283	0.89	0.01	78.2	21856	1347
	Total			100.0	19053	1016			100.0	27956	458
55-59 yrs	Outdoor	0.04	0.02	3.3	644	309	0.04	0.02	3.0	858	271
	In Transit	0.09	0.02	12.4	2466	463	0.09	0.02	20.1	5846	1050
	Indoor	0.89	0.04	84.3	16823	1445	0.89	0.04	76.9	22361	1392
	Total			100.0	19933	673			100.0	29064	72

Table 6. Summary of the mean simulated exposures, distincted for cumulative MEs and season. Results are shown as statistics calculated employment-distincted subpopulations within Milan-urban area

Subpopulation (Occupational status)	ME (cumulative)	Summer					Winter				
		Time use (fraction)		Exposure			Time use (fraction)		Exposure		
		Mean	SD	Mean (%)	Mean (pt/cm^3)	SD (pt/cm^3)	Mean	SD	Mean (%)	Mean (pt/cm^3)	SD (pt/cm^3)
Student	Outdoor	0.02	0.03	1.9	402	569	0.02	0.03	1.7	495	700
	In Transit	0.10	0.02	13.5	2868	431	0.10	0.02	20.7	6100	802
	Indoor	0.90	0.01	84.6	17954	392	0.90	0.01	77.6	22791	819
	Total			100.0	21224	254			100.0	29386	922
Employed	Outdoor	0.03	0.01	2.5	469	133	0.03	0.01	2.3	634	111
	In Transit	0.09	0.01	12.8	2383	165	0.09	0.01	19.7	5317	776
	Indoor	0.89	0.01	84.7	15752	472	0.89	0.01	78.0	21090	578
	Total			100.0	18603	175			100.0	27041	309
Self-employed	Outdoor	0.04	0.04	3.8	721	684	0.04	0.04	2.9	847	704
	In Transit	0.10	0.01	13.9	2636	255	0.10	0.01	23.2	6606	1073
	Indoor	0.88	0.03	82.3	15586	580	0.88	0.03	73.9	20982	767
	Total			100.0	18943	359			100.0	28435	1010
Housewife*	Outdoor	0.03	-	2.5	559	-	0.03	-	2.8	873	-
	In Transit	0.08	-	10.1	2262	-	0.08	-	16.0	4995	-
	Indoor	0.89	-	87.4	19564	-	0.89	-	81.2	25377	-
	Total			100.0	22386	-			100.0	31246	-
Retired**	Outdoor	0.10	-	6.8	1516	-	0.10	-	7.8	2561	-
	In Transit	0.13	-	17.3	3862	-	0.13	-	24.5	8012	-
	Indoor	0.77	-	75.9	16894	-	0.77	-	67.7	22157	-
	Total			100.0	22271	-			100.0	32730	-

**only Female subjecst; **only Male subjects*

Table 7. Summary of the mean simulated exposures, segregated for cumulative MEs and seasons. Results are calculated for subpopulations segregated by educational level (*i.e.*, year of scholarity).

Subpopulation (Educational Level)	ME (cumulative)	Summer					Winter				
		Time use (fraction)		Exposure			Time		Exposure		
		Mean	SD	Mean (%)	Mean (pt/cm^3)	SD (pt/cm^3)	Mean	SD	Mean (%)	Mean (pt/cm^3)	SD (pt/cm^3)
0–9 years	Outdoor	0.06	0.05	5.3	1001	850	0.06	0.05	4.2	1179	785
	In-Transit	0.09	0.01	13.1	2524	276	0.09	0.01	21.1	6006	1082
	Indoor	0.86	0.06	81.6	15,823	1914	0.86	0.06	74.7	21288	2447
	Total			100.0	19,348	788			100.0	28473	581
10–13 years	Outdoor	0.03	0.01	3.5	658	294	0.03	0.01	3.1	853	255
	In-Transit	0.09	0.01	12.9	2431	233	0.09	0.01	19.7	5438	1119
	Indoor	0.89	0.02	83.7	15841	879	0.89	0.02	77.2	21274	855
	Total			100.0	18929	352			100.0	27565	518
14–16 years	Outdoor	0.03	0.01	3.2	629	251	0.03	0.01	3.0	869	329
	In-Transit	0.10	0.01	13.6	2658	110	0.10	0.01	21.7	6201	1112
	Indoor	0.88	0.02	83.2	16277	1042	0.88	0.02	75.2	21428	1174
	Total			100.0	19563	681			100.0	28497	267
≥17years	Outdoor	0.02	0.01	2.2	418	217	0.02	0.01	2.0	556	231
	In-Transit	0.09	0.01	12.4	2346	149	0.09	0.01	19.1	5236	397
	Indoor	0.90	0.01	85.4	16116	518	0.90	0.02	78.8	21576	856
	Total			100.0	18880	153			100.0	27,368	228

4.9. REFERENCES

1. Moschandreas, D.J. Models of human exposure to environmental contaminants. In Encyclopedia of Environmental Health; Nriagu, J.O., Ed.; Elsevier: Amsterdam, Netherlands, 2011; pp. 820–830.

2. Kruize, H.; Hanninen, O.; Breugelmans, O.; Lebret, E.; Jantunen, M. Description and demonstration of the EXPOLIS simulation model: Two examples of modeling population exposure to particulate matter. J. Expos. Anal. Environ. Epidem. 2003, 13: 87–99.

3. Duan, N. Models for human exposure to air pollution. Environ. Int. 1982, 8, 305–309.

4. Ott, W.R. Total human exposure: An emerging science focuses on humans as receptors of environmental pollution. Environ. Sci. Technol. 1985, 19: 880–886.

5. Duan, N. Stochastic microenvironmental models for air pollution exposure. J. Expos. Anal. Environ. Epidem. 1991, 1: 235–257.

6. Ryan, P.B. An overview of human exposure modeling. J. Expos. Anal. Environ. Epidem. 1991, 1: 453–474.

7. Ott, W.R. Exposure estimates based on computer generated activity patterns. J. Toxicol.-Clin. Toxicol. 1984, 21: 97–128.

8. Ott, W.; Thomas, J.; Mage, D.; Wallace, L. Validation of the simulation of human activity and pollutant exposure (SHAPE) model using paired days from the Denver, Colorado carbon monoxide field study. Atmos. Environ. 1988, 22: 2101–2113.

9. Lioy, P.J. Assessing total human exposure to contaminants—A multidisciplinary approach. Environ. Sci. Technol. 1990, 7: 938–945.

10. Duan, N.; Mage, D.T. Combination of direct and indirect approaches for exposure assessment. J. Expos. Anal. Environ. Epidem. 1997, 7: 439–470

11. Buonanno, G.; Stabile, L.; Morawska, L. Personal exposure to ultrafine particles: The influence of time-activity patterns. Sci. Total Envir. 2014, doi:10.1016/j.scitotenv.2013.09.016.

12. Broich, A.V.; Gerharz, L.E.; Klemm, O. Personal monitoring of exposure to particulate matter with a high temporal resolution. Environ. Sci. Pollut. Res. 2012, 19: 2959–2972.

13. Hanninen, O.; Kruize, H.; Lebret, E.; Janutnen, M. EXPOLIS simulation model: PM2.5 application and comparison with measurements in Helsinki. J. Expos. Anal. Environ. Epidem. 2003, 13: 74–85.

14. Letz, R.; Ryan, B.P.; Spengler, J.D.; Estimated distributions of personal exposure to respirable particles. Environ. Monit. Assess. 1984, 4: 351–359.

15. Jerrett, M.; Arain, A.; Kanaroglou, P.; Beckerman, B.; Potoglou, D.; Sahsuvaroglu, T.; Morrison, J.; Giovis, C. A review and evaluation of intraurban air pollution exposure models. J. Expo. Sci. Environ. Epidem. 2004, 15: 185–204.

16. Buonanno, G.; Giovinco, G.; Morawska, L.; Stabile, L. Tracheobronchial and alveolar dose of sub-micrometer particles for different population age groups in Italy. Atmos. Environ. 2011, 45: 6216–6224.

17. Buonanno, G.; Morawska, L.; Stabile, L.; Wang, L.; Giovinco, G. A comparison of sub-micrometer particle dose between Australian and Italian people. Environ. Pollut. 2012, 169: 183–189.

18. Ott, W. Concepts of human exposure to air pollution. Environ. Int. 1982, 7: 179–196

19. Fugas, M. Assessment of Total Exposure to an Air Pollutant. In Proceedings of the International Conference on Environmental Sensing and Assessment (ICESA), Las Vegas, NV, USA, 14-19 September 1975.

20. Klepeis, N.E. An introduction to the indirect exposure assessment approach: Modeling human exposure using microenvironmental measurements and the recent national human activity pattern survey. Environ. Health Perspect. 1999, 107: 365–374.

21. Freijer, J.I.; Bloemen, H.J.T.H.; Loos, S.; Marra, M.; Rombout, P.J.A.; Steentjes, G.M.; van Veen, M.P. Modelling exposure of the Dutch population to air pollution. J. Hazard. Mater. 1998, 61: 107–114.

22. Morawska, L.; Ristovski, Z.; Jayaratne, E.R.; Keogh, D.U.; Ling, X. Ambient nano and ultrafine particles from motor vehicle emissions: Characteristics, ambient processing and implications on human exposure. Atm. Environ. 2008, 42: 8113–8138.

23. Zhu, Y.; Hinds, W.C.; Kim, S.; Sioutas, C.; Concentration and size distribution of ultrafine particles near a major highway. J. Air Waste Manage. Assoc. 2002, 52: 1032–1042.

24. Ragettli, M.S.; Corradi, E.; Braun-Fahrländer, C.; Schindler, C.; de Nazelle, A.; Jerrett, M.; Ducret-Stich, R.E.; Künzli, N.; Phuleria, H.C. Commuter exposure to ultrafine particles in different urban locations, transportation modes and routes. Atmos. Environ. 2013, 77: 376–384.

25. Lonati G.; Ozgen, S.; Luraghi, I.; Giugliano, M. Particle number concentration at urban microenvironments. Chem. Eng. Trans. 2010, 22: 137–142.

26. Cattaneo, A.; Garramone, G.; Taronna, M.; Peruzzo, C.; Cavallo, D.M. Personal exposure to airborne ultrafine particles in the urban area of Milan. J. Phys. Conf. Ser. 2009, doi:10.1088/1742-6596/151/1/012039

27. Hertel, O.; Hvidberg, M.; Ketzel, M.; Storm, L.; Stausgaard, L. A proper choice of route significantly reduces air pollution exposure—A study on bicycle and bus trips on urban streets. Sci. Total Environ. 2008, 389: 58–70.

28. ExpoFacts. Available online: http://expofacts.jrc.ec.europa.eu/ (accessed on 10 October 2014).

29. Spinazzè, A.; Cattaneo, A.; Garramone, G.; Cavallo, D.M. Temporal variation of size-fractionated particulate matter and carbon monoxide in selected microenvironments of the Milan urban area. J. Occup. Environ. Hyg. 2013, 10: 652–662.

30. Schlitt, C.; Garramone, G.; Cattaneo, A.; Peruzzo, C.; Taronna, M.; Pulvirenti, S.; Vercelli, F.; Cavallo, D.M. Fine and Ultrafine Particle Levels Determined during Everyday Activities: The PM-CARE Project. In Proceedings of 11th International Conference on Indoor Air Quality and Climate: Indoor Air, Copenhagen, Denmark, 17-22 August 2008.

31. Cattaneo, A.; Taronna, M.; Garramone, G.; Peruzzo, C.; Schlitt, C.; Consonni, D.; Cavallo, D.M. Comparison between personal and individual exposure to urban air pollutants. Aerosol Sci. Technol. 2010, 44: 370–379.

32. Rodes, C.E.; Thornburg, J.W. Breathing zone exposure assessment. In Aerosols Handbook: Measurement, Dosimetry, and Health Effects; Ruzer, H., Ed.; CRC Press: Boca Raton, FL, USA, 2005; pp. 61–74.

33. Binnig, J.; Meyer, J.; Kasper, G. Calibration of an optical particle counter to provide PM2.5 mass for well-defined particle materials. Aerosol Sci. 2007, 38: 325–332.

34. Lonati, G.; Ozgen, S.; Ripamonti, G.; Cernuschi, S.; Giugliano, M. Pedestrian exposure to size-resolved particles in Milan. J. Air Waste Manage. Assoc. 2011, 61: 1273–1280.

35. Marcazzan, G.M.; Ceriani, M.; Valli, G.; Vecchi, R. Source apportionment of PM10 and PM2.5 in Milan (Italy) using receptor modeling. Sci. Total Environ. 2003, 317: 137–147.

36. Giugliano, M.; Lonati, G.; Butelli, P.; Romele, L.; Tardivo R.; Grosso, M. Fine particulate (PM2.5–PM1) at urban sites with different traffic exposure. Atmos. Environ. 2005, 39: 2421–2431.

37. Vecchi, R.; Marcazzan,G.; Valli, G.; Ceriani, M.; Antoniazzi, C. The role of atmospheric dispersion in the seasonal variation of PM1 and PM2.5 concentration and composition in the urban area of Milan (Italy). Atmos. Environ. 2004, 38: 4437–4446.

38. Kaur, S.; Nieuwenhuijsen, M.J.; Colvile, R.N. Fine particulate matter and carbon monoxide exposure concentrations in urban street transport microenvironments. Atmos. Environ. 2007, 41: 4781–4810.

39. Adams, H.S.; Nieuwenhuijsen, M.J.; Colvile, R.N.; McMullen, M.A.S.; Khandelwal, P. Fine particle (PM2.5) personal exposure levels in transport microenvironments, London, UK. Sci. Total Envir. 2001, 279: 29–44.

40. Van Wijnen, J.H.; Verhoeff, A.P.; Jans, H.W.; van Bruggen, M. The exposure of cyclists, car drivers and pedestrians to traffic-related air pollutants. Int. Arch. Occup. Environ. Health 1995, 67: 187–193.

41. Kaur, S.; Nieuwenhuijsen, M.J. Determinants of personal exposure to PM2.5, ultrafine particle counts, and CO in a transport microenvironment. Environ. Sci. Technol. 2009, 43: 4737–4743.

42. McCreanor, J.; Cullinan, P.; Nieuwenhuijsen, M.J.; Stewart-Evans, J.; Malliarou, E.; Jarup, L.; Harrington, R.; Svartengren, M.; Han, I.K.; Ohman-Strickland, P.; Fan Chung, K.; Zhang, J. Respiratory effects of exposure to diesel traffic in persons with asthma. N. Engl. J. Med. 2007, 357: 2348–2358.

43. Ott, W.R. A physical explanation of the lognormality of pollutant concentrations. J. Air Waste Manage. Assoc. 1990, 40: 1378–1383.

44. Manigrasso, M.; Stabile, L.; Avino, P.; Buonanno, G. Influence of measurement frequency on the evaluation of short-term dose of sub-micrometric particles during indoor and outdoor generation events. Atmos. Environ. 2013, 67: 130–142.

45. Morgan, M.G.; Henrion, M. Uncertainty: A Guide to Dealing with Uncertainty in Quantitative Risk and Policy Analysis; Cambridge University Press: New York, NY, USA, 1990.

46. Cullen, A.C.; Frey, H.C. Probabilistic Techniques in Exposure Assessment: A Handbook for Dealing with Variability and Uncertainty in Models and Inputs; Plenum Press: New York, NY, USA, 1999; pp. 243–271.

5. EXPOSURE CHARACTERIZATION TO ULTRAFINE PARTICLES IN URBAN MICROENVIRONMENTS: A MULTI-METRIC APPROACH

At the beginning of the study, our hypothesis was that visiting certain microenvironments (MEs) is one of the most important determinants of personal exposure to ultrafine particles (UFP) and that moving between microenvironments significantly differentiates exposure. The overall aim of this study is to perform relevant exposure measurements to extend our knowledge on environmental exposure to UFPs in urban environments; the previously published methods for this appear inadequate. The UFP concentrations in different urban MEs were measured by personal monitoring in repeated sampling campaigns along a fixed route. The measurement runs were performed on one-week periods and at different times of day (AM: 08.00 - 10.30; PM: 16.00 - 18.30) and repeated in different periods of the year (winter, spring, summer, and autumn) for a total of 56 runs (> 110 hours). Measurements included on-line monitoring of the UFP particle number concentration (PNC), mean diameter (mean-d) and lung-deposited surface-area (LDSA). Additionally, the PNC, particle mass concentration (PMC) profiles for quasi-ultrafine particles (QUFP; $PM_{0.25}$) were estimated. A significant seasonal difference in the PNC and PMC, mean diameter and surface area was observed as well as between different times of the day and days of the week. In addition, differences in the UFP concentrations were also found in each ME, and there were specific mean-diameter and surface area concentrations. In general, the mean particle diameters showed an inverse relationship with the PNC, while the LDSA had the opposite behaviour. Appreciable differences among all microenvironments and monitoring periods were observed; the concentration patterns and variations seemed related to the typical sources of urban pollutants (traffic), proximity to sources and time of day. The highest exposures were observed for walking or biking along high-trafficked routes and while using public buses. The UFP exposure levels in modern cars, equipped with high-efficiency filters in air reciruculation mode, were significantly lower.

5.1. INTRODUCTION

Human exposure to ultrafine particles (UFP) in urban transport microenvironments (MEs) is of particular interest because it has been estimated that transport environments provide a significant proportion of the total daily exposure [1]. In fact, individuals may encounter a significant contribution to their daily exposure when commuting in traffic; the levels of most air pollutants are particularly high along busy roads, and their peak concentrations are typically registered during commute hours [2, 3]. Therefore, time spent in transit represents a high-exposure period compared to various daily activities, especially in metropolitan areas, even though individuals usually travel for no more than 6 - 8% of the day [4]. Moreover, the UFP concentrations are characterized by high spatial and temporal variability in urban microenvironments [5, 6]; recent studies have documented the dependence of the UFP levels on several urban factors (e.g., traffic volume, the environment and meteorological characteristics) [7 - 11]. The highest UFP concentrations were found in the vicinity of the primary sources (e.g., near busy roads) where the particle number concentrations are typically between 10^4 and 10^6 particles/cm^3 [12], while the UFP concentrations decrease rapidly with distance from the emission sources [13, 14]. Therefore, the highest urban UFP concentrations are generally expected when moving along busy streets or in their immediate environments, while lower concentrations are usually detected in indoor environments and urban green areas.

Because of these important differences in the space and time of the UFP concentrations between urban micro-environments, fixed monitoring stations are not capable of depicting the full spatial distribution of air pollution over the extent of an urban area [4]. Furthermore, it has been demonstrated that the use of fixed-monitoring measurements for assessing general exposure levels to pollutants may lead to significant underestimations in the exposure of particular population subgroups [15, 16]. Therefore, mobile measurements are frequently applied as an efficient tool, capable of describing human exposures with a high spatial and temporal resolution, even in complex urban environments [10, 13, 17 - 21]. This is the case for some in-transit MEs involving transient aerosol dynamics (characterized by very rapid aerosol generation and dilution processes), for which measurements should be performed with sampling frequencies that are high enough to track the steep changes that aerosols undergo [22]. Mobile measurements are also applied for further developments, such as mapping the spatial distribution of air pollutants [14], characterizing the local source contributions to ambient air pollution and developing models [23, 24]. Furthermore, at the present time, there is insufficient information to determine which exposure metrics – size-resolved number, surface area, and mass concentration - are the most relevant to human health outcomes. Therefore, it is currently unclear how exposure to UFP should most appropriately be monitored and regulated. Nevertheless, there is strong toxicity-based evidence that the nanoaerosol surface-area is an appropriate exposure metric for nanoaerosols [25 - 28] and that the biological response depends more on the surface-area of particles deposited in the lungs [26, 27, 29, 30] than on the other possible metrics of exposure. However, there are also indications that the PNC within specific particle size ranges might be an important indicator of the health effects of UFP exposures [31 - 33]. Furthermore, the number of sub-100-nm particles dominates the extent of respiratory deposition [34]. This clearly suggests that knowledge of the particle size distributions, which can dramatically change in urban environments [35], is important for accurately estimating UFP uptake [36]. Therefore, it is expected that particles within a nanometer size range have a biological behaviour that is more closely associated with the PNC, mean-d and surface area than with the PMC. In any case, the concentration was usually selected as a reference metric because it was expected to provide more accurate data, when compared to continuously measured mass concentrations, if the latter are not properly corrected with simultaneous gravimetric data [37].

In view of the potential for covering large areas in a city, we performed mobile monitoring campaigns to investigate the time and spatial variations of airborne UFP concentrations and characteristics. The originality of the present work lies in the continuous, time-resolved personal monitoring of the particle number concentration (PNC), mean diameter (mean-d) and surface area (LDSA: lung deposited surface area) with a study design allowing for measurements of the urban microenvironmental UFP concentrations with high temporal resolution. In addition to the PNC, the particle mass concentration (PMC) profiles were estimated through the use of an estimation method, based on the particulate mass density factors, which were defined by the contextual personal sampling of quasi-ultrafine particles (QUFP; $PM_{0.25}$) and subsequent gravimetrical analysis.

5.2. METHODS

Study Area and Monitoring Protocol

The measurements were performed in Como, Italy (45°49′00″N, 9°05′00″E). Como is a medium-sized provincial town (84000 inhabitants, 37.34 inhabitants/km^2) in the northern part of Italy. Experimental data were collected within the central area of the city over four, one-week periods in four different seasons during the year 2014. The average daily meteorological conditions for the study periods are reported in *table 1* (source: ARPA, Agenzia Regionale Protezione Ambiente, Lombardia).

A fixed route (17 km long) was defined (*figure 1*) and the average travelling time for the entire route was approximately 120 minutes. The major part of the mobile route was located in the residential areas, but streets with differing configuration and with differing traffic dynamics were included in the designed route. The results presented in this study mainly focus on a selection of urban microenvironments (ME) or modes of transportation (*table 1*), which were assumed to reflect the most relevant variations of exposures with respect to the mode of transportation (walk/bike, car, or bus) and traffic density (High traffic areas – HT; Low- or no- traffic areas - LT). An indoor environment (office) was also included. More specifically, measurements were performed along a fixed route, according to a sequential protocol that started and finished at the office. The study design first included a car (2012 Toyota Yaris, petrol-fueled) journey around the city centre (6.3 km); the measurements were collected at the passenger's seat. In-car ventilation settings were set for all runs as follows: windows closed, circulation fan on and recirculation (RC) fan on, and the fan speed was kept low. The study protocol then included a bicycle journey (2.8 km), and a pedestrian route was established, crossing the city centre approximately in the S-N direction for an overall distance of 4 km and considering variations in the mode of transport traffic density with busy roads versus traffic-limited areas and urban parks. Pedestrian and bike journeys were combined into two MEs, the first (qualitatively) related to high traffic condition (Bike/Walk – HT) and the other related to low or no traffic (Bike/Walk – LT). Finally, a bus (diesel-fuel with a diesel particulate filter) journey (2.1 km, N-S direction) was taken to return to the starting point; in-bus ventilation conditions (windows open, ventilation and air conditioning settings) were not standardized. All circumstances in which the operator was exposed in other unspecified ME or situations (e.g., waiting at the bus stop) are classified as 'undefined ME' (Other). The study design was also influenced by practical and organizational circumstances. However, the monitoring protocol was designed to collect data for at least 5 minutes in each selected ME and to assess the daily, weekly and seasonal variations in the UFP concentrations and characteristics. Data were collected daily at two times, morning ('AM': 08:00–10:30) and afternoon ('PM': 16:00–18:30), and measurements were carried out over one-week periods (four repetitions: winter, spring, summer, and autumn).

Materials

Portable monitors were synchronized before each sampling campaign and were additionally equipped with a GPS system to register the measurement locations. A time-activity diary (with a resolution of 1 second) was also used to accurately separate the concentration data as a function of

the different monitored environments. Data were continuously collected with a high sampling frequency (averaging time: 10 seconds). All instruments were placed into a backpack that was carried by one investigator; because the sampling inlets were placed in strict correspondence with the breathing zone (the hemisphere of 30 cm radius extending in front of the face) [38], the study results can appropriately refer to the standardized concept of personal exposure.

The concentrations of airborne UFP were measured using a miniature diffusion size classifier (DSC) and a portable condensation particle counter (CPC). Both of these instruments can provide real-time measurement of particles, although each type of instrument has its own sensitivity to specific particle characteristics. The DSC used for this study (DiSCmini, Matter Aerosol AG, Wohlen AG, Swiss) is a compact, battery-powered handheld instrument that measures the number and average size (mean diameter and lung-deposited surface area) in the size range of approximately $10 < Dp < 700$ nm according to the manufacturer (an impactor is used to remove particles larger than 700 nm). DiSCmini is based on unipolar charging of the aerosol, which is followed by detection in two electrometer stages [39, 40]. DiSCmini can detect particle concentrations of 10^3–10^6 pt/cc; the detection limits are presented in a dedicated publication [40]. The experimental results showed that the DiSCmini performances correspond fairly well (within 20%) to traditional instruments used for UFP measurements; also, they are reproducible and in agreement with standard condensation particle counters and SMPS-systems [40 - 42]. A portable CPC was also used in this study (P–Trak Ultrafine Particle Counter model 8525; TSI Inc., Shoreview, MN, USA) to perform a field comparison with the DSC. The CPC quantifies the particle (size range: 0.02 to 1 μm) concentration in the interval of 0-5 x 10^5 pt/cm^3 using isopropanol to artificially enlarge particles through condensation of vapours on the particle surface. It should be noted that the lower detection limit of DSC in respect to CPC would introduce a bias that would not affect the conclusions [42]. Furthermore, the particle size range for both instruments is beyond the upper limit of ultrafine particles that are 100 nm; therefore, UFP data also include submicrometer particles with dimensions >100 nm, although their concentration is assumed to be very low compared to those smaller than 100 nm. Information on the instrumentation is added to avoid erroneous interpretation due to differences in instrumentation performances. In fact, although, portable and easy–to–use devices are usually characterized by a worse metrological performance than the commonly used standard counters in aerosol research in terms of the accuracy, minimum detectable particle diameter and maximum measurable concentrations [43]. The portable rapid-response instruments used in this study have an adequate capacity to measure short-term variations in the PNC in urban environments in spite of the lack of accuracy at high concentrations [20, 40].

By means of DSC, UFP continuous profiles were characterized from 3 variables, the particle number concentration (PNC, particle/cm^3), mean diameter (mean-d, nm), and lung-deposited surface-area (LDSA, μm^2/cm^3). The LDSA concentration is defined as the particle surface area concentration per unit volume of air, weighted by the deposition probability in the lung and calculated according to ICRP report 66 [34]; the size range between 16 and 240 nm typically contains the majority of the LDSA and PNC for ambient aerosols. In this range, the DSC provides a good approximation of the LDSA (even without any diameter-dependent correction). For particles larger than approximately 400 nm, the PNC decreases and LDSA may be severely underestimated; however, under normal ambient conditions, the contribution of these larger particles to the total

LDSA is expected to be small. Therefore, the LDSA can be estimated with reasonable accuracy using the miniature DSC, and it is strictly dependent of the PNC for the 16-240 nm size range [40].

The personal air temperature (T) and relative humidity (RH) values were also continuously measured via a portable sensor (Hobo U12, Onset Computer Corporation, Bourne, MA, USA; Accuracy T = ± 0.35°C; RH = ± 2.5%). The T and RH values were measured with an acquisition time of 10 seconds and recorded via an internal data logger.

The gravimetric determination of the $PM_{0.25}$ was conducted in every sampling session; the aim was to characterize the mean exposure expressed in mass concentrations along the fixed route defined by the experimental protocol. A Personal Cascade Impactor Sampler (PCIS), developed for the analysis of size-segregated particulate matter, was used [44]. A PCIS is a miniaturized cascade impactor, which operates at a flow rate of 9 L/min and consists of four impaction stages with cut-off diameters of 2.5, 1.0, 0.5, and 0.25 μm (PTFE s/PTFE filters; diameter: 25 mm; porosity: 0.8 μm), which is followed by an after-filter for particles < 0.25 μm (PTFE w/PMP ring; diameter: 37 mm; porosity: 2 μm). Personal samples were collected in the breathing zone of operators; each sampling covered two measurement runs (AM and PM) to define a daily mean gravimetric concentration of $PM_{0.25}$. Mass concentrations ($\mu g/m^3$) were then determined by gravimetric analysis in accordance with the reference methods [45 - 46] and with the accepted standard practice. The net PM mass on the filters was measured by weighing the conditioned filters before and after sampling with a microbalance in a temperature- and relative humidity-controlled (20± 1 °C; 50 ± 5%) environment (Activa Climatic; Aquaria, Lacchiarella, MI, Italy). The quality of the weighing procedure was assessed using the ASTM D 6552 method [47]. The mass limit of detection (LOD) for $PM_{0.25}$ was estimated following a method proposed by ASTM [47] and was approximately 1.3 μg (approximately 0.48 $\mu g/m^3$ for a 5-hour sampling at 9 l/min).

The $PM_{0.25}$ mass concentrations were used to estimate the daily mean mass density factors (ρ) for Quasi-UFP (QUSP). These were then used to estimate the continuous QUFP mass concentrations profiles (PMC), based on the PNC and mean-d continuous profiles, which were obtained using the DSC, according to the general relationship described by equation 1 (where M is the particle mass concentration, V is the particle volume, N is particle number concentration, and ρ is the mass density of particles).

$$M = V \cdot \rho \cdot N \qquad \textit{(eq. 1)}$$

Therefore, $PM_{0.25}$ data were first combined with the particle volume (calculated from the mean diameter) and daily average number concentration values to obtain the mean ρ values for each sampling day and then an overall mean ρ value (*table 2*). Once the ρ values for each sampling day were defined, eq.1 was used to calculate the PMC continuous profile for each sampling session.

Data treatment and analysis

The collected data were examined and handled to provide for the exclusion of zero, unreliable and missing data (casewise deletion), and they were synchronized in function of their acquisition interval following good practice in statistics [48 - 50], as follows. The concentration distributions

were truncated above the 99th percentile and below the 1st percentile to prevent unrealistically high and low concentration values.

Statistical analysis of the collected data was performed via SPSS Statistics 20.0 (IBM, Armonk, NY, USA); it consisted of tests analysing the data distribution (Kolmogorov–Smirnoff test) and assessing the differences among groups (Kruskal–Wallis one–way ANOVA – KW; Mann-Whitney U-test - MW) and Analysis of Variance ANOVA with the Bonferroni post-hoc test. Pearson's correlation was used to explore the relationship between the considered variables. Finally, multiple linear regression analyses were performed on Log_{10}-transformed variables. In every model, the different UFP measured metrics (PNC, mean-d and LDSA) were included as dependent variables, and, as predictors, spatial (ME), temporal (measurement session, day and season) and continuously measured microenvironmental variables (temperature and relative humidity).

5.3. RESULTS

UFP indoor and outdoor monitoring was performed within the Como urban area for a wide monitoring period, consisting of a total of 56 runs (> 110 hours) performed on 28 days in the period from February – October 2014. The average run durations for each ME/mode of transportation are reported in *table 1*. In total, N = 39001 data observations were collected; after data cleaning, N = 37351 data were used for the statistical analysis. The results are presented in text as the mean ± standard deviation (mean ± SD).

Spatial variability

The results of the multi-metric sampling monitoring, segregated for ME, are provided in *table 3*. The lowest UFP concentration in Como was found indoors. The highest mean UFP concentrations were observed in the Bus ME and were one order of magnitude higher than indoors. The mean UFP concentration in walk/bike - LT was more than 40% lower than that for the traffic related route Walk/Bike - HT, which was the second most polluted ME. A large variability was observed in Car ME. Non-classified MEs (Other) also had high UFP concentrations. In general, the mean particle diameters had an inverse relationship with the PNC, while the LDSA had a direct relationship with the PNC (*figure 2*). Therefore, larger particles and reduced LDSA were primarily found in car and indoor MEs (*table 2*), while the distribution shifted towards smaller particles, with a higher LDSA in Walk/Bike - HT ME and Bus ME.

Temporal variability

For all ME/transportation modes, the UFP number was higher during the morning sessions than in the afternoon (*table 4*). Similarly, lower UFP concentrations were observed in the weekend compared to during a typical working day (*table 5*). Furthermore, a clear seasonal variation was observed (*table 6*); as expected, the UFP concentrations were higher in winter than in summer, while the UFP levels were similar in spring and autumn. The average UFP particle diameters followed an opposite trend compared to the UFP concentration, showing larger average particle sizes for the sampling times with lower UFP number concentrations and vice-versa. By contrast, the LDSA showed a direct trend with the UFP concentration with a wider surface area for sampling times and higher UFP number concentrations and vice-versa. Therefore, the average particle

number was higher during the weekend, while the LDSA were higher on weekdays (*table 5*). Accordingly, during AM sessions, smaller average particle sizes and higher LDSA were measured compared to PM (*table 4*). Similarly, seasonal trends were found, with larger mean-d and lower LDSA concentrations in summer than in winter (*table 6*).

PMC estimation

The results of the estimated PMC are reported in *table 2*. The lower concentration in Como was found indoors, while the highest mean concentrations were estimated for the in-bus travels. The mean PMC concentration in walk/bike - LT was approximately 40% lower than for the traffic related route Walk/Bike - HT, which was the second most polluted ME. A large variability was estimated in the Car ME, while non-classified MEs (Other) had high UFP concentrations. The estimated mean PMC was similar during the morning and afternoon sessions, but the latter showed a wider variability. Similarly, lower PMCs were estimated in the weekend compared to during a typical working day. Furthermore, the estimated seasonal mean PMCs were higher in winter than in summer, as expected, but higher estimates were observed in spring and autumn.

Statistical Analysis

Non-Parametric tests (Kruskal-Wallis one-way ANOVA- "KW" / Mann-Whitney U-test - "MW") and ANOVA with post-hoc Bonferroni test (performed on Log-transformed data) indicated statistically significant differences ($p < 0.001$) among the groups of temporal (season, day, and period) and spatial (ME) variables. *Figure 2* reports Pearson's correlation coefficients between different metrics used in the description of UFP concentrations, and the percentage variation of Pearson's correlation in each investigated ME. Significant correlations ($p < 0.01$) were found between each metric, with the highest between the PNC and LDSA ($r = 0.924$) and the lowest between the PMC and mean-d ($r = 0.190$). The correlation factor between the PNC and LDSA was very stable and almost constant, even when considering data from each single ME, while the correlation factors showed very high variations, considering ME-specific data, with percentage variations up to ±80 % (LDSA vs Mean-d).

Multiple linear regression analysis (*table 7*) substantially confirmed the findings of univariate analyses; significant relationships were found between each of the UFP measured metrics and temporal and spatial variables; furthermore, the ME and microclimatic parameters (T and RH) had a significant relationship with the UFP metrics. Meteorological variables were excluded from the regression analysis due to their poor/lack of variability (rain) and because they showed a low correlation with the UFP concentration (wind velocity). Accordingly, the adjusted r-square for the models containing UFP metrics as the dependent variable ranged from 0.114 to 0.217. The considered variables explained little (from 10 to 20%) of the total variability in the UFP concentrations. In particular, significant increases in the PNC, PMC and LDSA (and, consequently, a significant decrease in the mean diameter) were found for the ME predictor; by contrast, significant decreases for the same variables were found as function of temperature.

Finally, the comparison of the DSC and the CPC during environmental monitoring showed that both devices measured similar particle numbers with a high time resolution, allowing both devices to identify the same peak episodes. However, in spite of the good relationship between the two techniques (CPC vs DSC), demonstrated by correlation ($r_{pearson} = 0.668$; $p < 0.001$) and regression

analysis (r^2 0.447; m = 0.4956; q = 3730.5), the CPC particle counts were significantly lower, as already showed in previous studies [40, 41, 51-53]. The underestimation was the most pronounced in Car and Bus MEs (*figure 3*). The underestimation effect has been explained by the size-dependent efficiency (different cut-off diameters for detection), and it has been hypothesized that semi-volatile compounds of freshly emitted particles are not effectively detected by the CPC [52]. Finally because a previous study reported that the DiSCmini provides accurate particle number concentrations and geometric mean diameters at traffic-influenced sites, making it a useful tool for personal exposure assessment in such settings [41], the DSC was selected as a reference measurement method for the PNC metric and for estimating the PMC in this study (*table 2*).

5.4. DISCUSSION

This study supports the strong microenvironmental variability in the UFP, as outlined in previous studies that used mobile UFP measurements. The spatial inhomogeneity of the UFP remains a key challenge for assessing the UFP concentrations. This inhomogeneity is generally largest in urban transport MEs, especially those close to roadsides, which is normally found by multiple-site intra-city PNC monitoring studies that have up to an order of magnitude difference with respect to the minimum average PNCs [36]. For instance, spatial differences in the average PNCs between sites within a city were highly variable [43, 54 - 56] and rapid decreases with distance from the emission sources were observed [13, 14]. This study provides an overview of the UFP concentrations in different transport microenvironments (modes of transport) during different times of the day, week and year in a medium-sized town. Higher UFP concentrations were observed during bus travel and while walking or cycling along a high-traffic route, compared to private transportation and low-traffic routes. The levels were higher for all travel modes during weekday mornings (corresponding to rush hour) and in winter. The large observed spatial variability is a product of numerous factors, affecting their emission and dispersion. Furthermore, some of the spatial variations may be caused by seasonal (e.g., temperature inversion) effects that significantly increase the PNCs during cold months [57]. Temperature is known to affect the UFP concentrations through the condensation or evaporation of semivolatile compounds [54]. Therefore, the highest urban UFP concentrations are generally expected to occur while moving along busy streets or in their immediate vicinity, while lower concentrations are usually detected in indoor environments and urban green areas. Some of the observed variability can be attributed to other local factors, including the traffic volume, fuel type, urban morphology, climate, dispersion conditions specific to individual sampling locations, and uncertainty in the measured data due to manual (e.g., data collection and handling) and mechanical (e.g., instrument calibration) errors, which are difficult to generalize [36].

Particle Number Concentrations (PNCs)

Some studies have examined the UFP exposures across different urban areas and MEs, both using mobile and fixed site measurements. In a recent study [58], a review of European studies on commuter exposure to UFP was performed. On this basis, we concluded that the mean PNC measured along traffic routes in this study (*table 3*, "Walk/Bike - HT") were similar to the levels reported in other studies about cyclist exposure in other European studies [10, 59 - 61]. Nevertheless, some other studies showed higher PNCs for cyclists [61 - 64]. Cyclist exposures along low traffic routes, defined in a Dutch study [64], were also stably higher than those measured

in this study (Walk/Bike – LT). It should be noted that the aforementioned studies did not include exposure during walking, but many other studies have defined PNCs for traffic-related pedestrian routes, which are mostly in the order of 3 to 6 x 10^4 pt/cm^3 [65- 68]. Traffic-free pedestrian routes were studied in a previous study in Milan, Italy [65], which had a median UFP concentration of 14600 ± 12000 pt/cm^3. All of the aforementioned PNCs for pedestrian exposure, were higher than those reported in the present study, both for traffic (Walk/Bike - HT) and traffic-free zones (Walk/Bike –LT). Furthermore, the mean in-vehicle PNC measured in this study was well below those reported in other European studies [59, 65 - 69]. Therefore, although dependent on several factors (e.g., route choice, traffic intensity, car isolation, and fuel), the levels measured in the present study (*table 3*) are largely lower than the average PNCs reported for car rides in Europe. In this respect, it must be considered that the study in-car condition (windows closed, circulation fan on and recirculation fan on) are assumed to ensure that the outside air entered the in-cabin environment through a manufacturer installed filter (and, secondly, through leaks in windows and doors); the last of these should be ensure the minor infiltration of freshly emitted UFP in the well-encapsulated vehicle cabins of modern cars [71, 72]. Furthermore, studies on personal exposure to air pollutants during commuting by car have typically concluded that travelling by car involves exposure to relatively high PM exposure concentrations; however, the exposures will differ greatly according to the traffic intensity, speed and type of ventilation inside the car [58]. Therefore, reducing the number of vehicles, especially of older (more polluting) vehicles, may represent a recommendation for reducing the UFP exposure other than improving the air pollution in the urban environment. Furthermore, the maximum possible protection against outdoor particles can be obtained in relatively new cars equipped with manufacturer-installed particle filtration systems and when the fan and recirculation are both activated [71]; however, the reduction varies significantly between vehicles. The extent of the protection will depend on the efficiency of the installed filters, and the use of recirculation appears to be an effective method for reducing exposure to on-road UFP [73]. By contrast, the highest UFP levels in this study were measured for bus travel, which is in accordance with some other studies, reporting the in-bus UFP mean concentrations at the same order of magnitude (2 x 10^4 pt/cm^3 or higher) [54, 62 -66, 69, 71]. The elevated in-bus UFP concentrations observed in previous studies were primarily explained by the self-pollution of diesel-powered buses without particulate filters [75]. In contrast, lower values were expected in conditions that were similar to those reported in the present study (in the presence of low traffic density and for travels with newer buses) [64; 71]. Major differences compared with other studies were found for indoor MEs; the mean indoor (office environment) PNC levels observed in this study were well below to those reported for offices in Milan (Italy) in previous studies [65, 66, 68]. In any case, it must be considered that the indoor MEs considered in this study were characterized by the absence of relevant sources of UFP (e.g., laser printers). The aforementioned differences could also be attributed to the fact that Milan, located approximately 50 km away from Como, is a city of with approximately 1.2 million of inhabitants with a large urbanized area in the surroundings, leading to a high density of combustion sources and resulting in higher UFP background levels with respect to Como [76]. In summary, the PNCs were in agreement with those reported in the literature for similar studies, with the exception of indoor and car MEs.

Particle Mass Concentrations (PMCs)

The ambient particle mass concentrations are usually dominated by particles larger than 100 nm and, in the absence of strong local sources, are characterized by minor spatial variability within city environments [77]. Nevertheless, the PMCs estimated in this study (derived from the $PM_{0.25}$ sampling) showed some spatial variability in the traffic MEs. The mean PMC levels estimated in the Bus MEs were similar to those reported in the traffic-related pedestrian routes, and they were approximately 80% higher than those estimated during car driving and pedestrian traffic-free routes. Furthermore, as expected, all of these values were well above the indoor-estimated PMCs. The personal PMC values estimated in this study (derived from the $PM_{0.25}$ mean values) were similar or higher to those observed in the other studies. For example, a study in France reported QUFP ($PM_{0.29}$) concentrations of 6.7 ± 1.4 $\mu g/m^3$ for an urban-traffic sector [78]. Other studies, performed in California, with a reported QUFP ($PM_{0.18}$) concentration ranging between 2.6 and 7.3 $\mu g/m^3$ in an urban site [79] and 2.6 ± 0.5 $\mu g/m^3$ in an urban-traffic environment [80]. Other studies showed higher urban concentrations, reporting QUFP concentrations ($PM_{0.25}$) of 16.1 ± 2.8 in the urban area of Los Angeles [81] and between 7.2 and 11.5 $\mu g/m^3$ outdoors or between 6.0 and 10.3 $\mu g/m^3$ in the residential indoor environments of the same basin [82].

Mean diameter

The UFP level had an inverse association with the particle size, which remained almost stable regardless of the transportation mode or time of day. Few studies have quantified the particle size diameters in different transportation modes. A previous study on pedestrian exposure reported a mode diameter of 30 – 40 nm [43], while an average particle size of 48 nm was defined for pedestrian UFP exposure in Ragettli et al., 2013 [10]. In the present study, the mean average sizes for pedestrian/cyclist UFP exposure were 64.0 nm (traffic-related route) and 70.1 nm (traffic-free route). For in-bus MEs, the particle sizes were lower (60.0 ± 12.6 nm), while in-car MEs showed similar or larger particle size (72.5 ± 17.3 nm) (*table 3*). The last of these is likely due to the lower infiltration of smaller particles in the well-encapsulated vehicle cabins of modern cars, considering the ventilation settings and air filtration, which play a major role in the mitigating the in-cabin exposure to freshly emitted UFP [71, 72].

Surface area

The scientific literature about urban measurements of the particle surface area concentrations is limited. The average weekly LDSA of up to 89 $\mu m^2/cm^3$ decreased to approximately 35 $\mu m^2/cm^3$ during weekends at a busy freeway in Portugal [83]. The mean LDSA was reported at level up to 153 $\mu m^2/cm^3$, on average, at the Los Angeles freeway, while an urban background site was characterized by average concentrations of approximately 53 $\mu m^2/cm^3$ [84]. Although not directly comparable, the present results had the same order of magnitude. Traffic-related MEs showed a mean LDSA (Walk/Bike – HT, Bus) similar to those reported above, while traffic-free routes (Bike/Walk – LT) were characterized by a lower LDSA comparable to those measured in Los Angeles at the urban background site [84].

Temporal variability

As already reported, in addition to the mode of transport, temporal factors (time of day, day of the week and season) significantly affect the UFP concentrations [2, 65, 83, 84]. In this respect, particle concentrations show great daily variability related to the meteorological variables and thermodynamic conditions of the atmosphere, especially with respect to the mixing layer height [85]. In this respect, daily variations in the UFP concentrations were likely influenced by differences in the traffic volumes and corresponding emissions, which is in accordance with the pattern reported in another study [83]. With respect to seasonal trends, other studies in Milan showed a strong seasonal variation in the air pollutant concentrations, which was also due to differences in the average dispersion conditions of the atmosphere in summer and winter [86]. Ambient particulate matter shows a strong seasonal pattern, which is more evident for the fine and ultrafine particle-sizes that have higher winter values. This is primarily linked to differences in the average thermodynamic and meteorological conditions of the atmosphere and then to variations in the type or number of emitting sources [87]. In this respect, it is worth noting that, in this study, the PNC mean concentration was almost two times higher in winter than in summer, while the PMCs had less variation among seasons. This discrepancy should be explained by the contextual seasonal variations in the size and mass density of the UFP (*table 2*).

Strengths and limitations

Some assumptions and limits must be considered in interpreting this study's results. Although the categories of the microenvironments were chosen to reflect common the urban activities and general trends, the specific locations were selected according to a systematic and technical protocol; therefore, the ME evaluated in this study may not directly correspond to similar microenvironments elsewhere.

The discrepancies between the UFP levels and spatial variability may be due to differences in the sampling times and methods, which may affect the absolute temporal and spatial patterns of UFP. For example, the site type classification and source characteristics are also expected to play a role in transport MEs [10], and the measurements should be performed with frequencies that are high enough to track the steep changes that the aerosols undergo [22]. In this respect, the comparability of the UFP levels between other studies might be impaired by the different measurement devices used and may, primarily, be due to the different particle cut-off diameters

Although the variability in the quantitative data on traffic fluxes in Como is not available, it should be noted that rush hours occurred in the first part of the morning sessions and in the last period of the afternoon, and this mismatch of rush hours in the two sampling sessions could have introduced a bias in the comparison of exposures during the two periods of the day. Similarly, measurements were collected during four, one-week periods, which may not be fully representative of the seasonal variability. In this respect, while microenvironmental differences due to temperature and relative humidity were considered, other meteorological parameters (wind direction and speed, height of the mixing layer, etc.) could not be considered.

Finally, the algorithm used to transform particle numbers to mass-assumed particles were spherical [88] and had a calculated mean mass density of 2.09 ± 0.96 g/cm^3. In fact, particles are not

usually perfectly spherical [89] and other authors suggested different mass density factors equal to 1.3 or 1.65 g/cm^3 [88, 90]. In any case, variations in the particle density with size [88] and time of day [91] are likely; therefore, using different values for the particle density will change the estimated mass values without affecting the correlations between the number of particles and estimated mass [92]. In conclusion, the PMC estimations in this study were not expected to provide accurate PMC values; they were only useful indicators of the PMC variability in different MEs.

Despite the aforementioned limitations, this study has several strengths. First, the study provides comprehensive information on the average particle number and mass concentrations, sizes and surface area in various microenvironments within urban areas by means of personal monitoring, which is the gold standard in exposure assessment studies. Furthermore, this research detected UFP levels and average particle sizes in four seasons on the same route and with high sampling frequency (per a 110-hour sampling) to evaluate the variability in human exposure in urban environments for different microenvironments and routes. In summary, the study provides important insights into the UFP exposure in urban environments, which should be considered in developing additional and larger studies on population exposure. For example, the UFP concentration was higher in specific transport MEs and for particular modes of transportation, where the average particle size is smaller and the surface area concentration is higher, supporting the idea that traffic-related exposure has impacts health. Furthermore, the UFP concentrations were specific for the time (period of the day, week and season) and area of sampling. Therefore, once validated, the findings derived from this study may represent an important tool in the definition of health and social implications of UFP exposure (for example, exposure can be reduced by avoiding traffic-related ME) as well as provide complete and accurate exposure assessment data for risk assessors, including exposure metrics that are mostly relevant as health effects indicators (exposure monitoring stations should be placed in different urban areas with different road and traffic characteristics [10]).

5.5. CONCLUSIONS

In this study, mobile monitoring campaigns, consisting of repeated runs along a fixed route over four, one-week periods, were carried out in a small-sized provincial town in the northern part of Italy to evaluate the ultrafine particle concentration in urban microenvironments. Some general conclusions can be drawn. This study provided seasonal information on the average UFP concentration in various microenvironments. As expected, the highest mean exposures, well above the indoor and traffic-free microenvironmental concentrations, were obtained for some traffic-related MEs. The environmental UFP concentrations also showed significant differences between the time of day, week and season. Significant temporal and spatial differences were also found when considering the mean UFP diameter and surface area. The mean UFP diameter, which had an inverse relationship with the UFP concentration, was stably higher for indoor MEs than for traffic-related MEs. In contrast, the mean surface area showed a direct relationship with the UFP concentrations. Higher surface areas were measured in traffic-related MEs. In conclusion, temporal and micro-environmental patterns have to be considered as significant determinants of UFP exposure in urban environments. Future studies should focus on the 24-h personal UFP multi-metric monitoring to evaluate the contribution of every-day activity to total daily personal exposures to UFP.

5.6. ACKNOWLEDGEMENTS

A special acknowledgment to dr. Damiano R. Scocca for his fundamental contribution in the environmental sampling activity and to Dr. Matteo Bonzini for the supervision in statistical analysis.

This chapter is based on:

- Spinazzè A., Cattaneo A., Scocca D.R., Cavallo, D.M. (2014)
  Particelle Ultrafini in ambiente urbano: esposizione personale e caratterizzazione secondo diversi parametri di misura.
  Atti del Atti del 31° Congresso Nazionale AIDII" (ISBN 978-88-86293-24-2), pp 284-289

5.7. FIGURES

Figure 1: Como's urban area monitoring route (adapted from Mappy, © Mappy – AND, GeoSignal, Teleatlas 2014); Legend: IN = indoor (office); Walk/Bike routes in busy streets (dotted lines); Walk/Bike routes in traffic-free routes (continuous line); Car travel (dash-dot line); and Bus journey (dash- double dot line)

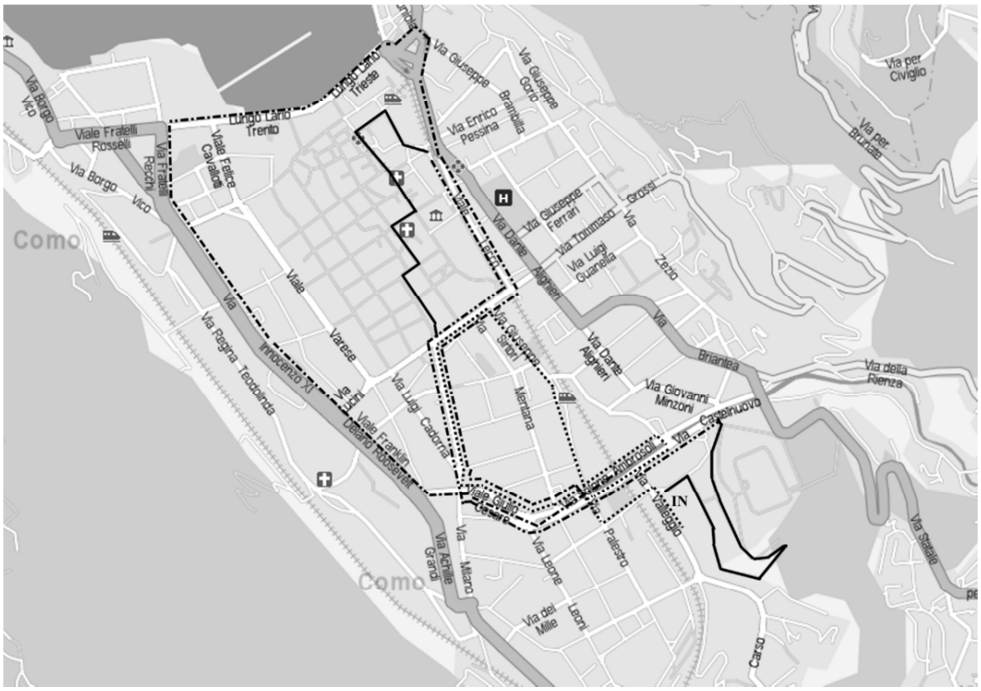


Figure 2: Pearson's Correlation coefficient (r) for the total dataset available (bar plot) and its variation [%] in each ME (scatter plot)

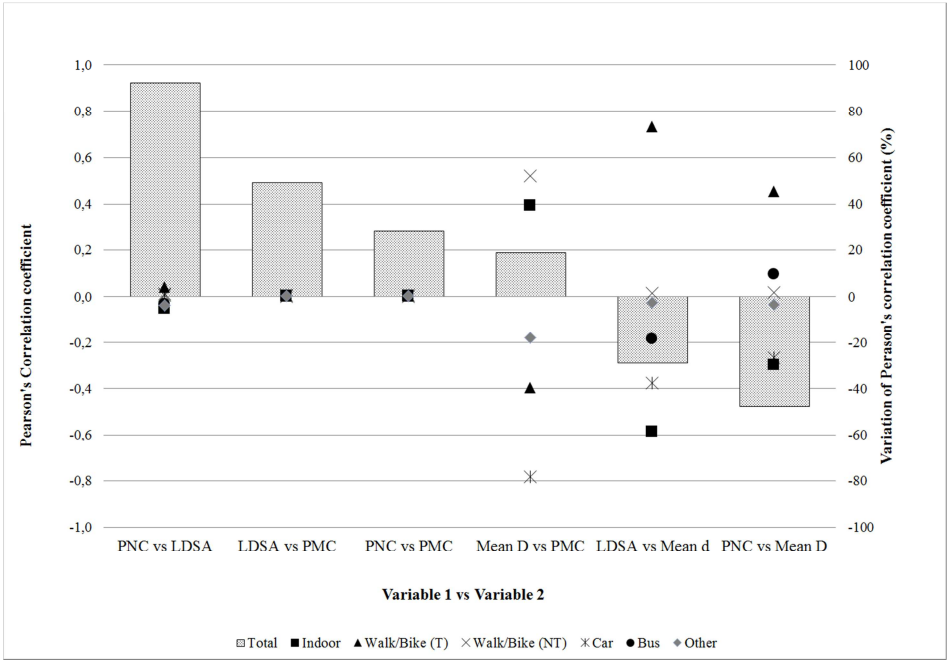
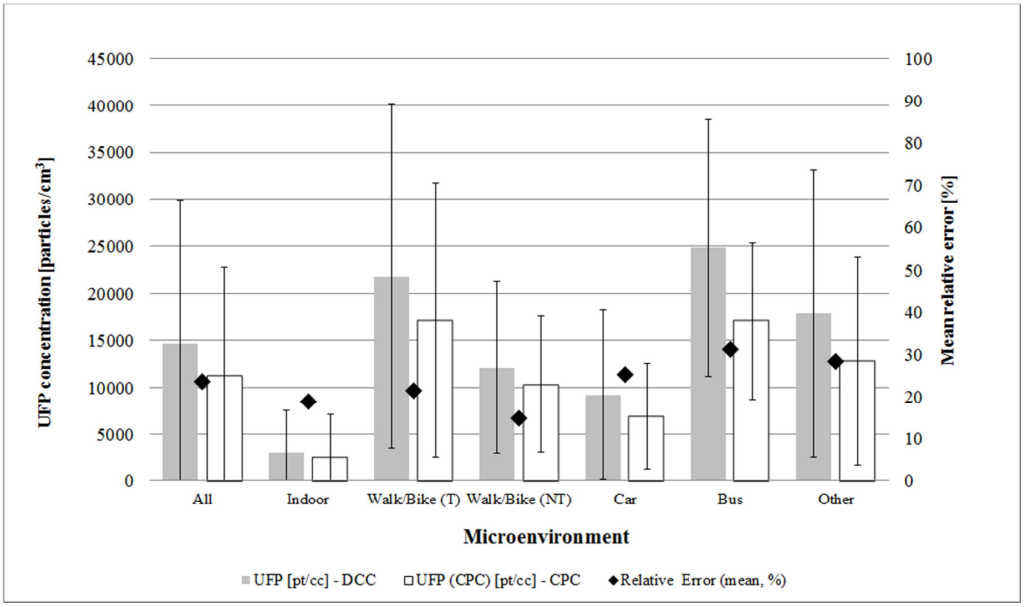


Figure 3: UFP number concentration [mean ± SD; particles/cm^3] measured using a miniature DSC (grey bars) and CPC in the study microenvironments. The mean relative error between the two measurement techniques is also reported (diamonds). Error bars = standard deviation



5.8. TABLES

Table 1. Microenvironmental measurement characteristics: total number of valid data (N), total sampling time [hours], measurement time for each session (mean, standard deviation). Meteorology conditions during sampling sessions: Rain [mm], Temperature [T, °C], Relative Humidity [RH, %] and Wind Velocity [m/s]

Microenvironmental Measurement					
ME	**N**	**Total time [h]**	**Time [min]**		
			Mean		**SD**
Indoor	6939	19.3	24		7
Walk/Bike - HT	11385	31.6	12		6
Walk/Bike - LT	5504	15.3	9		3
Car	5527	15.4	7		3
Bus	2662	7.4	9		1
Other	5334	14.8	17		6
Meteorology					
Sampling campaign (date)	**Statistics**	**Rain [mm]**	**T [°C]**	**RH [%]**	**Wind velocity [m/s]**
Winter	*Mean*	0.1	9.0	81.4	1.0
(18/24-02-14)	*SD*	0.8	2.1	19.6	0.7
Spring	*Mean*	0.0	26.9	57.4	2.3
(5/11-06-14)	*SD*	0.0	5.7	15.9	1.0
Summer	*Mean*	0.0	27.0	68.6	2.2
(14/20-07-14)	*SD*	0.0	4.3	17.5	0.8
Autumn	*Mean*	0.0	19.0	83.8	1.5
(25-09-14/1-10-14)	*SD*	0.0	3.0	13.7	0.6

Table 2. Summary of the monitoring results and PMC estimation, segregated for each sampling season, day, session and ME. Ultrafine particles are characterized by the following different measurement metrics: particle number concentration (PNC; particle/cm^3), mean diameter (mean-d; nm) and surface-area (LDSA; $\mu m^2/cm^3$) as well as time-weighted gravimetric mass concentration [$PM_{0.25}$]. Following the relationship described in eq.1, the mass density factor (ρ; g/cm^3) and continuous particle mass concentration profiles (PMC, ug/m^3) were estimated.

	UFP [pt/cm^3]		Mean-d [nm]		LDSA [$\mu m^2/cm^3$]		$PM_{0.25}$ [$\mu g/m^3$]		ρ [g/cm^3]		PMC [ug/m^3]			
Season	Mean	DS	Mean	DS	Mean	DS	Mean	DS	Mean	DS	Mean	SD	Median	IQR
Winter	22270	19836	55.8	12.0	62.4	51.82	12.7	4.8	2.12	0.82	10.6	14.1	8.1	8.7
Spring	11589	10897	69.8	19.4	40.79	31.97	14.1	1.6	2.56	1.03	13.6	27.1	11.7	9.1
Summer	10447	10578	72.7	21.3	37.16	30.81	9.5	2.6	1.83	1.08	8.6	11.8	7.0	6.4
Autumn	14867	15369	70.6	21.0	48.75	41.87	13.8	3.6	1.84	0.93	10.9	17.3	8.6	8.6
Day														
Mon	16642	17510	64.3	18.0	50.87	44.86	13.3	1.8	2.3	1.16	11.4	18.7	9.3	8.0
Tue	16687	17143	64.1	17.8	52.81	48.48	12.4	4.0	1.9	0.28	10.7	15.3	8.0	7.8
Wed	14437	14346	64.2	14.7	45.92	38.59	14.0	5.1	2.4	0.99	11.5	15.9	9.2	8.3
Thu	16191	15261	59.9	15.4	48.31	39.39	14.0	5.6	2.9	1.67	13.4	30.4	9.4	10.3
Fri	15466	15191	68.4	14.8	51.64	43.33	13.1	1.6	1.8	0.41	11.3	18.9	8.9	7.7
Sat	14220	15013	69.2	21.4	45.78	38.83	10.8	3.7	1.7	0.70	9.1	8.5	7.4	9.0
Sun	9009	9466	81.5	27.0	33.7	25.84	10.6	4.3	1.6	0.75	9.1	14.8	7.5	8.8
Session														
AM	16522	16793	66.4	20.0	51.44	43.55	12.6	3.8	2.09	0.96	10.9	13.4	8.8	7.8
PM	12972	13483	68.3	19.9	42.98	37.92					10.9	22.4	8.4	9.5
ME														
Indoor	2984	4501	70.9	22.5	9.95	10.87	12.6	3.8	2.09	0.96	2.6	2.9	1.5	2.4
Walk/Bike (T)	21778	18302	64.0	20.1	66.21	44.77					14.2	26.5	11.1	7.7
Walk/Bike (NT)	12091	9176	70.1	19.4	41.88	23.19					10.7	9.6	9.6	6.7
Car	9159	9048	72.5	17.3	33.46	27.48					9.5	9.0	7.3	5.1
Bus	24819	13673	60.0	12.6	78.24	36.49					16.1	14.7	13.7	10.3
Other	17852	15318	65.8	19.8	57.75	43.41					13.8	22.5	10.2	8.3

Table 3. Summary of monitoring results, segregated for each ME. Ultrafine particles are characterized by the following metrics: the particle number concentration (PNC; particle/cm^3), mean diameter (mean-d; nm) and surface-area (LDSA; µm^2/cm^3). The results are calculated among all sampling sessions (SD = standard deviation, IQR = interquartile range).

Metric	Statistics	ME					
		Indoor	**Walk/Bike – HT**	**Walk/Bike - LT**	**Car**	**Bus**	**Other**
PNC [pt/cm^3]	*Mean*	2984	21778	12091	9159	24819	17852
	SD	4501	18302	9176	9048	13673	15318
	Median	1769	15346	8896	6195	21619	12562
	IQR	2352	19345	8756	5722	15794	14770
Mean-d [nm]	*Mean*	70.9	64.0	70.1	72.5	60.0	65.8
	SD	22.5	20.1	19.4	17.3	12.6	19.8
	Median	69.6	60.2	66.5	70.8	59.5	63.5
	IQR	27.0	23.1	23.1	22.4	13.7	21.3
LDSA [µm^2/cm^3]	*Mean*	9.95	66.21	41.88	33.46	78.24	57.75
	SD	10.87	44.77	23.19	27.48	36.49	43.41
	Median	6.07	51.76	35.20	25.57	71.88	44.63
	IQR	9.50	46.96	22.37	18.31	42.82	38.36

Table 4. Summary of the UFP monitoring results, segregated for each sampling session. Ultrafine particles are characterized by the following metrics: the particle number concentration (PNC; particle/cm^3); mean diameter (mean-d; nm) and surface-area (LDSA; µm^2/cm^3). The results are calculated among all sampling sessions (SD = standard deviation, IQR = interquartile range).

Metric	Statistics	Session	
		AM	**PM**
PNC [pt/cm^3]	*Mean*	16522	12972
	DS	16793	13483
	Median	10816	8652
	IQR	16101	12513
Mean-d [nm]	*Mean*	66.36	68.33
	DS	20.03	19.92
	Median	62.60	66.60
	IQR	22.50	23.80
LDSA [µm^2/cm^3]	*Mean*	51.44	42.98
	DS	43.55	37.92
	Median	38.76	34.15
	IQR	42.54	38.60

Table 5. Summary of the UFP monitoring results, segregated for the day of the week. Ultrafine particles are characterized by the following metrics: the particle number concentration (PNC; particle/cm^3), mean diameter (mean-d; nm) and surface-area (LDSA; $\mu m^2/cm^3$). The results are calculated among all sampling sessions (SD = standard deviation, IQR = interquartile range).

Metric	Statistics	Day						
		Mon	Tue	Wed	Thu	Fri	Sat	Sun
PNC [pt/cm^3]	*Mean*	16642	16687	14437	16191	15466	14220	9009
	SD	17510	17143	14346	15261	15191	15013	9466
	Median	10537	11324	10204	11141	9943	8914	6085
	IQR	17818	16261	13238	14982	16093	14609	8579
Mean-d [nm]	*Mean*	64.27	64.08	64.19	59.93	68.36	69.18	81.49
	SD	18.03	17.76	14.74	15.36	14.85	21.42	26.97
	Median	62.20	60.80	62.70	60.00	67.80	69.30	77.60
	IQR	22.70	22.80	16.80	19.20	19.70	30.80	38.80
LDSA [$\mu m^2/cm^3$]	*Mean*	50.87	52.81	45.92	48.31	51.64	45.78	33.70
	SD	44.86	48.48	38.59	39.39	43.33	38.83	25.84
	Median	39.47	39.64	37.00	37.70	40.98	37.30	29.87
	IQR	48.43	44.29	38.22	43.32	49.04	42.90	23.01

Table 6. Summary of the monitoring results, segregated for each season. Ultrafine particles are characterized by the following metrics: the particle number concentration (PNC; particle/cm^3), mean diameter (mean-d; nm) and surface-area (LDSA; $\mu m^2/cm^3$). The results are calculated among all sampling sessions (SD = standard deviation, IQR = interquartile range).

Metric	Statistics	Season			
		Winter	**Spring**	**Summer**	**Autumn**
PNC [pt/cm^3]	*Mean*	22270	11589	10447	14867
	SD	19836	10897	10578	15369
	Median	17049	8403	7618	10222
	IQR	26242	8969	9078	15386
Mean-d [nm]	*Mean*	55.75	69.75	72.65	70.59
	SD	12.00	19.35	21.30	20.96
	Median	55.10	69.30	70.30	66.60
	IQR	15.00	23.90	24.10	24.70
LDSA [$\mu m^2/cm^3$]	*Mean*	62.40	40.79	37.16	48.75
	SD	51.82	31.97	30.81	41.87
	Median	50.70	33.46	31.79	38.59
	IQR	69.00	28.97	27.94	42.51

Table 7. Summary of the regression model results, segregated for the considered dependent variables. Ultrafine particles are characterized by the following metrics (used as dependent variables for the linear regression model): the particle number concentration (PNC; particle/cm^3), mean diameter (mean-d; nm) and surface-area (LDSA; $\mu m^2/cm^3$). The results show the standardized coefficients for each independent variable with the standardized beta (Beta), 95% confidence interval for beta - with upper and lower bounds - (95% C.I.) and p value. The last two rows provide an overview of the linear regression model for each combination of dependent variables and set of predictors, presenting the R squared value (r^2), standard error (Std. Error) and p value.

.

Dependent Variable (metric)	**PNC (pt/cm^3)**				**Mean-d (nm)**				**LDSA ($\mu m^2/cm^3$)**			
Independent Variables (Predictors)	Beta	95% C.I. Upper	Lower	p	Beta	95% C.I. Upper	Lower	p	Beta	95% C.I. Upper	Lower	p
Season	-0.019	-0.013	-0.003	0.001	0.052	0.005	0.007	<0.0001	-0.006	-0.007	0.002	0,342
Day	-0.137	-0.036	-0.031	<0.0001	0.248	0.015	0.016	<0.0001	-0.079	-0.020	-0.015	<0.0001
Session	0.035	0.024	0.044	<0.0001	0.013	0.001	0.006	0.015	0.043	0.028	0.047	<0.0001
ME	0.304	0.045	0.048	<0.0001	-0.045	-0.002	-0.001	<0.0001	0.326	0.044	0.046	<0.0001
Temperature	-0.338	-1.398	-1.302	<0.0001	0.413	0.402	0.426	<0.0001	-0.250	-0.954	-0.863	<0.0001
Relative Humidity	-0.026	-0.179	-0.064	<0.0001	0.187	0.205	0.233	<0.0001	0.027	0.06	0.167	<0.0001
Dependent Variable (metric)	**PNC (pt/cm^3)**				**Mean-d (nm)**				**LDSA ($\mu m^2/cm^3$)**			
Regression model statistics	r^2	Std. Error		p	r^2	Std. Error		p	r^2	Std. Error		p
	0.209	0.436		<0.0001	0.171	0.402		<0.0001	0.217	0.109		<0.0001

5.9. REFERENCES

1. Lonati G.; Ozgen, S.; Luraghi, I.; Giugliano, M. Particle number concentration at urban microenvironments. Chem. Eng. Trans. 2010, 22: 137–142.

2. Morawska, L.; Ristovski, Z.; Jayaratne, E.R.; Keogh, D.U.; Ling, X. Ambient nano and ultrafine particles from motor vehicle emissions: characteristics, ambient processing and implications on human exposure. Atmos. Environ. 2008, 42: 8113-8138.

3. Moreno, T.; Querol, X.; Alastuey, A.; Viana, M.; Gibbons, W. Profiling transient daytime peaks in urban air pollutants: city centre traffic hotspot versus urban background concentrations. J Environ. Monit. 2009; 11: 1535–42.

4. Kaur, S.; Nieuwenhuijsen, M.J.; Colvile, R.N. Fine particulate matter and carbon monoxide exposure concentrations in urban street transport microenvironments (Review). Atmos. Environ. 2007; 41(23): 4781–810.

5. Moore, K.; Krudysz, M.; Pakbin, P.; Hudda, N.; Sioutas, C. Intra-community Variability in Total Particle Number Concentrations in the San Pedro Harbor Area (Los Angeles, California). Aerosol Sci. Technol. 2009, 43: 587–603.

6. Hudda, N.; Cheung, K.; Moore, K.F.; Sioutas, C. Inter-community Variability in Total Particle Number Concentrations in the Eastern Los Angeles air Basin. Atmos Chem Phys 2010, 10: 11385–11399.

7. Boogaard, H.; Montagne, D.R.; Brandenburg, A.P.; Meliefste, K.; Hoek, G. Comparison of short-term exposure to particle number, PM_{10} and soot concentrations on three (sub) urban locations. Sci. Total Environ. 2010, 408: 4403-4411.

8. Hoek, G.; Beelen, R.; Kos, G.; Dijkema, M.; van der Zee, S.C.; Fischer, P.H.; Brunekreef, B. Land use regression model for ultrafine particles in Amsterdam. Environ. Sci. Technol. 2011, 45: 622-628.

9. Morawska, L.; Ristovski, Z.; Jayaratne, E.R.; Keogh, D.U.; Ling, X. Ambient nano and ultrafine particles from motor vehicle emissions: characteristics, ambient processing and implications on human exposure. Atmos. Environ. 2008, 42: 8113 - 8138.

10. Ragettli, M.S.; Corradi, E.; Braun-Fahrl€ander, C.; Schindler, C.; de Nazelle, A.; Jerrett, M.; Ducret-Stich, R.E.; Künzli, N.; Phuleria, H.C. Commuter exposure to ultrafine particles in different urban locations, transportation modes and routes. Atmos. Environ. 2013, 77: 376-384.

11. Rivera, M.; Basagana, X.; Aguilera, I.; Agis, D.; Bouso, L.; Foraster, M.; Medina-Ramon, M.; Pey, J.; Kunzli, N.; Hoek, G.; Spatial distribution of ultrafineparticles in urban settings: a land use regression model. Atmos. Environ. 2012, 54: 657-666.

12. Nikolova, I.; Janssen, S.; Vrancken, K.; Vos, P.; Mishra, V.; Berghmans, P. Size Resolved Ultrafine Particles Emission Model - A Continuous Size Distribution Approach. Sci. Total. Environ. 2011, 409: 3492–3499.

13. Zhu, Y.; Fung, D.C.; Kennedy, N.; Hinds, W.C.; Eiguren-Fernandez A. Measurements of Ultrafine Particles and Other Vehicular Pollutants inside a Mobile Exposure System on Los Angeles freeways. J. Air Waste Manage. Assoc. 2008, 58: 424–434

14. Hagler, G.; Thoma, E.D.; Baldauf, R.W. High resolution Mobile Monitoring of Carbon Monoxide and Ultrafine Particle Concentrations in a Near-road Environment. J. Air Waste Manage. Assoc. 2010, 60: 328–336.

15. Adams, H.S.; Nieuwenhuijsen, M.J.; Colvile, R.N.; McMullen, M.A.S.; Khandelwal, P. Fine particle ($PM_{2.5}$) personal exposure levels in transport microenvironments, London, UK. Sci. Total Environ. 2001, 279: 29–44.

16. Gulliver, J.; Briggs, D.J. Personal exposure to particulate air pollution in transport microenvironments. Atmos. Environ. 2004, 38:1–8.

17. Berghmans, P.; Bleux, N.; Int Panis, L.; Mishra, V.K.; Torfs, R.; Van Poppel, M. Exposure Assessment of a Cyclist to PM_{10} and Ultrafine Particles. Sci. Total Environ. 2009, 407: 1286–1298

18. Westerdahl, D.; Fruin, S.; Sax, T.; Fine, P.M.; Sioutas, C. Mobile Platform Measurements of Ultrafine Particles and Associated Pollutant Concentrations on Freeways and Residential Streets in Los Angeles. Atmos. Environ. 2005, 39: 3597–3610.

19. Kaur, S.; Nieuwenhuijsen, M.; Colvile, R. Personal Exposure of Street Canyon Intersection Users to $PM_{2.5}$, Ultrafine Particle Counts and Carbon Monoxide in Central London, UK. Atmos. Environ 2005, 39: 3629–3641.

20. Cattaneo A.; Garramone, G.; Taronna, M.; Peruzzo, C.; Cavallo, D.M. Personal exposure to airborne ultrafine particles in the urban area of Milan. J. Phys. Conf. Ser. 2009; IOP Publishing, p. 012039.

21. Peters, J.; Theunis, J.; Van Poppel, M.; Berghmans, P. Monitoring PM_{10} and ultrafine particles in urban environments using mobile measurements. Aerosol and Air Quality Research 2013, 13(2): 509-522.

22. Manigrasso, M.; Stabile, L.; Avino, P.; Buonanno, G. Influence of measurement frequency on the evaluation of short-term dose of sub- micrometric particles during indoor and outdoor generation events. Atmos. Environ. 2013, 67: 130-142.

23. Zwack, L.M.; Paciorek, C.J.; Spengler, J.D.; Levy, J.I. Characterizing Local Traffic Contributions to Particulate Air Pollution in Street Canyons Using Mobile Monitoring Techniques. Atmos. Environ. 2011, 45: 2507–2514

24. Ragettli, M.S.; Ducret-Stich,R.E.; Foraster, M.; Morelli, X.; Aguilera, I.; Basagaña, X.; Corradi, E.; Ineichen, A.; Tsai, M.Y.; Probst-Hensch, N.; Rivera, M.; Slama, R.; Künzli, N.; Phuleria, H.C. Spatio-temporal variation of urban ultrafine particle number concentrations. Atmos. Environ. 2014, 96: 275-283

25. Oberdörster, G. Toxicology of ultrafine particles: in vivo studies. Philosophical Transactions of the Royal Society of London. Series A: Mathematical, Physical and Engineering Sciences, 2000, 358(1775): 2719-2740.

26. Brown, D. M.; Wilson, M. R.; MacNee, W.; Stone, V.; Donaldson, K. Size-dependent proinflammatory effects of ultrafine polystyrene particles: a role for surface area and oxidative stress in the enhanced activity of ultrafines. Toxicol. Appl. Pharm. 2001, 175(3): 191-199.

27. Tran, C.L.; Buchanan, D.; Cullen, R.T.; Searl, A.; Jones, A.D.; Donaldson, K. Inhalation of poorly soluble particles. II. Influence of particle surface area on inflammation and clearance. Inhal. Toxicol. 2000, 12(12): 1113-1126.

28. Lison, D.; Lardot, C.; Huaux, F.; Zanetti, G.; Fubini, B. Influence of particle surface area on the toxicity of insoluble manganese dioxide dusts. Arch. Toxicol. 1997, 71(12): 725-729.

29. Donaldson, K.; Beswick, P.H.; Gilmour, P.S. Free radical activity associated with the surface of particles: a unifying factor in determining biological activity? Toxicol. Lett. 1996, 88(1): 293-298.

30. Hamoir, J.; Nemmar, A.; Halloy, D.; Wirth, D.; Vincke, G.; Vanderplasschen, A; et al. Effect of polystyrene particles on lung microvascular permeability in isolated perfused rabbit lungs: role of size and surface properties. Toxicol. Appl. Pharm. 2003, 190(3): 278-285.

31. Oberdörster, G.; Ferin, J.; Lehnert, B.E. Correlation between particle size, in vivo particle persistence, and lung injury. Environm. Health Persp. 1994, 102(S5), 173.

32. Beck-Speier, I.; Dayal, N.; Karg, E.; et al. Agglomerates of ultrafine particles of elemental carbon and TiO_2 induce generation of lipid mediators in alveolar macrophages. Environ. Health. Perspect. 2001, 109: 613–618.

33. Peters, A.; Wichmann, H.; Tuch, T.; Heinrich, J.; Heyder, J.; Respiratory effects are associated with the number of ultrafine particles. Am. J. Respir. Crit. Care Med. 1997, 155: 1376–1383.

34. ICRP. ICRP publication 66: human respiratory tract model for radiological protection. A report of a task group of the International Commission on Radiological Protection; 1994. p. 1–482.

35 Dall' Osto, M.; Thorpe, A.; Beddows, D.C.S.; Harrison, R.M.; Barlow, J.F.; Dunbar, T.; et al. Remarkable dynamics of nanoparticles in the urban atmosphere. Atmos. Chem. Phys. 2011, 11: 6623–6637.

36 Kumar, P.; Morawska, L.; Birmili, W.; Paasonen, P.; Hu, M.; Kulmala, M.; Harrison, R,M.; Norford, L.; Britter, R. Ultrafine particles in cities. Environ. Int. 2014, 66: 1-10.

37. Binnig, J.; Meyer, J.; Kasper, G. Calibration of an optical particle counter to provide PM2.5 mass for well-defined particle materials. J. Aerosol. Sci. 2007, 38: 325–332.

38. Rodes C.E.; Thornburg, J.W. Breathing zone exposure assessment. In: Aerosols handbook: Measurement, dosimetry, and health effects. L.S. Ruzer, N.H. Harley (ed) pp. 61–74. Boca Raton: CRC Press, 2005.

39. Fierz, M.; Burtscher, H.; Steigmeier, P.; Kasper, M. Field Measurement of Particle Size and Number Concentration with the Diffusion Size Classifier (DiSC). SAE 2008, 01: 1179.

40. Fierz, M.; Houle, C.; Steigmeier, P.; Burtscher, H. Design, Calibration, and Field Performance of a Miniature Diffusion Size Classifier, Aerosol Sci. Tech. 2011, 45(1) 1-10.

41. Meier R.; Clark, K.; Riediker, M. Comparative testing of a miniature diffusion size classifier to assess airborne ultrafine particles under field conditions. Aerosol Sci. Technol. 2013, 47(1): 22–28

42. Kaminski, H.; Kuhlbusch, T.A.J.; Rath, S.; Götz, U.; Sprenger, M.; Wels, D.; Polloczek, J.; Bachmann, V.; Dziurowitz, N.; Kiesling, H-J.; Schwiegelshohn, A.; Monz, C.; Dahmann, C.; Asbach C. Comparability of mobility particle sizers and diffusion chargers J. Aerosol Sci. 2013, 57: 156–178

43. Buonanno G.; Fuoco, F.C.; Stabile, L. Influential parameters on particle exposure of pedestrians in urban microenvironments. Atmos. Environ. 2011, 45: 1434–1443.

44. Misra, C.; Singh, M.; Shen, S.; Sioutas, C.; Hall, P.M. Development and evaluation of a personal cascade impactor sampler (PCIS). J. Aerosol Sci. 2002, 33(7): 1027-1047.

45. UNI EN 14907:2005: Ambient air quality - Standard gravimetric measurement method for the determination of the $PM_{2,5}$ mass fraction of suspended particulate matter

46. UNI EN 12341 "Air quality - Determination of the PM10 fraction of suspended particulate matter Reference method and field test procedure to demonstrate reference equivalence of measurement methods"

47. American Society of Testing and Materials (ASTM): ASTM Standard Practice for Controlling and Characterizing Errors in Weighing Collected Aerosols (ASTM D 6552). [Standard] West Conshohocken, Pa.: ASTM, 2000.

48. Robakiewicz P.; Ryder, E.F. Statistic: Detecting differences Among groups. In Current protocols in protein Science (commonly used techniques). Supplement 21: A.3 G.1-A.3G.22 New York: John and Wiley & Sons, Inc., 2000.

49. Norman, G.; Streiner, D.L. Biostatistica: Quello che avreste voluto sapere. Milano: Casa Editrice Ambrosiana – Zanichelli, 2000

50. Miller, J.N.; Miller, J.C. Statistics and Chemiometrics for Analytical Chemistry. Fifth Edition. Harlow. Pearson Education Ltd, 2005.

51. Karanasiou, A.; Viana, M.; Querol, X.; Moreno, T.; de Leeuw, F. Assessment of personal exposure to particulate air pollution during commuting in European cities - Recommendations and policy implications. Sci. Total Environ. 2014, 490: 785-797

52. Int Panis, L.; de Geus, B.; Vandenbulcke, G.; Willems, H.; Degraeuwe, B.; Bleux, N.; et al. Exposure to particulate matter in traffic: a comparison of cyclists and car passengers. Atmos. Environ. 2010, 44(19): 2263–2270.

53. Berghmans, P.; Bleux, N.; Panis, L.I.; Mishra, V.K.; Torfs, R.; Van Poppel, M.; Exposure assessment of a cyclist to PM_{10} and ultrafine particles. Sci. Total Environ. 2009, 407: 1286–1298.

54. Strak, M.; Boogaard, H.; Meliefste, K.; Oldenwening, M.; Zuurbier, M.; Brunekreef, B.; et al. Respiratory health effects of ultrafine and fine particle exposure in cyclists. Occup. Environ. Med. 2010, 67(2):118–124.

55. de Nazelle, A.; Fruin, S.; Westerdahl, D.; Martinez, D.; Ripoll, A.; Kubesch, N.; et al. Travel mode comparison of commuters' exposures to air pollutants in Barcelona. Atmos. Environ. 2012; 59: 151–9.

56. Kaur, S.; Clark, R.; Walsh, P.; Arnold, S.J.; Colvile, R.N.; Nieuwenhuijsen, M. Exposure visualization of ultrafine particle counts in a transport microenvironment. Atmos. Environ. 2006, 40: 386–398.

57. Zuurbier, M.; Hoek, G.; Oldenwening, M.; Lenters, V.; Meliefste, K.; van den Hazel, P.; et al. Commuters' exposure to particulate matter air pollution is affected by mode of transport, fuel type, and route. Environ. Health. Perspect. 2010, 118(6): 783–789.

58. Spinazzè, A.; Cattaneo, A.; Garramone, G.; Cavallo, D.M. Temporal variation of size-fractionated particulate matter and carbon monoxide in selected microenvironments of the Milan urban area. J. Occup. Environ. Hyg. 2013, 10: 652–662.

59. Cattaneo, A.; Garramone, G.; Taronna, M.; Peruzzo, C.; Cavallo, D.M. Personal exposure to airborne ultrafine particles in the urban area of Milan. J. Phys. Conf. Ser. 2009, doi:10.1088/1742-6596/151/1/012039

60. Schlitt, C.; Garramone, G.; Cattaneo, A.; Peruzzo, C.; Taronna, M.; Pulvirenti, S.; Vercelli, F.; Cavallo, D.M. Fine and Ultrafine Particle Levels Determined during Everyday Activities: The PM-CARE Project. In Proceedings of 11th International Conference on Indoor Air Quality and Climate: Indoor Air, Copenhagen, Denmark, 17-22 August 2008.

61. Lonati, G.; Ozgen, S.; Luraghi, I.; Giugliano, M. Particle number concentration at urban microenvironments. Chem. Eng. Trans. 2010, 22: 137-142.

62. Dennekamp, M.; Mehenni, O.; Cherrie, J.; Seaton, A. Exposure to ultrafine particles and PM2.5 in different microenvironments. Ann. Occup. Hyg. 2002, 46: 412–414.

63. Diapouli, E.; Grivas, G.; Chaloulakou, A.; Spyrellis, N. PM_{10} and ultrafine particles counts in vehicle and On-road in the Athens area. Water Air Soil Pollut. Focus 2008, 8(1): 89–97.

64. Zhu, Y.; Eiguren-Fernandez, A.; Hinds, W.C.; Miguel, A.H. In-cabin commuter exposure to ultrafine particles on Los Angeles freeways. Environ. Sci. Technol. 2007, 41(7): 2138-2145.

65. Qi, C.; Stanley, N.; Pui, D.Y.H.; Kuehn, T.H. Laboratory and on-road evaluations of cabin air filters using number and surface area concentration monitors. Environ. Sci. Technol. 2008, 42: 4128-4132.

66. Knibbs, L. D.; de Dear, R. J.; Morawska, L. Effect of cabin ventilation rate on ultrafine particle exposure inside automobiles. Environ. Sci. Technol. 2010, 44(9): 3546-3551.

67. Asmi, E.; Antola, M.; Yli-Tuomi, T.; Jantunen, M.; Aarnio, P.; Mäkelä, T.; et al. Driver and passenger exposure to aerosol particles in buses and trams in Helsinki, Finland. Sci. Total Environ. 2009, 407(8): 2860–2867.

68. Kaur, S.; Nieuwenhuijsen, M.; Colvile, R. Personal exposure of street canyon intersection users to $PM_{2.5}$, ultrafine particle counts and carbon monoxide in Central London, UK. Atmos. Environ. 2005, 39(20): 3629.

69. Knibbs, L.K.; Cole-Hunter, T.; Morawska, L. A review of commuter exposure to ultrafine particles and its health effects. Atmos. Environ. 2011, 45: 2611–2622.

70. Lonati, G.; Crippa, M.; Gianelle, V.; Van Dingenen, R. Daily patterns of the multi-modal structure of the particle number size distribution in Milan, Italy. Atm. Environ. 2011; 45(14): 2434-2442.

71. Birmili, W.; Tomsche, L.; Sonntag, A.; Opelt, C.; Weinhold, K.; Nordmann, S.; et al. Variability of aerosol particles in the urban atmosphere of Dresden (Germany): effects of spatial scale and particle size. Meteorol. Z. 2013; 22: 195–211.

72. Mbengue, S.; Alleman, L.Y.; Flament, P. Size-distributed metallic elements in sub-micronic and ultrafine atmospheric particles from urban and industrial areas in northern France. Atmos. Res. 2014, 135–136: 35–47

73. Ning, Z.; Geller, M.D.; Moore, K.F.; Sheesley, R.; Schauer, J.J.; Sioutas, C. Daily variation in chemical characteristics of urban ultrafine aerosols and inference of their sources. Environ. Sci. Technol. 2007, 41: 6000–6006.

74. Ntziachristos, L.; Ning, Z.; Geller, M.D.; Sheesley, R.J.; Schauer, J.J.; Sioutas, C. Fine, ultrafine and nanoparticle trace element compositions near a major freeway with a high heavy-duty diesel fraction. Atmos. Environ. 2007, 41: 5684–5696.

75 Daher, N.; Hasheminassab, S.; Shafer, M.M.; Schauer, J. J.; Sioutas, C. Seasonal and spatial variability in chemical composition and mass closure of ambient ultrafine particles in the megacity of Los Angeles. Env. Sci. Process. Impact. 2013, 15: 283–295.

76. Arhami, M.; Minguillón, M. C.; Polidori, A.; Schauer, J. J.; Delfino, R. J.; Sioutas, C. Organic compound characterization and source apportionment of indoor and outdoor quasi-ultrafine particulate matter in retirement homes of the Los Angeles Basin, Indoor Air, 2010, 20: 17–30.

77. Albuquerque, P.C.; Gomes, J.F.; Bordado, J.C. Assessment of exposure to airborne ultrafine particles in the urban environment of Lisbon, Portugal. J. Air Waste Manage. Assoc. 2012, 62: 373–380

78. Ntziachristos, L.; Polidori, A.; Phuleria, H.; Geller, M.D.; Sioutas, C. Application of a diffusion charger for the measurement of particle surface concentration in different environments. Aerosol Sci. Technol. 2007, 41: 571–580

79. Marcazzan, G.M.; Ceriani, M.; Valli, G.; Vecchi, R. Source apportionment of PM_{10} and $PM_{2.5}$ in Milan (Italy) using receptor modeling. Sci. Total Environ. 2003, 317: 137–147.

80. Vecchi, R.; Marcazzan, G.; Valli, G.; Ceriani, M.; Antoniazzi, C. The role of atmospheric dispersion in the seasonal variation of PM_1 and $PM_{2.5}$ concentration and composition in the urban area of Milan (Italy). Atmos. Environ. 2004, 38: 4437–4446.

81. Cattaneo, A.; Peruzzo, C.; Garramone, G. et al. Airborne particulate matter and gaseous air pollutants in residential structures in Lodi Province, Italy. Indoor Air 2011, 21: 489–500.

82. Mishra, V.K.; Kumar, P.; Van Poppel, M.; Bleux, N.; Frijns, E.; Reggente, M.; et al. Wintertime spatio-temporal variation of ultrafine particles in a Belgian city. Sci. Total Environ. 2012; 431: 307–13.

83. Mejıa, J.F.; Morawska, L.; Mengersen, K. Spatial variation in particle number size distributions in a large metropolitan area. Atmos. Chem. Phys. 2008; 8: 1127–1138.

84 Buonanno, G.; Fuoco, F.C.; Morawska, L.; Stabile, L. Airborne particle concentrations at schools measured at different spatial scales. Atmos. Environ. 2013, 67: 38–45.

85. Matson, U.; Ekberg, L.E.; Afshari, A. Measurement of Ultrafine Particles: A Comparison of Two Handheld Condensation Particle Counters. Aerosol Sci. Technol. 2004, 38: 487–495.

86. Zhu, Y.F.; Yu, N.; Kuhn, T.; Hinds, W.C. Field Comparison of P-Trak and Condensation Particle Counters. Aerosol Sci. Technol. 2006, 40: 422–430.

87. Fierz, M.; Houle, C.; Steigmeier, P.; Burtscher, H. Design, Calibration, and Field Performance of a Miniature Diffusion Size Classifier. Aerosol Sci. Technol. 2011, 45:1–10.

88. Wittmaack, K. (2002). Advanced evaluation of size-differential distributions of aerosol particles. J. Aerosol Sci. 33(7): 1009-1025.

89. Taylor, M.A., 2002. Quantitative measures for shape and size of particles. Powder Technol. 124, 94–100.

90. Tuch, T., Mirme, A., Tamm, E., Heinrich, J., Heyder, J., Brand, P., Roth, C., Wichmann, H.E., Pekkanen, J., Kreyling, W.G. Comparison of two particle size spectrometers from ambient aerosol measurements in environmental epidemiology. Atm. Environ. 2000, 34: 139–149.

91. Morawska, L., Johnson, G., Ristovski, Z.D., Agranovski, V. Relation between particle mass and number for sub-micrometer airborne particles. Atm. Environ. 1999, 33: 1983–1990.

92. Tittarelli, A., Borgini, A., Bertoldi, M., De Saeger, E., Ruprecht, A., Stefanoni, R., Tagliabue, G.; Contiero, P.; Crosignani, P. Estimation of particle mass concentration in ambient air using a particle counter. Atm. Environ. 2008, 42(36): 8543-8548.

6. OCCUPATIONAL EXPOSURE TO ENGINEERED NANOPARTICLES AND NANOMATERIALS: DEVELOPMENT OF AN EXPOSURE ASSESSMENT STRATEGY

The most extensive exposures to engineered nanoparticles (NP) are likely to occur at workplace. Until research moves forward to characterize more broadly the potential hazards of nanoparticles and to provide a scientific basis for appropriate control of nanomaterials in the workplace, current and future workers may be at risk from occupational exposures.

This chapter describes the development of an instrumental approach for controlling NP exposures, which takes into account the major potential route of exposure and factors that may influence biological activity and potential toxicity of nanomaterials and incorporates a risk management approach.

Two different methods were generally used to measure and assess occupational exposures to engineered nanoparticles (NP): the first method involved off-line gravimetric analysis of filter samples collected with Low Pressure Impactor. The second method used hand-held, direct-reading instruments to obtain a time series of particle number concentrations (PNC), mean diameter and surface-area concentration for NP.

This approach is applicable to any occupational or environmental setting; further, the possibility of controlling exposures, even if in the presence of uncertainty, permit to define a conceptual framework for occupational risk management applied to manufactured (engineered) nanomaterials (MNM). The risk management framework incorporates primary approaches based on the traditional industrial hygiene hierarchy of controls involving elimination or substitution, engineering controls, administrative controls, and use of personal protective equipment. It also includes valuable secondary approaches involving health surveillance and medical monitoring.

6.1. INTRODUCTION

Manufactured (engineered) nanomaterials (MNM) are commonly defined as materials designed and produced to have structural features with at least one dimension of 100 nm or less. Such materials typically possess nanostructure-dependent properties, which make them desirable for commercial or medical applications. However, these same properties might lead to nanostructure-dependent biological activity that differs from the bulk properties of the constituent chemicals and compounds. Thus, there is an increased concern that the emergence and proliferation of nanotechnology will result in workers being exposed to engineered nanoparticles (NP, particles with diameters < 100 nm), mainly through inhalation [1, 2]. The most extensive exposures to NP are likely to occur at workplace. Until research moves forward to characterize more broadly the potential hazards of nanoparticles and to provide a scientific basis for appropriate control of nanomaterials in the workplace, current and future workers may be at risk from occupational exposures.

In the traditional risk framework, risk management decisions concerning occupational safety and health rely on site-specific risk assessment and information about the effectiveness of available measures to mitigate exposure. There is a mounting evidence that some MNM may impose a health hazard to humans health; nevertheless, the target organs and endpoints and the specific dose-response relationship are not clearly delineated [3]. The assessment and control of the potential exposure of workers to MNM and NP become crucial in occupational health and safety in order to minimize the risk of the workers; nevertheless, because of the uncertainty in the hazard assessment of MNM, traditional methods to measure particulate exposures involving the measurement of mass concentration of health-related fractions of particles in the worker's breathing zone may be inadequate for engineered nanoparticles. In occupational settings, the regulatory-approved method [4] for the exposure assessment of aerosols involves gravimetric analysis of a filter sample to measure mass concentration. The main exceptions to this methodology are particle-number-based metrics for exposure, which can be determined using a large number of commercially-available instruments even though they are not yet subject of regulations. It should be noted that NP often have a negligible mass when compared with larger particles that are collected with regulatory-approved, mass-based samplers [5]. Several OSH institutes agreed on a convention [6] for the measurement of NP in order to permit comparison of measurements. The core points of this convention includes that (I) the PNC should be measured in the range form approximately 10 to 600 nm, (II) the entire particle size distribution should be measured and (III) a concentration range of up to 1 x 10^8 particle/cm^3 should be covered. Further, as discussed in the previous chapter, there is evidence that other metrics such as surface-area concentrations may be better descriptors for the biological effects of NP [7] and have been shown to scale more closely with the toxic effects of nanoparticle inhalation [8, 9]. Reflecting the current state of the art, it is possible to conclude that effective approaches for measuring exposure to a wide range of MNM and NP require methods for measuring aerosol number, surface area and mass concentration.

The most widespread method that evolved to determine airborne nanoaerosol PNC as a function of particle size is based on electrical mobility analysis of the particles [10] measured via SMPS (Scanning Mobility Particle Sizers). These techniques usually comprise three main components: (I) a particle charger that charges particle depending on their size, (II) a mobility analyzer that classifies the particles on polarity according to their electrical mobility and (III) a particle counter that determines the number concentration of mobility-classified particles.

In the absence of occupational exposure to NP, exposure is often put into perspective by comparing it with the UFP exposure to workers at the workplace or with the "ambient" or "background" concentration of ultrafine particles [11]. Following this measurement strategy, the identification of the source of NP is in general fairly easy. However, the identification of the individual particles by further chemical methods and imaging methods still remains a challenge. *Brouwer et al. (2009)* [12] discuss the literature available till early 2009 with emphasis on possible ways to cope with the problem of background distinction: a major finding of most studies is that during production and handling of nanoparticles the workplace PNC of NP is close to background concentration in companies. This background nanoaerosols consists of ubiquitous ambient particles and of ultrafine particles from various sources (e.g.

vehicular traffic). In contrast, during sporadic events (e.g. leakage in the production line) very high PNC (up to 130000 pt/cm^3) have been encountered. In summary, most of the studies are more explorative in character and focused on the potential for emission of manufactured NP. To our knowledge, no work-shift average was presented based on PNC or Surface-area concentration. The most widespread used instrumentation for measurement of the PNC and size distribution was the SMPS, but no standard methods has been agreed on to produce official reference PNC [10]. Furthermore, due to the encountered rather low concentrations of NP in comparison to those of UFP at workplace, additional methods to determine the morphology and chemical composition of the nanoparticles are necessary in order to overcome the problem of background distinction. Further, the SMPS method showed some limitations (the technique is too bulky, complicated to use and too expensive for routine operation). Mass determination (and chemical characterization) does not provide information on particle concentration, dimension, agglomeration status and surface but it can act as a surrogate measure if data on size distribution or specific surface area are available [13]. The use of conventional impact techniques for determining NMs exposure is limited as the limit impact size range is from 200 and 300 nm, anyway. With low pressure impactors, it is possible to measure particles of up to 10 nm as static samplers; though their dimensions and complexity do not allow a personal use. However, a personal cascade impactor is available with a lower aerosol cut point of 250 nm [14], allowing an approximation of nanoaerosol mass concentration in the worker's breathing zone. All this samplers enable the sampling of materials deposited onto membranes and already divided into particle size fractions, thus they enable off-line investigations on UFP and NP through chemical analysis and electron and scanning probe microscopies. However, the gravimetric measure, although deriving from the traditional monitoring approach, is very little sensitive to NM made of NP.

Smaller handheld instruments can however be successfully used to monitor the efficiency of engineering controls which up to now have proven to efficiently control the exposure of worker against nanoparticles at the workplace. Several portable, affordable and easy to operate instruments are available to measure PNC. Condensation Particle Counters (CPCs) provide particle number concentrations between about 10 and 1000 nm, Optical Particle Counters (OPCs) provide PNC by size in multiple channels. The lower detection limit of many portable OPCs is approximatively 300 nm, with upper limits ranging from 10 to 20 µm. Without currently available field-portable instrument capable of sizing and counting particles < 100 nm, NIOSH has suggested determining PNC using a combined CPC and OPC method (count-difference method: number concentrations of particle <300 nm are calculated by subtracting OPC-measured number concentrations from CPC-measured concentrations) [15]. Therefore, this technique has been established as a proxy for assessing NP exposures, despite there are several possible errors associated with use of the count-difference method to estimate NP PNC: firstly, the response of an OPC is strongly dependent in particle refractive index (RI) and shape; thus, incorrect estimates of PNC by size can results when the shape and RI of measured particles as having a smaller than actual diameter [16]. Further, since most OPCs are unable to detect particle smaller than 300 nm, number concentrations measured with this method are not true estimates of NP concentrations but actually "very fine particles" number concentration, which are necessarily higher. However, other data reduction

approaches might be used to leverage the additional size distribution data provided by an OPC to estimate nanoparticle number concentrations [17].

The use of Condensation Particle Counter (CPC) is relatively easy and can be extended without great difficulty for particles of up to 3 nm. These instruments are widely used to measure ultrafine particles in the urban atmosphere [18-21]. As these devices are not size selective (except initial selection), it is difficult to distinguish the different sources of UFP generated by processes from those present in the background. Nevertheless, the adoption of this measurement process carried out in the vicinity of potential sources has been put forward for the raw identification of nanoaerosols emitted by sources in the workplace [22]. Such devices can be typically used in a static way only. Instruments providing information on particle total number and size are commercially available today and allow to measure the size distribution of particles with a range from 3 to 800 nm [23]. These techniques allow the determination of nano-range particles but they are not able to distinguish single NPs from those formed by agglomerates of smaller particles. The need to measure the surface area of NPs as it is shown to be more correlated to the potential biological effects: at present, the instrument which allows the measurements of aerosol surface-area is the epiphaniometer, nevertheless, more recent devices (diffusion chargers), might find a wider use, as they use the same principles of the previous instrumentation, although they operate by generation and adhesion of positive unipolar ions to the aerosol particles surface [24]. Further, a recent version of this instrument provided data which were well correlated to the surface area of particles deposited in the human respiratory tract [25].

In summary, despite strong evidence suggest that nanoaerosols should be primarily monitored with respect to PNC and surface-area concentration, it is also necessary to consider the characterization of exposure against aerosol mass, until further information is available. In addition, some studies have shown that there may be critical particle sizes influencing the fate and toxicity of respirable particles in the lungs [26, 27]. For each of these exposure metrics, but particularly in the case of mass concentrations, size-selective sampling will need to be employed to ensure only particle within the relevant size range are sampled [28]. However, currently, no standard sampling methods are available for the assessment of exposure to airborne nanoaerosols; even more so, every attempt to estimate exposure to UFP and NP requires the use of multiple sampling and assessment techniques. Some monitoring and characterization methods allow exposure assessments for NP and nanoaerosols in terms of mass, concentration and surface area, representing the basis for the development of new standards for the exposure characterization. Estimates and characterization of exposure to nanoaerosols are deeply limited by the lack of efficient instrumentation for personal sampling and, therefore, the combined use of devices for in-situ assessments and offline sampling analysis represents, today, the best tool for the estimation of personal exposure; nevertheless, most of instrumentations available, are expected to be adapted in terms of compactness, portability and costs for routinary applications in the workplace. Finally, an increasing number of studies are indicating that exposure to engineered nanoparticles may present an inhalation health risk that is not adequately addressed by conventional exposure evaluation methods. Before appropriate standards are developed, advances are needed in identifying

nanoaerosol attributes critical to environmental and occupational health, as well as the implementation of instruments and strategies capable of measuring exposure against these attributes. The monitoring and characterization techniques discussed in this chapter aim to evaluate nanoaerosol exposure in terms of mass, surface-area and/or number concentration in occupational settings.

6.2. METHODS

An experimental setup has been developed and then tested in a selected company. The aim was to measure the mass distribution, the number concentration and the surface-area concentration of engineered nanoparticles in occupational settings. Airborne NP were measured and characterized using a combination of different techniques: Condensation Particle Counters (CPC), Optical Particle Counters (OPCs), Diffusion size classifier (DiSC) and a Low Pressure cascade Impactor both for fixed-site and personal sampling.

In every campaign, the measurements were conducted during an entire work-shift, following a detailed protocol. A pilot study was conducted prior to the start of sampling to evaluate the sampling protocols for potential sources of error.

NP measurement and samples should be collected approximately in the same position during each sampling sessions. Fixed-site sampling devices were placed at a distance of approximately 1.50 m above the ground, which corresponded to the breathing zone of the occupants. Portable devices were also used in order to perform personal sampling on workers. Simultaneously, airborne NP were measured outdoors and in a control area (office).

Instruments

NP sampling and gravimetric analysis

Low Pressure Impactor (DLPI - Dekati® Low Pressure Impactor) has been used to measure the mass distribution of the airborne particles by post-gravimetric analysis. DLPI is a cascade low pressure impactor which classifies airborne particles into 13 size fractions at a sampling flow rate of 30 l/min. The size classification is made from 30 nm up to 10 μm with evenly distributed impactor stages. An additional back-up filter can be used to collect particles smaller than 30 nm. The operating principle of the DLPI is based on inertial size classification and gravimetric or chemical analysis of the collected particles. The DLPI features low inter-stage wall losses, a small particle collection area and robust yet easy to use operation. After each sampling, the impactors were cleaned following the manufacturer's instructions, and a continuous control of impactor's pressure were performed during the sampling period, in order to improve the accuracy of the sampling volume estimates. Sampling were conducted by mean of greased filters (Polycarbonate membranes with a 0.8 μm pore size and 25 mm diameter, greased with Apiezon-L). The mass of collected particles on membrane filters was determined gravimetrically following a standard operating procedure. Before weighing, each greased filter was conditioned at 50% ± 5% relative humidity and 20 °C ± 1 °C for a minimum of 24 hours in a climatic cabinet (Activa Climatic

Cabinet, Aquaria). The filters were weighed three times (every 20″) by a micro-balance with a precision of 1 μg (Gibertini Micro 1000, Gibertini Elettronica s.r.l., Novate Milanese, Milan, Italy), ensuring a standard deviation < 3 μg. An electrical C-shaped ionizer (HAUG GmbH & CO. KG, Osnabruck, Germany) was used to eliminate electrostatic charges from the filter surfaces. This procedure was repeated before and after each sampling, and the mass of the PM was determined by differential weighing. Two laboratory blanks were always weighed under the same conditions to verify possible anomalies during the conditioning. Prior to the analysis, the micro-balance was auto-calibrated, and the calibration check was performed using certified standard weights, allowing deviations from the true value < 3 μg. Particles mass concentrations ($\mu g/m^3$) were then determined by gravimetric analysis, in accordance to reference methods [29, 30] and in accordance with accepted standard practice. The net PM mass on filters was measured by weighing the conditioned filters before and after sampling with a microbalance in a temperature- (20 ± 1 °C) and relative humidity- (50 ± 5%) controlled environment. The quality of the weighing procedure was assessed using the ASTM D 6552 method [31]. Mass limit of detection (LOD) for $PM_{0.25}$ was estimated following a method proposed by ASTM [31].

NP on-line measurements

Numeric concentrations of airborne UFP were measured using a Miniature Diffusion Size Classifier (DSC) and Condensation Particle Counters (CPC). Both these instruments are able to provide real-time measurement of particles, although each type of instrument has its own sensitivity to specific particle characteristics. The DSC used for this study (DiSCmini, Matter Aerosol AG, Wohlen AG, Swiss) is a compact, battery-powered handheld instrument which measures the number and average size (mean diameter and lung-deposited surface area) in the size range of about 10 < Dp < 700 nm according to the manufacturer (an impactor is used to remove particles larger than 700 nm). DiSCmini is based on unipolar charging of the aerosol, followed by detection in two electrometer stages [32, 33]. DiSCmini can detect particle concentrations of 10^3–10^6 pt/cm^3; the detection limits are presented in a dedicated publication [33]. Experimental results showed that DiSCmini performances correspond fairly well (within 20%) to traditional instruments used for UFP measurements, also in terms of reproducibility and agreement with standard condensation particle counters [33]. A portable CPC was also used in this study (P–Trak Ultrafine Particle Counter model 8525; TSI Inc., Shoreview, MN, USA), in order to perform a field comparison with the DSC. The CPC quantifies the particles (size range: 0.02 to 1 μm) number concentration in the interval 0 - 5 x 10^5 pt/cm^3, using isopropanol to artificially enlarge particles through condensation of vapors on the particle surface. It should be observed that the lower detection limit of DSC in respect to CPC, would introduce a bias that would not affect the conclusions [34] . Further, particle size range for both instruments is beyond the upper limit of ultrafine particles of 100 nm, thus UFP data include also particles with dimension >100 nm (sub-micrometer particles), although their number concentration is assumed to be very low with respect to those smaller than 100 nm.

Information on the instrumentation is added to avoid erroneous interpretation due to differences in instrumentation performances. In fact, although, portable and easy–to–use

devices are usually characterized by worse metrological performance than standard counters commonly used in aerosol research in terms of accuracy, minimum detectable particle diameter and maximum measurable concentrations, the portable rapid-response instruments used in this study have shown adequate capacity to measure short-term variation of PNC [34]; further, the DiSCmini is attended to be less accurate than CPC, but in general, the DiSCmini should in our opinion be a useful tool in any application where ease of use and portability are more important than high accuracy [33].

Numeric concentrations of airborne particles were also measured using optical particle counters (OPC), which are able to provide real-time measurement of particles. OPCs allow measurement of particle number concentration (PNC) separated as a function of their size, by detecting the light scattered by individual particles. The size range normally measured by OPCs is 0.3 to 20 μm, while the maximum number concentration is dependent on particle size. This instrument measures the total number of particles per cubic centimeter of air (pt/cm^3) according to six specific size cut-points: 300 nm, 500 nm, 1 μm, 2.5 μm, 5.0 μm, and 10.0 μm. The OPC used in this study (mod. Handheld 3016, Lighthouse Worldwide Solutions, Fremont, CA; Counting Efficiency: 50% at 0.3 μm; 100% for particles > 0.45 μm) uses an active sampling mode. It is based on the principle of light scattering of a linear radiation produced by a diode laser focused on the air flow to measure PNC. Each signal is counted and classified into 6 different dimensional fractions (0.3–0.5, 0.5–1.0, 1.0–2.5, 2.5–5.0, 5.0–10.0, > 10.0 μm). The timing of signal processing is extremely fast and allows measurements with high monitoring frequency (10 second-weighted average concentration). Immediately before the study, all the instruments were calibrated by factory-supplied services.

The CPCs and DSC were set to record a sample every second, whereas the OPCs were set to sample every 10 seconds

Experimental protocol

The aim of this protocol was to characterise the exposure concentration associated with each working task and working place, as well as the general workplace concentration. An initial assessment involved identifying the potential source(s) of engineered nanomaterial emissions by reviewing the type of process and work practices. When available, literature was reviewed to gain an understanding of the engineered nanomaterial used, including their physicochemical properties (e.g. size range, shape, chemical composition, solubility). Once potential sources of emissions have been identified, an observational survey of the production area was made to identify the processes/tasks that require air sampling. Then, the frequency and duration of each operation and the type of equipment used for handling and containment of the material were defined. Further, the process points where containment is deliberately breached were identified.

Background concentrations

In order to determine the influence of background particle concentration on measurements made for the engineered nanomaterial of interest, the average airborne PNC were measured with the CPC and OPC before the processing or handling of nanomaterial begins, in two locations (outdoors and in an office area of the company, used as a "control room"). Measurements of background PNC are repeated after the active processing, manufacturing, or handling of the nanomaterial has ended. An average background number concentration is then subtracted from the measurements made during processing, manufacturing, or the handling of engineered nanomaterials. This approach of "adjusting" the process/task-based measurements is acceptable only if the background particle number concentration remains relatively stable throughout the measurement period and emissions from the process under investigation are sufficiently elevated above background [35]. Once background particle number concentrations have been determined, specific measurements are made with all instruments simultaneously at different locations. Airborne particle number concentrations are determined and compared to background to determine mean exposures. Measurement data can also be used to identify processes, tasks, locations, and personnel.

Source-Specific Area Sample

One filter-based, size-selective air sample (DLPI) was collected at process/task locations where process operations involved engineered nanomaterial emissions or application. Gravimetrical analysis of the samples result in size-selective mass concentration. Further, electron microscopy analysis of filter allows the determination of the particle size range and degree of agglomeration of the aerosol collected. The source-specific air samples were collected as close as possible to the emission source to increase the probability of detecting any release of engineered nanomaterials and to identify locations most likely to result in worker exposure. Therefore, results from this type of sampling should not be interpreted as representative of full-shift worker exposure, but as an indicator of NP release. Sampling duration generally matched the length of time necessary to complete a process or task.

Microenvironmental Area Measurements

A pair of on-line NP air concentration measurements (CPC and OPC) were collected (I) at process/task location where process operations involved engineered nanomaterial emissions or application (in correspondence of DLPI sampling) and (II) at process/task location where process nanomaterials were not directly involved. Sampling duration generally matches the length of time necessary to complete a work shift. Since this techniques provides an high-frequency continuous measurement of workplace NP PNC. Results from this type of measurement, should be interpreted as an indicator of workplace's micro-environments exposures. Further, continuous measurements obtained with the CPC and OPC can also be used to indicate that NP is emitted from a specific process or task. The micro-environmental sampling is thus assumed to allow measurement of exposure concentrations without losing accuracy with respect to breathing zone (personal) measurements [36].

Personal Air Monitoring.

Personal breathing zone samples were collected using the portable diffusion charger classifier described above (DSC). Personal measurements were collected in the breathing zone of workers for whom high exposures were expected (worst-case exposure scenario). Typically, each sampling campaign consisted of (I) one personal monitoring for measurement of workers' exposure (DiSCmini), and of a number of fixed site sampling points (micro-environmental sampling), as described above. A time-activity diary was also used in order to accurately separate the on-line concentration data as a function of the different monitored environments and working task.

6.3. RESULTS

The experimental protocol described above has been used to evaluate potential exposure to engineered nanoparticles in a selected company; a summary of those findings is presented in the next chapter of this thesis.

6.4. DISCUSSION

Because the size range of nanomaterials in the selected facilitiy was often unknown, it was postulated that using these particle sizing and counting instruments would provide a semiquantitative indication of the nature and magnitude of potential exposures for each process or task. The instruments used simultaneously, alongside each other, provide a comprehensive evaluation of the aerosol being sampled. For example, a high particle number concentration on the CPC and DiSCmini, in combination with a high particle count in the small size range (300–500 nm) on the OPC, would indicate the possible presence of nanoscale particles. Conversely, a low CPC count, in combination with a high OPC count in the larger size range (> 1.0 μm) would indicate the presence of large particles and/or agglomerates [35]. Overall, these direct-reading, real-time instruments appear useful in identifying sources and tasks that contribute to the exposure to NP in the work environment.

6.5. CONCLUSIONS

The presented protocol was intended to be useful in determining potential exposure to engineered nanomaterials and nanoparticles in the workplace with complementary approaches. Direct-reading instruments were used to evaluate the total particle number concentrations relative to background and the mean size characteristics of the particles (mean diameter, surface-area concentration). Then filter-based air samplings were performed for qualitative analysis of particle size, shape, and morphology (by electron microscopy analysis) and for the determination of mass concentrations; further, direct-reading instruments were also been used to evaluate potential personal exposure to engineered NP. Beyond exposure assessment purposes, these information may also be used to determine whether engineering controls are effective in preventing release of the engineered nanomaterials into the workplace atmosphere. Results obtained from the application of this protocol in occupational settings from selected companies, are presented in the following chapter as case-studies.

6.6. ACKNOWLEDGEMENTS

A special acknowledgment to Andrea Cattaneo, PhD for his fundamental contribution in the development of the sampling protocol and strategy.

This chapter is based on:

- Spinazzè A., Del buono L., Cattaneo A., Cavallo, D.M. (2014)
  Valutazione e gestione del rischio da esposizione a nanomateriali
  Atti del Atti del 31° Congresso Nazionale AIDII" (ISBN 978-88-86293-24-2), pp 272-278

6.7. REFERENCES

1. Aitken, R.J.; Creely, K.S.; Tran, C.L. Nanoparticles: An occupational hygiene review. HSE Books. 2004

2. Balbus, J.M.; Florini, K.; Denison, R.A.; Walsh, S.A. Protecting workers and the environment: An environmental NGO's perspective on nanotechnology. J. Nanopart. Res. 2007, 9(1): 11-22.

3. Murashov, V.; Engel, S.; Savolainen, K.; Fullam, B.; Lee, M.; Kearns, P. Occupational safety and health in nanotechnology and organisation for economic cooperation and development. J. Nanopart. Res. 2009, 11: 1587–1591

4. National Insititute of Occupational Safety and Health (NIOSH): method 0500, Particulates not Otherwise Regolated, Total. In NIOSH Manual of Analytical Methods (NMAM), 4th ed. DHHS (NIOSH) Publication 94-113, NIOSH, 1994

5. Mark, D. Occupational exposure to nanoparticles and nanotubes. In Nanotechnology: conseguences for human health and the environment, R.E. Hester and R.M. Harrison (eds). Cambrige, UK: The Royal Society of Chemistry, 2007. pp. 50-80

6. Riediger, G,; Möhlmann, C. Ultrafeine Aerosole an Arbeitsplätzen – Konventionen und Beispiele aus der Praxis. Gefahrstoffe – Reinhalt Luft 2001, 61: 429–434

7. Maynard, A.D.; Aitken, R.J. Assessing exposure to airborne nanomaterials; current abilities and future requirements. Nanotoxicology 2007, 1: 26–41

8. Wittmaack, K. In search of the most relevant parameter for quantifying lung inflammatory response to nanoparticle exposure: particle number, surface area, or what?. Environ. Health. Persp. 2007, 115(2): 187-194.

9. Monteiller, C.; Tran, L.; MacNee, W.; Faux, S.; Jones, A.; Miller, B.; Donaldson, K. The pro-inflammatory effects of low-toxicity low-solubility particles, nanoparticles and fine particles, on epithelial cells in vitro: the role of surface area. J. Occup. Env. Med. 2007, 64(9): 609-615.

10. Asbac, C.; Kaminski, H.; Fissan, H.; Monz, C.; Dahmann, D.; Mülhopt, S.; Paur, HR.; Kiesling, H.J.; Herrmann, F.; Voetz, M.; Kuhlbusch, T.A.J. Comparision of four mobility particle sizers with different time resolution for stationary measurement. J. Nanopart. Res. 2009, 11: 1593–1609

11. Berges, M. G. M. Exposure during Production and Handling of Manufactured Nanomaterials, in Nanomaterials (ed Deutsche Forschungsgemeinschaft (DFG)), Wiley-VCH Verlag. GmbH & Co. KGaA, Weinheim, Germany. 2013 DOI: 10.1002/9783527673919.ch2

12. Brouwer, D.; van Duuren-Stuurman, B.; Berges, M.; Jankowska, E.; Bard, D.; Mark, D. From workplace air measurement results towards estimates of exposure? Development of a strategy to assess exposure to manufactured nano-objects. J. Nanopart. Res. 2009, 11: 1867–1881

13. ISO/TR. International Standardization Organisation. Workplace atmospheres - Ultrafine, nanoparticle and nano-structured aerosols - Inhalation exposure characterization and assessment. Technical Report ISO/TR 27628/2007

14. ISO. International Standardization Organisation. Health and Safety Practices in Occupational. Settings Relevant to Nanotechnologies. Technical Report ISO 12885/2008.

15. National Institute for Occupational Safety and Health (NIOSH): Approaches to Safe Nanotechnology, managing the health and safety concerns associated with engineered nanomaterials. DHHS (NIOSH) Pub. No. 2009-125. Cincinnati, Ohio: NIOSH, 2009

16. Heim, M.; Mullins, B. J.; Umhauer, H.; Kasper, G. Performance evaluation of three optical particle counters with an efficient "multimodal" calibration method. J.Aerosol Sci. 2008, 39(12): 1019-1031.

17. Schmoll, L. H.; Peters, T. M.; O'Shaughnessy, P. T. Use of a condensation particle counter and an optical particle counter to assess the number concentration of engineered nanoparticles. J. Occup. Environ. Hyg. 2010, 7(9): 535-545.

18. Hind, W.C. Aerosol Technology: Properties, Behavior, and Measurement of airborne particles. Second edition, 2nd ed. Wiley-Interscience, New York, 1999.

19. Kim, S.; Shen, S.; Sioutas, C.; Zhu, Y.; Hinds, W. Size distribution and diurnal and seasonal trends of ultrafine particles in source and receptor sites of the Los Angeles Basin. J. Air Waste Manage. Assoc. 2002, 52: 297-307

20. Aalto, P.; Hämeri, K.; Paatero, P.; Kulmala, M.; Bellander ,T.; Berglind, N.; et al. Aerosol particle number concentration measurements in five European cities using TSI-3022 condensation particle counter over a three-year period during health effects of air pollution on susceptible subpopulations J. Air Waste Manage. Assoc. 2002, 55: 1064–1076.

21. Marconi, A.; Cattani, G.; Cusano, M.; Ferdinandi, M.; Inglessis, M.; Viviano, G.; Settimo, G.; Two years of fine and ultrafine particles measurements in Rome, Italy. J. Toxicol. Environ. Health. A. 2007, 70: 213-221.

22. Brouwer, D.H.; Gijsbers, J.H.; Lurvink, M.W. Personal exposure to ultrafine particles in the workplace: exploring sampling techniques and strategies. Ann. Occup. Hyg. 2004, 48(5): 439-453.

23. Flagan, R.C. Electrical techniques. In: Aerosol measurement: principles, techniques and applications, Baron PA and Willeke K. John Wiley &Sons, New York, 537-568, 2001.

24. Keller, A.; Fierz, M.; Siegmann, K.; Siegmann, H.C.; Fillippov, A. Surface science with nanosized particles in a carrier gas. J. Vacuum. Sci. Technol. 2001,19(1): 1-8.

25. Wilson, W.E. Use of the electrical aerosol detector as an indicator for the total particle surface area deposited in the lung. Proceedings of the 2004 Air and Waste Management Association Conference.

26. Li, N.; Sioutas, C.; Cho, A.; Schmitz, D.; Misra, C.; Sempf, J.; et al. Ultrafine particulate pollutants induce oxidative stress and mitochondrial damage. Environ. Health. Persp. 2003, 111(4): 455.

27. Hofmann, W.; Sturm, R.; Winkler-Heil, R.; Pawlak, E. Stochastic model of ultrafine particle deposition and clearance in the human respiratory tract. Radiat. Prot. Dosim. 2003, 105(1-4): 77-79.

28. ISO. International Standardization Organisation. Air Quality – Particle size fraction definitions for health-related sampling, ISO 7708:1995, 1995.

29. UNI EN 14907:2005: Ambient air quality - Standard gravimetric measurement method for the determination of the PM2,5 mass fraction of suspended particulate matter.

30. UNI EN 12341 “Air quality - Determination of the PM10 fraction of suspended particulate matter Reference method and field test procedure to demonstrate reference equivalence of measurement methods”.

31. American Society of Testing and Materials (ASTM): ASTM Standard Practice for Controlling and Characterizing Errors in Weighing Collected Aerosols (ASTM D 6552). [Standard] West Conshohocken, Pa.: ASTM, 2000.

32. Fierz, M.; Burtscher, H.; Steigmeier, P.; Kasper, M. Field Measurement of Particle Size and Number Concentration with the Diffusion Size Classifier (DiSC). SAE 2008, 01: 1179.

33. Fierz, M.; Houle, C.; Steigmeier, P.; Burtscher, H. Design, Calibration, and Field Performance of a Miniature Diffusion Size Classifier, Aerosol Sci. Tech. 2011, 45(1): 1-10.

34. Cattaneo A.; Garramone, G.; Taronna, M.; Peruzzo, C.; Cavallo, D.M. Personal exposure to airborne ultrafine particles in the urban area of Milan. J. Phys. Conf. Ser. 2009; IOP Publishing, p. 012039.

35. Methner, M.; Hodson, L.; Geraci, C. Nanoparticle Emission Assessment Technique (NEAT) for the Identification and Measurement of Potential Inhalation Exposure to Engineered Nanomaterials — Part A. J. Occup. Environ. Hyg., 2009, 7:3, 127-132

36. Cattaneo, A.; Taronna, M.; Garramone, G.; Peruzzo, C.; Schlitt, C.; Consonni, D.; Cavallo, D.M. Comparison between personal and individual exposure to urban air pollutants. Aerosol. Sci. Technol. 2010, 44: 370–379.

7. OCCUPATIONAL EXPOSURE TO ENGINEERED NANOPARTICLES AND NANOMATERIALS: A CASE STUDY – APPLICATION OF NANOSTRUCTURED TITANIUM DIOXIDE

The case study here presented, refers to a company devoted to the application of nano-sized titanium dioxide (nano-TiO_2) onto concrete building materials (e.g. pavement blocks). Workers' exposure to nano-TiO_2 was investigated by an environmental multi-parametric monitoring protocol (*chapter 6*) in order to characterise the exposure concentration associated with each working task and working place, as well as the general workplace concentration.

7.1. INTRODUCTION

The case study here presented refers to a company devoted to the application of nano-sized titanium dioxide (nano-TiO_2) onto concrete building materials (e.g. pavement blocks).

Characteristics of nano-TiO_2

Titanium dioxide (TiO_2) powders have been commonly used as white pigments because they are inexpensive, chemically stable, harmless, and have no absorption in the visible region, leading to their white color [1]. Heterogeneous photocatalysis with TiO_2 as catalyst is a rapidly developing field in environmental engineering; the impulse of the use of TiO_2 as photocatalyst was given by Fujishima and Honda in 1972 [2]; more recently, technical developments allowed to develop TiO_2 photocatalytic capabilities for the decomposition of pollutants: the addition of a photocatalyst to ordinary building materials, creates "environmental friendly" materials by which air pollution or pollution of the surface itself can be diminished. Existing applications may be found for example in water purification, air conditioning (air purification), self-cleaning glazing, ceramic tiles and textiles. Heterogeneous photocatalysis with TiO_2 results in a total reduction of a broad gamma of chemical and biological pollutants; further, besides the air purifying and antiseptical action, TiO_2 is also used to obtain a self-cleaning material [3]. Over the past decade, commercial photocatalytic concrete products have been introduced and used as building materials [4 -6].

Toxicity of nano-TiO2

Some concerns were raised regarding the health aspects linked to the presence of nano-sized TiO_2 needed for production of this kind of materials. In fact, as reported in a recent review [7], while TiO_2 has been conventionally considered as a low toxicity material, nano-TiO_2 exhibits different physicochemical properties compared to the bulk material, which would be expected to alter its biological properties correspondingly. To date, a full risk assessment for the possible routes of exposure to TiO_2 NPs requires further data: current understanding on nano-TiO_2 toxicity largely depends on a limited number of experimental animal or cell culture studies, where extrapolation to humans and to the actual levels of exposure is required. Many studies have been conducted in vitro and in vivo to investigate the genotoxicity of TiO_2 NP but the results are conflicting and doses employed were high. Certain

reproductive and developmental toxicities in experimental animals or cell cultures have been observed in a few in vivo and in vitro studies. Whether human exposure to TiO_2 NP causes reproductive and developmental toxicities is unclear. Epidemiological studies thus far have not been able to detect an association between the occupational exposure to TiO_2 particles and an increased risk for cancer. In summary, although TiO_2 NP have been studied extensively in recent years, there is still much remaining to be elucidated concerning their possible health effects, to support risk assessment and management. However, the results currently available imply that nano-TiO_2 exhibit greater toxicity than TiO_2 in bulk form: these data should not be ignored, and development of prevention strategies to protect worker's health appears to be a precautionary course of action. Under conditions of occupational exposure, inhalation of nano-TiO_2 is normally the principal route for entry into the human body, even if the dermal route should not be ignored. Pulmonary inflammatory responses and lung cancers are the most important adverse effects observed in experimental animals due to nano-TiO_2 exposures. When only realistic doses are considered, as in the case of some inhalation studies, inflammatory responses are still one of the prominent observed effect.

The production process

TiO_2, in combination with light, reacts with pollutants: to increase the efficiency of the photocatalyst, its presence at the surface of the material is crucial. The goal of the production process is to have as much TiO_2 as possible at the surface of the material, without the risk of losing it by abrasion or weathering. The most efficient way to apply the TiO_2 is in a thin layer on the cementitious material: application in concrete tiles is therefore very suitable.

The production process in the study company was organised as follows: (I) raw material (concrete) supply and mixing, (II) production of concrete tile by extrusion, (III) racking, (IV) curing, and (V) drying. The concrete by-products then undergo a quality control (visual/manual inspection) just before the application of the photocatalyst by mean of a surface treatment with a water-based TiO_2 solution (99% water and 1% nano TiO_2) through an automatic electrostatic spraying system. Finishing operations, quality control and storage of the complete modules ends the production processes. All of these tasks were performed within a single building organised to accommodate different but not segregated areas for each specific activity. The production line was fully automated with minimal human intervention; only maintenance operations and quality control before and after the application of nano-TiO_2 required manual interventions: the concrete by-products, deposited on a metal rack, moved on a rail through different process phases and leave the production line when completely treated and packaged.

It must be considered that the nano-TiO_2 deposition processes were conducted at room conditions in close correspondence of one or two workers by means of an automatic spraying system device. The nano-TiO_2 water-based solution is therefore applied as an airborne sprayed aerosol. Some concern was expressed in this regard, since MNM-sprays were identified as the most critical category of MNM-containing products with regard to human health, due to the direct exposure pathway via lungs and because of the high emission potential [8-11].

Experimental set-ups to assess human exposure for MNM containing sprays were recently proposed [12-17] and an attempt was made to model exposure from consumer sprays on the basis of particle number instead of mass concentration [18]. In this case study, special emphasis was given to evaluate a reliable exposure assessment and was placed on how to improve experimental and analytical setups in order to trade on the existing approaches for the exposure assessment of airborne aerosols, for a comprehensive NP exposure assessment.

Objectives and aims

The main objective of this case study was to assess occupational exposures to nano-TiO_2 of workers engaged in the production of concrete by-products, involving the application of TiO_2 nanoparticles. The assessment was performed through environmental monitoring, following the above described protocol (*chapter 6*). Results have been also used to evaluate the effectiveness of the prevention and protection strategies adopted in the production site.

7.2. METHODS

Exposure Assessment: sampling and analysis

The exposure assessment protocol was intended to be useful in determining potential exposure to MNM and NP in the workplace with complementary approaches. Measurement were performed in accordance with the sampling protocol outlined above (*chapter 6*). The measurement design consists of the combination of:

- direct-reading instruments (CPC, OPC, DSC), used to evaluate the total particle number concentrations relative to the background and the mean size-dependent characteristics of particles (mean diameter, surface-area concentration);

- filter-based air sampling: for the determination of mass concentrations and for a following qualitative analysis of particle size, shape, and morphology (by means of Scanning Electron Microscope analysis);

It must be remembered that, in this case study, the nano-TiO_2 is applied as an airborne sprayed aerosol of a water-based solution. Currently there are no agreed upon guidelines for how to study the release of substances from spray application. Therefore, this chapter proposes an additional experimental and analytical approach for studying exposure to nano-TiO_2 in sprayed aerosols, to be used for exposure assessment. In order to evaluate the specific needs for nano-TiO_2 containing sprays, it was first evaluated the experimental information resulting from direct-reading measurements, which were then used to estimate the personal exposure to nano-TiO_2 by means of an indirect method. Specifically, for the generated spray aerosols, the size distribution and particle number concentration of ultrafine particles/NP were determined continuously by condensation particle counters (CPC) and a diffusion charger classifier (DSC) (for the ultrafine component); the larger sizes (between 300 nm and 10 μm) were investigated by optical particle counters (OPC) (for the fine and coarse components). Measurement frequencies were set at 1 to 10 seconds, depending on the type of instrument, providing a continuous profile of particle number concentration. "*Real room*" measurements

have the disadvantage that they are usually conducted with "*real ambient air*", so that the particle background concentration is not standardized. While this setup is more relevant for assessing realistic exposure, it is not so helpful for understanding the behavior of the NP released from production process, and the latter is crucial for the generalization of results and extrapolation of nano-TiO_2 exposure profiles. Thus, it was decided to normalize the particle concentration of all experiments, subtracting the background concentration, in order to obtain "occupational-specific" exposures. Thus the obtained differential concentrations measurements of airborne aerosol, were used in order to estimate nano-TiO_2 concentration in the airborne aerosols of each dimensional fraction, according to the general relationship described by *equation 1* (where M_{TiO2} is the nano-TiO_2 mass concentration, V is the particle volume, N is particle number concentration, ρ is the mass density of particles).

$$M_{TiO2} = (V \cdot \rho \cdot N) \cdot 10^{-2} \qquad \textit{(eq. 1)}$$

In this regard, some issue must be outlined: firstly, particle volume were calculated starting from mean diameter of particles. Number concentration values were derived form a differential count (subtracting background concentrations), in order to obtain production-related aerosol concentration. Further, ρ value for aerosols were assumed to be equal to mass density factor of the nano-TiO_2 aqueous solution (ρ = 1.0 g/cm^3, as reported by the manufacturer of the nano-TiO_2 water solution); finally, a correction factor (equal to 10^{-2}) was applied because nano-TiO_2 was expected to be present at 1% on mass by mass basis in the aerosol (as well as in the nano-TiO2 solution).

In summary, the measuring strategy for the exposure assessment consists of:

- Measuring the background particle number concentration (natural and anthropogenic nanoparticles in the ambient and environmental air) and measuring the particle number concentration generated by the processing equipment without use of MNM or nanoproducts (PRNP).
- Measuring the total particles' number concentration during processing, including the use of MNM;
- Subtracting the background and the PRNs from the total concentration to obtain a "*occupational-concentration*" of NP
- Estimate nano-TiO_2 mass concentration contained in nano-sized aerosols.
- Calculating a 8hr-TWA exposures for NP and nano-TiO_2;
- Compare these with proper occupational exposure levels, if available.

7.3. RESULTS

To date, only few measurement campaign were performed. A summary of some preliminary results from these campaigns are reported hereafter. The multi-metric characterization of UFP concentrations (obtained by direct-reading techniques) for different sampling position and production activity are reported in *table 1*. The estimation of airborne nano-TiO_2 were performed for different work activities (*table 2*) starting from continuous measurements of UFP and PM. These concentrations were then used to estimate the mean

exposure to nano-TiO_2 for workers involved in different tasks (*table 3*). Results are then presented as "occupational-concentration" and "occupational-exposure" since background and PRNP were previously subtracted. Samples from filter-based size-fractionated PM were analyzed through electron and scanning probe microscope (ESEM - EDS); an example image is reported in figure 1.

7.4. DISCUSSION

The United States National Institute for Occupational Safety and Health (NIOSH) proposed a recommended exposure limit (REL) for TiO_2 nanoparticles at 0.3 mg/m^3 (300 µg/m^3), which was 10 times lower than the REL for TiO_2 [19]. Apart from the NIOSH recommended REL, to date, no occupational or environmental exposure limits for TiO_2 NP have been set by any other regulatory agency. Regarding NP number concentrations, "nano reference values" (NRVs) were also developed to provide provisional limit values in situations where recognized OELs and DNELs are not available [20]. NRVs represent a warning level: when they are exceeded, exposure control measures should be taken. As such they may help the employer to ensure compliance with legal duties to manage the health and safety aspects of MNM in the workplace according to the state of the art in technology and science. For nano-TiO_2 the NRV was set to 40000 particles/cm^3 (Class 2b: "Bio-persistent granular and fiber-form nanomaterials in the range of 1 and 100 nm"; density < 6.0 g/cm^3) [20].

Accordingly to the performed measurements and estimations, workers were exposed to substantially lower nano-TiO_2 levels than the REL of 300 µg/m^3, since estimate exposure are in two order of magnitude lower (about 10 µg/m^3). Similarly, workers' exposure to NP was found to be lower than the NRV. It must be considered that the use of NRVs requires measurement of the particles' number concentration and diameter and requires limited information about the identity of the processed (and measured) MNM.

Information regarding the mean size and surface-area of the nano-aerosols are reported in table 2. The morphology of TiO_2 nanoparticle was qualitatively characterized through a XL30 environmental scanning electron microscopy (ESEM-FEG Philips) under low pressure condition at 20 keV (figure 1).

Since measurements indicate that the 8-hr time weighted average (TWA) concentration of nano-TiO_2 and NP (corrected for the background concentration), is lower than the REL and NRV, respectively, some general consideration may be extrapolated: firstly, the recommendation is that reasonable measures must be taken (in order to further reduce the duration and intensity of exposure to nanoparticles as much as possible) and repeated exposure measurements are recommended whenever the process is altered. The expected concentration may be ascribed to nanoparticles generated by process-based MNM or by the processing equipment used.

In summary, some general aspects emerge from this case study. Firstly, for an accurate exposure assessment the particle size, the shape of the particles and the particle number

concentration need to be determined, since these all are well-known crucial factors for NP toxicity. Analytical concepts for the online measurement of particle identity need to be further developed and validated. Up to now, information about the chemical identity can only be obtained by offline analysis (e.g. electron microscopy and ICP-MS). Furthermore, for nanoparticle analysis, a combination of several techniques is needed to obtain information on particle size, shape and identity.

7.5. CONCLUSIONS

Although the number of measurements taken for this study is too small to yield statistically significant evidences, some general conclusions can be drawn. This study provides useful and valuable information on the average exposure to NP in a specific occupational setting. The research outcomes appear to be of particular interest, since the exposure of workers in real working conditions was assessed with a multi-metric approach.

The following results were obtained, since from these preliminary phases of the study:

- Characterization of mass, size distribution, morphology, chemical properties of NP.
- TWA samples of PM were collected by active sampling in the fixed-site position in which the highest occupational exposures to NP were expected. These samples were subjected to gravimetric determinations and ESEM-FEG analysis for off-line NP characterization.
- Assessment of occupational exposure to NP, based on the study of concentrations (in mass and number): exposure levels expressed were obtained with the combination of high time resolution at personal level and fixed-site monitoring. Concurrent information of ultrafine particle levels in offices and outdoors were also obtained, to a better interpretation of exposure results.
- The lung-deposited surface area concentrations and the mean nanoparticle diameter were simultaneously measured by personal sampling for the subjects for which the highest occupational exposures to NMP were expected.

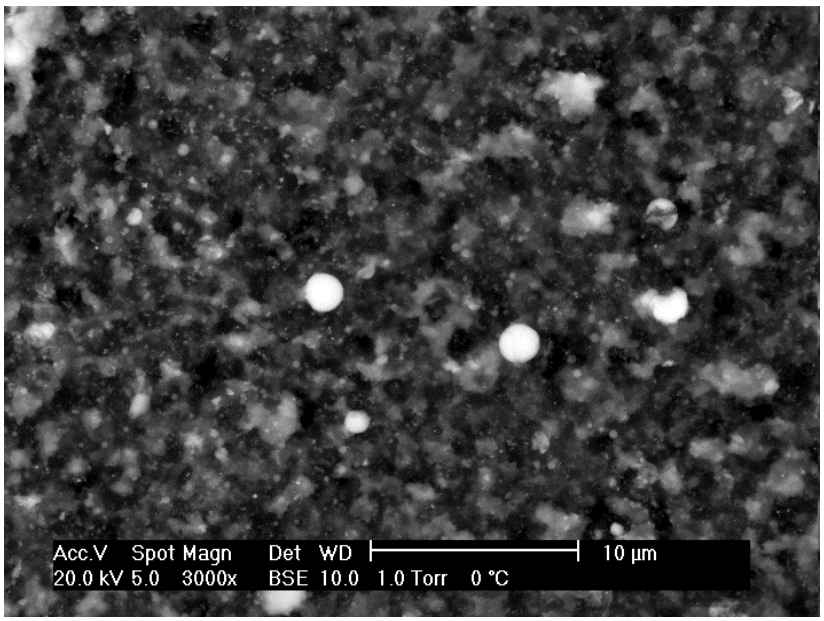
In summary, the study design allowed to obtain information on the levels of exposure and the sources of contamination at workplace, as well as on the risk management measures currently adopted in the study company to control exposures to NP.

7.6. ACKNOWLEDGEMENTS

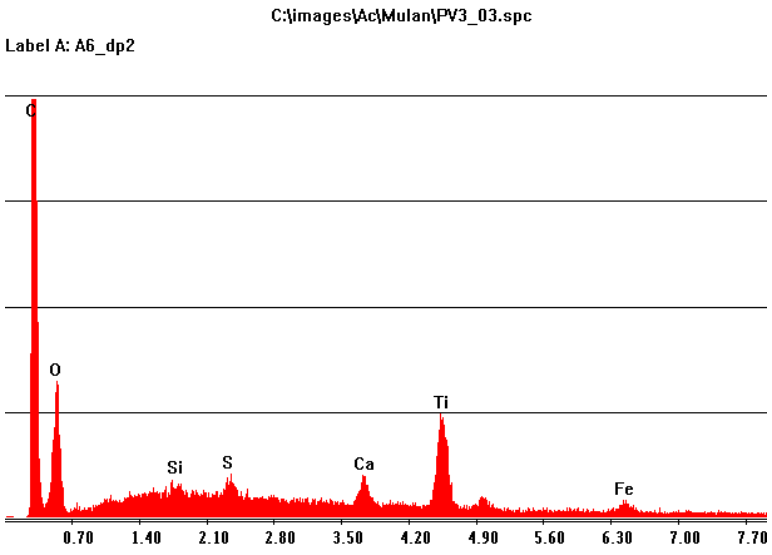
A special acknowledgment to dr. Marina Limonta for her contribution in environmental sampling campaigns and to Gabriele Carugati, PhD for his contribution in electron microscope analysis.

7.7. FIGURES

Figure 1. (a) Electron Microscopy (ESEM-FEG Philip; low pressure, 20 keV) images of TiO_2 particles collected by filter-based active sampling and (b) EDS spectrum of a TiO_2 NP.



(a)



(b)

7.8. TABLES

Table 1. Multi-metric characterization of UFP in different workplace location (*Office*: control area, *Work Area*: Production Facility, *Source-specific*: application of nano-TiO_2; *Personal*: personal sampling performed on worker involved in nano-TiO_2 application) and for different working (*None*, *Production*: application of nano-TiO_2; *Other*: other activities).

Sampling type	***Production***			***Other***		
Measured parameter	***Mean***	***Median***	***SD***	***Mean***	***Median***	***SD***
Fixed-site						
Office Conc. [pt/cm^3]	4613	4570	684	4431	4430	70
Work Area Conc. [pt/cm^3]	8817	8010	3171	7812	7860	468
Source- specific Conc. [pt/cm^3]	9656	8800	2653	8388	8265	659
Personal						
Concentration [pt/cm^3]	12247	10798	7456	12741	11562	4244
Mean diameter [nm]	50.17	49.5	5.93	44.42	43.9	5.59
LDSA [µm^2/cm^3]	33.23	30.01	16.39	30.99	27.98	10.81

Table 2. Size-fractionated PM concentration [gravimetric analysis; µg/m^3] and nano-TiO_2 concentrations [estimated; µg/m^3].

Sampling type	***PM [ug/m^3]***	***Nano-TiO_2 concentration [µg/m^3]***					
		Work task: Production			***Work Task: Other***		
Size- Fract. [µm]		***Mean***	***Median***	***SD***	***Mean***	***Median***	***SD***
Personal							
NP (< 0.1)	-	0.0005	0.0003	0.0001	0.0007	0.0004	2.4E-05
Fixed site (source-specific)							
NP (< 0.1)	4.77	0.0006	0.0005	5.7E-07	0.0003	0.0002	1.7E-07
0.3 -0.5	4.59	-	-	-	-	-	-
0.5 – 1.0	44.80	0.016	0.014	0.007	0.012	0.008	0.007
1.0 – 2.5	190.42	0.036	0.037	0.024	0.016	-	0.039
2.5 – 5.0	130.38	1.571	1.472	1.273	1.080	0.157	1.663
5.0 – 10.0	672.79	1.303	1.161	1.042	0.866	0.033	1.436
> 10.0	374.96	8.045	6.834	7.553	5.643	0.688	9.229

Table 3: Time weighted average exposure (TWA 8-hr) to nano-TiO_2 [ug/m^3] and NP [pt/cm^3], for typical workers profile (Worker 1: involved in the nano-TiO_2 application; worker 2: involved in multiple tasks).

Profile: Worker (1)	**Nano-TiO_2 application**		**Other (work area)**		**Other (Background)**		**Total**	
	Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*
Time [min]	350	39	110	31	20	5		
Concentration NP [pt/cm^3]	9656	2653	8817	3171	4613	4570		
Concentration TiO_2 [µg/m^3]	10.97	9.52	7.62	0.89	-	-		
8-hr TWA Exp. NP [pt/cm^3]	7041	216	2021	205	192	48	**9253**	**468**
8-hr TWA Exp. TiO_2 [µg/m^3]	8.00	0.77	1.75	0.06	-	-	**9.75**	**0.83**
Profile: Worker (2)	***Nano-TiO_2 application***		***Other (work area)***		***Other (Background)***		***Total***	
	Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*
Time [min]	90	5	370	25	20	5		
Concentration NP [pt/cm^3]	9656	2653	8817	3171	4613	4570		
Concentration TiO_2 [µg/m^3]	10.97	9.52	7.62	0.89	-	-		
8-hr TWA Exp. NP [pt/cm^3]	1811	28	6796	165	192	48	**8799**	**240**
8-hr TWA Exp. TiO_2 [µg/m^3]	2.06	0.10	5.87	0.05	-	-	**7.93**	**0.15**

7.9. REFERENCES

1. Hashimoto, K.; Irie, I.; Fujishima; A. TiO_2 Photocatalysis: A Historical Overview and Future Prospects. Jpn. J. Appl.Phys 2005, 44: 8269-8285.

2. Fujishima, A.; Honda, K.; Electrochemical Photolysis of water at a semiconductor electrode, Nature 1972, 238: 37-38.

3. Beeldens, A. An environmental friendly solution for air purification and self-cleaning effect: the application of TIO_2 as photocatalyst in concrete. Proceedings of Transport Research Arena Europe–TRA, 2006 Göteborg, Sweden.

4. Ancora, R.; Borsa, M.; Cassar, L. Titanium Dioxide Based Photocatalytic Composites and Derived Products on a Metakaolin Support. US Patent 8,092,586. January 10, 2012.

5. Cassar, L.; Cucitore, R.; Pepe, C. Cement-Based Paving Blocks for Photocatalytic Paving for the Abatement of Urban Pollutants. US Patent 7,960,042. June 14, 2011.

6. Cucitore, R.; Cangiano, S.; Cessar, L. High Durability Photocatalytic Paving for Reducing Urban Polluting Agents. US Patent 8,039,100. October 18, 2011.

7. Shi, H.; Magaye, R.; Castranova, V.; Zhao, J. Titanium dioxide nanoparticles: a review of current toxicological data. Part. Fibre. Toxicol. 2013, 10(1): 15.

8. Sung, J. H.; Ji, J. H.; Yoon, J. U.; Kim, D. S.; Song, M. Y.; Jeong, J.; Han, B. S.; Han, J. H.; Chung, Y. H.; Kim, J.; Kim, T. S.; Chang, H. K.; Lee, E. J.; Lee, J. H.; Yu, I. J. Lung function changes in Sprague- Dawley rats after prolonged inhalation exposure to silver nanoparticles. Inhalation Toxicol. 2008, 20 (6), 567−574.

9. Ganguly, K.; Upadhyay, S.; Irmler, M.; Takenaka, S.; Pukelsheim, K.; Beckers, J.; De Angelis, M. H.; Hamelmann, E.; Stoeger, T.; Schulz, H. Impaired resolution of inflammatory response in the lungs of JF1/Msf mice following carbon nanoparticle instillation. Respir. Res. 2011, 12.

10. Semmler-Behnke, M.; Kreyling, W. G.; Schulz, H.; Takenaka, S.; Butler, J. P.; Henry, F. S.; Tsuda, A. Nanoparticle delivery in infant lungs. Proc. Natl. Acad. Sci. U.S.A. 2012, 109 (13), 5092−5097.

11. Nørgaard, A. W.; Larsen, S. T.; Hammer, M.; Poulsen, S. S.; Jensen, K. A.; Nielsen, G. D.; Wolkoff, P. Lung damage in mice after inhalation of nanofilm spray products: The role of perfluorination and free hydroxyl groups. Toxicol. Sci. 2010, 116 (1), 216−224.

12. Hagendorfer, H.; Lorenz, C.; Kaegi, R.; Sinnet, B.; Gehrig, R.; von Goetz, N.; Scheringer, M.; Ludwig, C.; Ulrich, A. Size-fractionated characterization and quantification of nanoparticle release rates from a consumer spray product containing engineered nanoparticles. J.Nanopart. Res. 2010, 12 (7), 2481−2494.

13. Quadros, M. E.; Marr, L. C. Silver nanoparticles and total aerosols emitted by nanotechnology-related consumer spray products. Environ. Sci. Technol. 2011, 45 (24), 10713−10719.

14. Nazarenko, Y.; Han, T. W.; Lioy, P. J.; Mainelis, G. Potential for exposure to engineered nanoparticles from nanotechnology-based consumer spray products. J. Exposure Sci. Environ. Epidemiol. 2011, 21 (5), 515−528.

15. Bekker, C.; Brouwer, D. H.; van Duuren-Stuurman, B.; Tuinman, I. L.; Tromp, P.; Fransman, W. Airborne manufactured nano-objects released from commercially available spray products: Temporal and spatial influences. J. Exposure Sci. Environ. Epidemiol. 2014, 24, 74−81.

16. Nørgaard, A. W.; Jensen, K. A.; Janfelt, C.; Lauritsen, F. R.; Clausen, P. A.; Wolkoff, P. Release of VOCs and particles during use of nanofilm spray products. Environ. Sci. Technol. 2009, 43 (20), 7824−7830.

17. Chen, B. T.; Afshari, A.; Stone, S.; Jackson, M.; Schwegler-Berry, D.; Frazer, D. G.; Castranova, V.; Thomas, T. A. Nanoparticlescontaining spray can aerosol: Characterization, exposure assessment,and generator design. Inhalation Toxicol. 2010, 22 (13), 1072−1082.

18. Lorenz, C.; Hagendorfer, H.; von Goetz, N.; Kaegi, R.; Gehrig, R.; Ulrich, A.; Scheringer, M.; Hungerbuhler, K. Nanosized aerosols from consumer sprays: experimental analysis and exposure modeling for four commercial products. J. Nanopart. Res. 2011, 13 (8), 3377−3391.

19. NIOSH: Occupational Exposure to Titanium Dioxide. In Current Intelligence Bulletin 63. Cincinnati: National Institute for Occupational Safety and Health; 2011.

20. Cornelissen R, Jongeneelen F, van Broekhuizen P et al. (2012) Guidance working safely with nanomaterials and –products, the guide for employers and employees. FNV, VNO/NCW, CNV.

8. DISCUSSION AND FURTHER RESEARCH

8.1. DISCUSSION

The goals of this PhD project was firstly to perform an exposure assessment of UFP using innovative techniques and strategies to obtain a state-of-the-art and comprehensive picture of exposures to UFP in urban environments and ENP in occupational settings. The techniques discussed in this research work aimed to evaluate nanoaerosol exposure with a multi-metric approach, in order to characterize the exposure of general population and workers in terms of number and mass concentration, surface-area and mean size. These methods were developed and used for exposure characterization both in environmental and occupational settings.

8.1.1. Measuring Exposure to Ultrafine Particles in Urban Environments

Personal monitoring campaign

In a first part of the study (*chapter 3*) concentrations of UFP (along with size-fractionated PM and CO) were measured in the central area of a major Italian urban area (Milan) over three-week-long periods (one each during summer, autumn, and winter), with three monitoring sessions per day (63 runs; > 120 hours). Experimental data were collected continuously during each monitoring period along an established urban pathway. To assess the relevance of time and spatial factors affecting atmospheric concentrations data were collected while walking or moving by different private and public means of transport. Measurements were divided on the basis of different microenvironments (ME), seasons, days of the week, and periods of the day. Data analysis shows statistically significant differences across MEs and monitoring periods. The highest measured median UFP concentrations and data variability were observed for busy streets, walking or moving by motorized vehicles; the lowest concentrations were observed in park areas and in indoor environments. The highest concentrations were measured during working day morning monitoring sessions. Regarding seasonal variation, the highest median UFP concentrations were observed in winter. Appreciable differences among all MEs and monitoring periods were observed: concentration patterns and variations appear related to typical sources of urban pollutants (traffic), proximity to sources, and time of day. Continuous real-time monitoring provided information necessary to define the influence of a local source or changes in local circumstances on UFP concentrations. In addition, continuous monitoring allows for the evaluation of short-term particle concentrations, as well as temporal and spatial variability for the studied microenvironments.

Later (*chapter 5*), UFP concentrations in different urban microenvironments (ME) were measured by personal monitoring in repeated sampling campaigns, along a fixed route in a medium-sized Italian city (Como). The measurement runs were carried out on one-week periods and at different times of the day and repeated in different periods of the year (winter, spring, summer, autumn) for a total of 56 runs (more than 110 hours). As a new exposure assessment approach with respect to chapter 3, measurements included not only on-line monitoring of UFP Particle Number Concentration (PNC), but also mean diameter (mean-d)

and lung-deposited surface area (LDSA). In addition, particle mass concentration (PMC) profiles for quasi-ultrafine particles (QUFP; $PM_{0.25}$) were estimated. A significant seasonal difference in PNC and PMC, mean diameter and surface area was observed, as well as between different times of the day and days of the week. Moreover, differences in UFP concentrations were also found in each ME, for each of which specific mean diameter and surface area concentrations were found. In general, mean particle diameters showed an inverse relationship with PNC, while LDSA showed an opposite behavior. Appreciable differences among all microenvironments and monitoring periods were observed: concentration patterns and variations appear related to typical sources of urban pollutants (traffic), proximity to sources and time of day. The highest exposures were experienced while walking or biking along high-trafficked routes, and while using public buses. UFP exposures in modern cars, equipped with high-efficiency filters were significantly lower, also than those previously measured in Milan and reported in chapter 3.

Discussion

Personal measurements with portable fast response instrumentation are essential to accurately assess the exposure of the general population to air pollution, since UFP concentrations are characterized by high spatial and temporal variability especially in some specific urban microenvironments [1, 2]. Recent studies, have documented the dependence of UFP levels on several urban factors (e.g. traffic volume, the built environment and meteorological characteristics) [3 - 7]. Because of these important differences in space and time of UFP concentrations, fixed monitoring stations are not capable to depict the full spatial distribution of air pollution over the extent of an urban area [8 - 10]. For these reasons, the mobile measurements performed in this PhD thesis (*chapter 3 and chapter 5*) represent an efficient tool, capable to represent human exposures with a high spatial and temporal resolution, even in complex urban environments [11 - 16], for which measurements should be performed with high sampling frequencies, in order to track the steep changes that nanoaerosols undergo [17]. Furthermore, it must be considered that it is expected that particles within the nanometer size range demonstrate a biological behavior more closely associated with PNC, particle size and surface area [18 - 23], but that there is still insufficient information to determine which exposure metrics are most relevant to human health outcomes. In this regard, the originality of the present work lies in the continuous, time-resolved and multi-metric monitoring of UFP in urban environments (*chapter 5*). Thus, once validated, findings derived from this study provide complete and accurate exposure assessment data for risk assessors, including exposure metrics mostly relevant as health effects indicators.

8.1.2. Modeling Exposure to Ultrafine Particles for Population

Microenvironmental probabilistic exposure model

In *chapter 4* average daily ultrafine particles (UFP) exposure of adult Milan subpopulations, in different exposure scenarios (typical working day in summer and winter) were simulated using a microenvironmental stochastic simulation model. The basic concept of this kind of

model is that time-weighted average exposure is defined as the sum of partial microenvironmental exposures, which are determined by the product of UFP concentration and time spent in each microenvironment. Environmental concentrations were derived from previous experimental studies that were based on microenvironmental measurements in the city of Milan by means of personal or individual monitoring, while time-activity patterns were derived from literature (the EXPOLIS study). A total of 26 subpopulations were defined (on the basis of gender, and then for age, employment or educational status), and for each of them an exposure simulation was performed, both for summer and winter, thus generating 52 exposure profiles. On average, statistically significant differences ($p < 0.05$) were found in total UFP exposure as a function of season and subgroup characteristics (age, employment, education) but not as a function of gender ($p = 0.067$). Differences were also found among exposures simulated in the studied MEs. Further, on average, the total daily exposure indoors was characterized by the same statistically significant differences in relation to the same variables, but with a better statistical significance between genders ($p = 0.066$). In contrast, there were no statistically significant differences in outdoor total exposures as a function of season ($p = 0.088$) and of population characteristics such as age, employment or education ($p = 0.905$). In summary, differences were found as a function of (I) age, (II) employment status and (III) educational level; accordingly, the highest total exposures resulted for (I) 55–59 years old people, (II) housewives and students and (III) people with higher educational level (more than 10 years of scholarity). Thus, demographic and socio-demographic factors, as well as environmental patterns, have to be considered as major determinants of pollutant exposure in urban environments.

Discussion

Exposure modeling is recognized as a valuable and cost-effective tool for assessing potential population exposures to air pollution and represent an element of exposure assessment, which evaluates, qualitatively and quantitatively, the degree of intake or uptake that is likely to occur. Exposure models allow estimation of pollutant exposure for groups of people and time periods for which personal monitoring has not been conducted; models can be also used to combine information from different sources to produce estimates for population exposures that would be very expensive or impossible to perform [24, 25]. More generally, stochastic models describe parameters with frequency or probability distributions instead of single values.

The simulation model used in this thesis (*chapter 4*), following a “micro-environmental approach” [26 - 33], can be used to simulate population exposures for existing, past or scenario situations and (sub-)populations, by simulations based on the distributions of input parameters (measured concentrations and time-activity pattern). This methods appear well-suited to overcome the issue of achieving representative population samples, while understanding the role of exposure variation at the individual level.

8.1.3. Occupational Exposure to Engineered Nanoparticles and Nanomaterials

Traditional techniques and strategies for measuring exposure cannot directly be used for occupational settings involved in NP production or utilization, as the methodologies and the analytical instrumentations requires adjustment. For instance, for "traditional" pollutants, the mass is the only analytical focus. For MNM and NP the mass is somehow of less interest, because nanoparticles show high PNC even if their contribution to mass is very small. Therefore, the assessment of exposure via mass concentrations only is not suitable for MNM and NP. Accordingly, other metrics (such as the particle size, surface area and number concentrations) must be considered.

In *chapter 6* a multi-metric protocol to the study of NP in occupational settings was presented. This protocol was applied in a case study (*chapter 7*). Although the number of measurements taken for this thesis is too small to yield significant evidences, some general conclusions can be drawn: this study provides useful and valuable information on the average exposure to NP in a specific occupational setting. Firstly, for an accurate exposure assessment the particle size, the shape of the particles and the particle number concentration need to be determined, since these all are known factors for NP toxicity. Analytical concepts for the online measurement of particle identity need to be further developed and validated. Up to now, the identification of NP can only be achieved by offline analysis (e.g. electron microscopy and ICP-MS). Furthermore, a combination of several techniques is needed to obtain information on NP particle size, shape and identity, resulting in a comprehensive characterization of NP exposure. In summary, a novel study design and exposure assessment approach was developed and field-tested to obtain comprehensive information on the levels of exposure and the sources of NP contamination at workplace, that can be also used for risk management and exposure control.

8.1.4. Weakness and strengths

Weaknesses of this thesis were already presented for each phase of the study. Some assumptions and limits must be considered in the interpretation of results, since the study design implies some limitations in the generalizability of findings. Major limitations of the present study are summarized hereafter:

- *UFP measurement in urban ME*:
  Although the categories of microenvironments were chosen to reflect common urban activities and general trends, the specific locations were selected according to a systematic and technical protocol that may not be representative of the average concentrations across all the city or in other cities. Thus, measurements of exposure in urban environments may not correspond directly to similar microenvironments elsewhere.
- *Modeling UFP exposure:*
  The model used in this thesis should provide a sufficient understanding of exposures in urban areas. Still, it is clearly evident that exposure research in the urban microenvironment has numerous inferences and there are various factors that can

potentially affect personal exposure concentrations, especially when considering particular subpopulations. In the absence of validation, however, it is questionable what solution would approach the real exposure situation most accurately. The simplifications used in the selection of microenvironments and the selection of parametric distributions inevitably introduce uncertainties to the model structure. Model uncertainty is composed by the selection of the distributions, definition of the MEs and modeled activities, selection of averaging times and number of iterations, and generation of the random numbers, and so forth.

- *Occupational exposure assessment*
  The study design implies some limitations in the generalizability of findings: measurements were taken within a specific occupational setting, according to a systematic and technical protocol which comprises some intrinsic limitations in accuracy. Further, characterization exposure to NP in the workplace is still limited (especially for routinary applications) by the need of the combined use of multiple devices for in-situ assessments and offline sampling analysis. To date however, this solution represents the best strategy for a comprehensive exposure assessment.

Despite these limitations, this thesis have several strengths, since it provides important insights into UFP exposure in urban and occupational environments, that should be considered in developing additional and larger studies on population exposure:

- Continuous real-time monitoring provided the information necessary to define the influence of local sources or changes in local circumstances on UFP concentrations. In addition, continuous monitoring allowed for the evaluation of short-term particle concentrations and demonstrated temporal and spatial trends in the studied urban microenvironments.

- The simulation model, used to estimate the average daily UFP exposure of adult subpopulations in a major Italian urban area and in different exposure scenarios, has defined that demographic and socio-demographic factors (gender, age, profession, instruction level), as well as environmental patterns, have to be considered among major determinants of population exposure in urban environments.

- This research detected UFP levels, average particle sizes and their seasonal variability, as well as comprehensive information on average particle number and mass concentrations, sizes and surface area in various microenvironments within urban areas, which is fundamental to evaluate the variability of human exposure in urban environments and to support the relevance of traffic-related exposure for health.

- Regarding occupational exposure assessment, this study defined an experimental protocol, which was intended to be useful in determining potential exposure to engineered nanomaterials and nanoparticles in the workplace with complementary approaches. These information may also be used to determine whether engineering controls are effective in preventing release of the engineered nanomaterials in the workplace atmosphere.

Thus, findings derived from this study may represent an important tool in the definition of health and social implication of UFP exposure for general populations and to provide complete and accurate exposure assessment data for risk assessors, including exposure metrics mostly relevant as health effects indicators.

8.2. FURTHER RESEARCH

Results from mobile measurements (*chapter 3* and *chapter 5*) and model exposure simulation (*chapter 4*) are expected to be further developed, such as the mapping of the spatial distribution of air pollutants [e.g., 34], the characterization of the local source contributions to ambient air pollution and the development of novel models [e.g., 35, 36]. Furthermore, these kind of information can be used to predict exposure of a large group of individuals or for a subset of the population using only very limited input-data. A range of up-to-date exposures would be predictable based on the changing time-activity pattern of an individual. The growing field of spatial epidemiology can benefit also from GIS methods: for example, personal measurements using GPS and microenvironmental stochastic models are concepts that can be incorporated in epidemiological studies. As described earlier, the advantage of using personal exposure measurements to estimate (sub-)population exposure, will introduce advantages (and also errors) not present when using raw measurement data on fixed monitors. In fact, exposure assessment to pollutants like UFP (that are mainly traffic-related), will be improved by the use of detailed exposure assessment (using personal monitoring) or exposure models. In that way, this study could contribute to the design of future health studies aimed at investigating the relationship between observed health effects and traffic. In fact, exposure is still estimated using simple methodologies not completely or not accurately reflecting real exposure scenarios. As a result, exposure misclassification can arise and health effects may not be identified, the effect will be smaller than in reality or an erroneous effect will be detected [37 - 39]. Improving exposure estimates, e.g. by taking into account direct personal measurements, is identified as the way forward in large epidemiological studies [40], although it must considered that personal measurements are realistic for short term exposures, but not necessarily for longer term exposures or for historic exposures.

Further, some of the most important opportunities are expected to come from the pursuance of occupational exposure assessment (mentioned as case-study in *chapter 7*). In fact, the research outcomes might be particularly rewarding since the exposure of workers to NP in real working conditions will be assessed with a complete, multi-metric approach. The following outcomes are expected:

- NP samples will be subjected to morphological (SEM-FEG) and chemical (SEM-EDAX and ICP-MS) characterization for NP identification.

- An assessment of occupational exposure to NP, based on the study of concentrations (in mass and number) and other NP characteristics (surface-area, mean diameter) for two occupationally exposed populations (more than 10 exposed subjects and more than 5 controls for each company) will be carried out in the next future.

- The dermal absorption pathway will be also considered and, if relevant, dermal exposure will be estimated by direct techniques (dermal pads).

- Another expected outcome is the evaluation of the effectiveness of the risk mitigation options and strategies in use to suggest new risk management strategies in the cases in which risk control can be improved.

- Blood samples will be collected from voluntary workers, to consider the possibility of a future study aimed at assessing the health impact of ENP exposures in well-characterized occupational populations. The effects of ENP in human body that cannot be extrapolated from conventional toxicology should be considered in this perspective (e.g., epigenetic markers relative to DNA methylation, histone modifications, microRNAs expression).

In this regard, the author would like to report that the occupational exposure assessment to NP are now in progress in two selected companies in northern Italy, whose selection will permit to analyze two industrial processes involved in the production or utilization of NP and thus potentially implying NP occupational exposures during: (I) the application of nano-sized anatase (nano-TiO_2) on concrete building materials (e.g. pavement blocks) and (II) the production of graphene-based nanomaterials. The former was mentioned in this thesis as case-study (*chapter 7*), while the second refers to a technology company pursuing the development and marketing of innovative manufacturing processes for the production of a new generation of nanomaterials, and in particular graphene nanoplatelets (GNP).

As outlined before, workers' exposure to airborne NP will be investigated by an environmental multi-metric monitoring in order to characterize the exposure concentrations associated with each working task and working place, as well as the general workplace concentrations. In particular, it must be considered that graphene family materials, with a particular attention to graphene nanomaterials (GFNs), have been recently introduced among many fields of science and technology. The characteristic of these materials makes them very promising in many areas such as electrochemical devices, energy storage, catalysis, cell imaging, photochemotherapy, drug delivery, biosensors, contamination purification, extraction devices for chemical, biological, and environmental samples [41 - 44]. Given the potential occupational and public exposure to graphene due to its versatile applications, scientists are directing more attention toward investigating the biosafety aspect of these nanomaterials [45]. What is emerging from the available results is a variety of effects which

are strictly related to the nature of the graphene used. The size, layer number, chemical groups, and surface of graphene may have a strong impact on biological and toxicological responses [46]. Under conditions of occupational exposure, to date, no occupational or environmental exposure limits for GNP and GFNs have been set by any regulatory agency. To summarize, even though a number of studies have been conducted for the assessment of graphene toxicity, further research is still needed in this emerging field. The sources are still not sufficient to reach conclusions about the potential hazards connected with risk assessment and regulation. Undoubtedly, the peculiar physicochemical properties of GFNs, such as chemical structure, thickness, lateral size, surface charge, surface area, may have significant influence on biological/toxicological activity toward investigated cells and animals. Moreover, some of these parameters may also be measured by different techniques, which makes the results of studies almost impossible to compare. Consequently, further systematic studies which would address the role of GFNs parameters in determining adverse environmental and health impacts, as well as appropriate methods to characterize real occupational and environmental exposure values are urgently needed.

8.4. REFERENCES

1. Moore, K.; Krudysz, M.; Pakbin, P.; Hudda, N.; Sioutas, C. Intra-community Variability in Total Particle Number Concentrations in the San Pedro Harbor Area (Los Angeles, California). Aerosol Sci Technol 2009, 43: 587–603.

2. Hudda, N.; Cheung, K.; Moore, K.F.; Sioutas, C. Inter-community Variability in Total Particle Number Concentrations in the Eastern Los Angeles air Basin. Atmos Chem Phys 2010, 10: 11385–11399.

3. Boogaard, H.; Montagne, D.R.; Brandenburg, A.P.; Meliefste, K.; Hoek, G.; Comparison of short-term exposure to particle number, PM_{10} and soot concentrations on three (sub) urban locations. Sci Total Environ 2010, 408: 4403-4411.

4. Hoek, G.; Beelen, R.; Kos, G.; Dijkema, M.; van der Zee, S.C.; Fischer, P.H.; Brunekreef, B. Land use regression model for ultrafine particles in Amsterdam. Environ Sci Technol 2011, 45: 622-628.

5. Morawska, L.; Ristovski, Z.; Jayaratne, E.R.; Keogh, D.U.; Ling, X.; Ambient nano and ultrafine particles from motor vehicle emissions: characteristics, ambient processing and implications on human exposure. Atmos Environ 2008, 42: 8113 - 8138.

6. Ragettli, M.S.; Corradi, E.; Braun-Fahrl€ander, C.; Schindler, C.; de Nazelle, A.; Jerrett, M.; Ducret-Stich, R.E.; Künzli, N.; Phuleria, H.C.; Commuter exposure to ultrafine particles in different urban locations, transportation modes and routes. Atmos Environ 2013, 77: 376-384.

7. Rivera, M.; Basagana, X.; Aguilera, I.; Agis, D.; Bouso, L.; Foraster, M.; Medina-Ramon, M.; Pey, J.; Kunzli, N.; Hoek, G.; Spatial distribution of ultrafineparticles in urban settings: a land use regression model. Atmos Environ 2012, 54: 657-666.

8. Kaur, S.; Nieuwenhuijsen, M.J.; Colvile, R.N. Fine particulate matter and carbon monoxide exposure concentrations in urban street transport microenvironments (Review). Atmos Environ 2007; 41(23): 4781–810.

9. Adams, H.S.; Nieuwenhuijsen, M.J.; Colvile, R.N.; McMullen, M.A.S.; Khandelwal, P. Fine particle ($PM_{2.5}$) personal exposure levels in transport microenvironments, London, UK. Sci Total Environ 2001, 279: 29–44.

10. Gulliver, J.; Briggs, D.J. Personal exposure to particulate air pollution in transport microenvironments. Atmos Environ 2004, 38:1–8.

11. Zhu, Y.; Fung, D.C.; Kennedy, N.; Hinds, W.C.; Eiguren-Fernandez A. Measurements of Ultrafine Particles and Other Vehicular Pollutants inside a Mobile Exposure System on Los Angeles freeways. J Air Waste Manage Assoc 2008, 58: 424–434

12. Berghmans, P.; Bleux, N.; Int Panis, L.; Mishra, V.K.; Torfs, R.; Van Poppel, M. Exposure Assessment of a Cyclist to PM_{10} and Ultrafine Particles. Sci. Total Environ. 2009, 407: 1286–1298

13. Westerdahl, D.; Fruin, S.; Sax, T.; Fine, P.M.; Sioutas, C. Mobile Platform Measurements of Ultrafine Particles and Associated Pollutant Concentrations on Freeways and Residential Streets in Los Angeles. Atmos Environ 2005, 39: 3597–3610.

14. Kaur, S.; Nieuwenhuijsen, M.; Colvile, R. Personal Exposure of Street Canyon Intersection Users to $PM_{2.5}$, Ultrafine Particle Counts and Carbon Monoxide in Central London, UK. Atmos. Environ 2005, 39: 3629–3641.

15. Cattaneo A.; Garramone, G.; Taronna, M.; Peruzzo, C.; Cavallo, D.M. Personal exposure to airborne ultrafine particles in the urban area of Milan. J Phys Conf Ser 2009; IOP Publishing, p. 012039.

16. Peters, J.; Theunis, J.; Van Poppel, M.; Berghmans, P. Monitoring PM_{10} and ultrafine particles in urban environments using mobile measurements. Aerosol and Air Quality Research 2013, 13(2): 509-522.

17. Manigrasso, M.; Stabile, L.; Avino, P.; Buonanno, G. Influence of measurement frequency on the evaluation of short-term dose of sub- micrometric particles during indoor and outdoor generation events, Atmos Environ 2013, 67: 130-142.

18. Oberdörster, G. Toxicology of ultrafine particles: in vivo studies. Philosophical Transactions of the Royal Society of London. Series A: Mathematical, Physical and Engineering Sciences, 2000, 358(1775): 2719-2740.

19. Brown, D. M.; Wilson, M. R.; MacNee, W.; Stone, V.; Donaldson, K. Size-dependent proinflammatory effects of ultrafine polystyrene particles: a role for surface area and oxidative stress in the enhanced activity of ultrafines. Toxicology and applied pharmacology 2001, 175(3): 191-199.

20. Tran, C.L.; Buchanan, D.; Cullen, R.T.; Searl, A.; Jones, A.D.; Donaldson, K. Inhalation of poorly soluble particles. II. Influence of particle surface area on inflammation and clearance. Inhalation toxicology, 2000, 12(12): 1113-1126.

21. Lison, D.; Lardot, C.; Huaux, F.; Zanetti, G.; Fubini, B. Influence of particle surface area on the toxicity of insoluble manganese dioxide dusts. Archives of toxicology, 1997, 71(12): 725-729.

22. Donaldson, K.; Beswick, P.H.; Gilmour, P.S. Free radical activity associated with the surface of particles: a unifying factor in determining biological activity? Toxicology letters, 1996, 88(1): 293-298.

23. Hamoir, J.; Nemmar, A.; Halloy, D.; Wirth, D.; Vincke, G.; Vanderplasschen, A; et al. Effect of polystyrene particles on lung microvascular permeability in isolated perfused rabbit lungs: role of size and surface properties. Toxicol appl pharm 2003, 190(3): 278-285.

24. Hanninen, O.; Kruize, H.; Lebret, E.; Janutnen, M. EXPOLIS simulation model: PM2.5 application and comparison with measurements in Helsinki. J. Expos. Anal. Environ. Epidem. 2003, 13: 74–85.

25. Letz, R.; Ryan, B.P.; Spengler, J.D. Estimated distributions of personal exposure to respirable particles. Environ. Monit. Assess. 1984, 4: 351–359.

26. Duan, N. Models for human exposure to air pollution. Environ. Int. 1982, 8, 305–309.

27. Ott, W.R. Total human exposure: An emerging science focuses on humans as receptors of environmental pollution. Environ. Sci. Technol. 1985, 19: 880–886.

28. Duan, N. Stochastic microenvironmental models for air pollution exposure. J. Expos. Anal. Environ. Epidem. 1991, 1: 235–257.

29. Ryan, P.B. An overview of human exposure modeling. J. Expos. Anal. Environ. Epidem. 1991, 1: 453–474.

30. Ott, W.R. Exposure estimates based on computer generated activity patterns. J. Toxicol.-Clin. Toxicol. 1984, 21: 97–128.

31. Ott, W.; Thomas, J.; Mage, D.; Wallace, L. Validation of the simulation of human activity and pollutant exposure (SHAPE) model using paired days from the Denver, Colorado carbon monoxide field study. Atmos. Environ. 1988, 22: 2101–2113.

32. Lioy, P.J. Assessing total human exposure to contaminants—A multidisciplinary approach. Environ. Sci. Technol. 1990, 7: 938–945.

33. Duan, N.; Mage, D.T. Combination of direct and indirect approaches for exposure assessment. J. Expos. Anal. Environ. Epidem. 1997, 7: 439–470

34. Hagler, G.; Thoma, E.D.; Baldauf, R.W. High resolution Mobile Monitoring of Carbon Monoxide and Ultrafine Particle Concentrations in a Near-road Environment. J Air Waste Manage Assoc 2010, 60: 328–336.

35. Zwack, L.M.; Paciorek, C.J.; Spengler, J.D.; Levy, J.I. Characterizing Local Traffic Contributions to Particulate Air Pollution in Street Canyons Using Mobile Monitoring Techniques. Atmos. Environ 2011, 45: 2507–2514

36. Ragettli, M.S.; Ducret-Stich,R.E.; Foraster, M.; Morelli, X.; Aguilera, I.; Basagaña, X.; Corradi, E.; Ineichen, A.; Tsai, M.Y.; Probst-Hensch, N.; Rivera, M.; Slama, R.; Künzli, N.; Phuleria, H.C. Spatio-temporal variation of urban ultrafine particle number concentrations. Atmos Environ 2014, 96: 275-283

37. Özkaynak, H., Baxter, L. K., Dionisio, K. L., & Burke, J. Air pollution exposure prediction approaches used in air pollution epidemiology studies. J. Expo. Sci. Environ. Epidemiol. 2013, 23: 566-572

38. Setton, E., Marshall, J.D., Brauer, M., Lundquist, K.R., Hystad, P., Keller, P., Cloutier-Fisher, D. The impact of daily mobility on exposure to traffic-related air pollution and health effect estimates. J. Expo. Sci. Environ. Epidemiol. 2011, 21: 42-48.

39. Sheppard, L., Burnett, R.T., Szpiro, A., Kim, S.-Y., Jerrett, M., Pope, C.A., Brunekreef, B. Confounding and exposure measurement error in air pollution epidemiology. Air Qual. Atmos. Health 2012, 5: 203-216.

40. Fenske, R.A. Review: For good measure: Origins and prospects of exposure science. J. Expo. Sci. Environ. Epidemiol. 2010, 20: 493-502.

41. Bianco, A. All in the graphene family – A recommended nomenclature for two-dimensional carbon materials. Carbon. 2013, 65: 1-6.

42. De, M.; Chou, S.S.; Dravid, V.P. Graphene oxide as an enzyme inhibitor: modulation of activity of alpha-chymotrypsin. J. Am. Chem. Soc. 2011, 133(44): 17524-17527.

43. Allen, M.J., Tung, V.C.; Kaner, R.B. Honeycomb carbon: a review of graphene. Chem. Rev. 2010, 110(1): 132-145.

44. Guo, C.X., et al., Layered graphene/quantum dots for photovoltaic devices. Angew. Chem Int. Ed. Engl. 2010, 49(17): 3014-3017.

45. Hu, X.; Zhou Q. Health and ecosystem risks of graphene. Chem. Rev. 2013, 113(5): 3815-3835.

46. Bianco, A. Graphene: safe or toxic? The two faces of the medal. Angew. Chem. Int. Ed. Engl. 2013, 52(19): 4986-4997.

ACKNOWLEDGMENTS

This PhD thesis was performed in the framework of “The MULAN program” (MULtilevel Approach to the study of Nanomaterials health and safety), a project founded by Fondazione Cariplo (project 2011-2096) and coordinated by University of Milan, under the scientific supervision of Prof. Pier Alberto Bertazzi. Prof. Domenico Maria Cavallo has been charged for scientific coordination of the Environmental and Occupational Hygiene work-team at the University of Insubria, taking part in the project as Partner.

PERSONAL ACKNOWLEDGMENTS

I would like to thank Prof. Pietro Alberto Bertazzi, Prof. Domenico Maria Cavallo and Dr. Giovanni De Vito, for serving as my committee members.

I would like to express my special appreciation and thanks to my advisor Prof. Domenico M. Cavallo, which has been a mentor for me: his advice on both research as well as on my career have been priceless. Similarly, I would like to thank Dr. Andrea Cattaneo for encouraging my research and for allowing me to grow as a research scientist.

I would especially like to thank my friends and colleague Davide, Luca and Sabrina, for letting my PhD period be an enjoyable moment, and for their brilliant comments and suggestions. Similarly, I would like to thank all the friend and colleague from “Clinica del Lavoro” and University of Milan, for the valuable opportunity to work together as a team in so many different projects.

A special thanks to my family: words cannot express how grateful I am for all of the sacrifices that you’ve made on my behalf. I would like to thank my beloved Silvia who was always my support in the moments when there was no one to answer my queries.

Finally I would also like to thank all of my friends and colleague from “R3nzo Piano” study group, that actually has become my family: “Lo spirito continua!”

JOURNAL PUBLICATIONS, CONFERENCE ABSTRACTS AND AWARDS.

FULL ARTICLES

- Spinazzè, A.; Cattaneo, A; Monticelli, D.; Rovelli, S.; Recchia, S.; Fustinoni, S.; Cavallo, D.M. *Occupational exposure to arsenic and cadmium in thin-film solar cell production*. Submitted to The Annals of Occupational Hygiene (Manuscript ID: AnnHyg-14-0236; Date submitted 24/10/2014).

- Nørgaard, A. W.; Kofoed-Sørensen, V.; Mandin, C.; Ventura, G.; Mabilia, R.; Perreca, E.; Cattaneo, A.; **Spinazzè, A**.; Mihucz, V.G.; Szigeti, T.; de Kluizenaar, Y.; Cornelissen, E.; Trantallidi, M.; Carrer, P.; Sakellaris, I.; Bartzis, J.; Wolkoff, P. *Ozone-initiated Terpene Reaction Products in Five European Offices: Replacement of a Floor Cleaning Agent*. Environ. Sci. Technol. 2014. DOI: http://dx.doi.org/10.1021/es504106j.

- **Spinazzè A.**; Cattaneo A.; Peruzzo C.; Cavallo D.M. Modeling Population Exposure to Ultrafine Particles in a Major Italian Urban Area. Int. J. Environ. Res. Public Health. 2014; 11(10): 10641-10662.

- Rovelli, S.; Cattaneo, A.; Nuzzi, C.P.; **Spinazzè, A**.; Piazza, S.; Carrer, P.; Cavallo, D.M. Airborne Particulate Matter in School Classrooms of Northern Italy. Int. J. Environ. Res. Public Health. 2014; 11(2), 1398-1421.

- Cavallo D.M.; **Spinazzè, A;**. Campagnolo, D.; Cattaneo, A. Dalle emissioni fino alla stima dell'esposizione umana: rilevanza della competenza dell'igienista industriale. IJOEHY 2013 4(4):175-181.

- **Spinazzè, A**.; Cattaneo, A.; Garramone, G.; Cavallo, D.M. Temporal variation of size-fractionated particulate matter and carbon monoxide in selected microenvironments of the Milan urban area. J. Occup. Environ. Hyg. 2013; 10:11, 652-662, DOI: 10.1080/15459624.2013.831985

- Cavallo, D.M.; **Spinazzè**, A.; Cattaneo, A. "Microinquinanti negli ambienti di vita e di lavoro: definizioni, normativa di riferimento e aspetti applicativi". IJOHEY 2012; 3: 66-75

AWARDS

- "Vincenzo Cocheo" memorial - Best Poster, Young Industrial Hygienist AIDII - 30th Occupational Hygiene National Congress

- Prizewinner - project work "Studio di Esposizione Occupazionale a nanoparticelle", during the training course for PhD students "Innotal. Talenti per l'innovazione globale e la professionalizzazione" organized by IULM University, fondazione CRUI, CINECA, Assolombarda and Fondazione Cariplo.

CONFERENCE ABSTRACT

YEAR 2014

31st AIDII National Conference - Atti del 31° Congresso Nazionale AIDII" (ISBN 978-88-86293-24-2)

- **Spinazzè, A**.; Cattaneo, A.; Scocca, D.R.; Cavallo, D.M. *Particelle Ultrafini in ambiente urbano: esposizione personale e caratterizzazione secondo diversi parametri di misura.*
- **Spinazzè**, A.; Del Buono, L.; Scocca, D.R.; Cavallo, D.M. *Valutazione e gestione del rischio da esposizione a nanomateriali.*
- Del Buono, L.; **Spinazzè**, **A.**; Cattaneo, A.; Campagnolo, D.; Mandin, C.; Mabilia, R.; Bartzis, J.; Fossati, S.; Carrer, P.; Cavallo, D.M. *Progetto Officair: valutazione della qualità dell'aria in uffici moderni.*

YEAR 2013

76th SIMLII National Conference. Messina, Giardini Naxos - 9-11 ottobre 2013 - GIORNALE ITALIANO DI MEDICINA DEL LAVORO ED ERGONOMIA Volume XXXV - N. 4, Supplemento Ottobre-Dicembre 2013

- Di Mare, V.; Fossati, S.; Bartzis, J.; Bertetti, V.; Campagnolo, D.; Cattaneo, A.; Mandine, C.; **Spinazzè, A.**; Cavallo, D.M.; Carrer, P. *Esposizione a composti organici volatili e indicatori dello stato di salute della superficie oculare in lavoratori d'ufficio in edifici moderni. Risultati dal progetto OFFICAIR.*
- Dell'Ombra, N.; Fossati, S.; Bartzis, J.; Campagnolo, D.; Cattaneo, A.; Di Mare, V.; Koppen, G.; Mandine, C.; **Spinazzè**, A.; Cavallo, D.M.; Carrer, P. *Inquinanti indoor e parametri infiammatori nell'esalato condensato di lavoratori d'ufficio. Risultati dallo studio di intervento del progetto OFFICAIR.*

30th AIDII National Conference - "Atti del 30° Congresso Nazionale AIDII" (ISBN 978-88-86293-22-8)

- **Spinazzè,** A.; Cattaneo, A.; Tecce, N.; Cavallo, D.M. *Elementi di gestione del rischio da esposizione a cadmio e arsenico in un'azienda produttrice di moduli fotovoltaici.*
- **Spinazzè**, A.; Cattaneo, A.; Taronna, S.; Cavallo, D.M. *Esposizione individuale a particolato atmosferico, particelle ultrafini e monossido di carbonio in microambienti urbani.*
- Del Buono, L.; **Spinazzè**, **A.**; Cattaneo, A.; Campagnolo, D.; Principi, M.; Carrer, P.; Cavallo, D.M. *Studio dei determinanti dell'inquinamento indoor in uffici del settore bancario.*
- Campagnolo, D.; Cattaneo, A.; **Spinazzè**, A.; Del Buono, L.; Mabilia, R.; Mihucz, V.G.; Mandin, C.; Somaini, A.; Carrer, P.; Cavallo, D.M. *Studio della qualità dell'aria in uffici moderni.*
- Cavallo, D.M.; Cattaneo, A.; **Spinazzè, A.** *Dalle emissioni alle immissioni fino alla stima dell'esposizione: rilevanza della competenza dell'Igienista Occupazionale.*
- Rovelli, S.; Cattaneo, A.; Nuzzi, C.P.; **Spinazzè, A**.; Piazza, S.; Carrer, P.; Cavallo, D.M. *Valutazione delle concentrazioni di particolato atmosferico e caratterizzazione della distribuzione dimensionale delle polveri nelle scuole del Nord Italia.*
- Rovelli, S.; Cattaneo, A.; Nuzzi, C.P.; **Spinazzè**, A.; Peverelli, G.; Cavallo, D.M. *Valutazione delle concentrazioni atmosferiche di polveri aerodisperse provenienti da attività di demolizione e caratterizzazione del contenuto in silice libera cristallina.*

Conference on Environment & Health / Conference of ISEE, ISES and ISIAQ, Basel, Switzerland: 19 - 23 August 2013 (Abstracts: Environ Health Perspect; http://dx.doi.org/10.1289/ehp.ehbasel13)

- **Spinazzè, A.**; Cattaneo, A.; Cavallo, D.M. *Individual exposure to size-fractionated particulate matter and carbon monoxide in selected micro-environments of the urban area of Milan.*
- Del Buono, L.; **Spinazzè**, A.; Campagnolo, D.; Cattaneo, A.; Cavallo, D.M. *Indoor Air Quality in Bank Offices.*
- Rovelli, S.; Cattaneo, A.; Nuzzi, C.P.; **Spinazzè**, A.; Piazza, S.; Fanetti, A.C.; Carrer, P.; Cavallo, D.M. *Airborne particulate matter in school classrooms of Northern Italy*.

YEAR 2012

29th AIDII National Conference -"Atti del 29° Congresso Nazionale AIDII" (ISBN 978-88-86293-20-4)

- **Spinazzè, A.**; Cattaneo, A.; Rovelli, S.; Limonta, A.; Nuzzi, C.P.; Cavallo, D.M. *Composti organici volatili e anidride carbonica all'interno di edifici scolastici.*
- Del Buono, L.; **Spinazzè, A.**; Cattaneo, A.; Nuzzi, C.P.; Cavallo, D.M. *Qualità dell'aria in uffici del settore bancario.*
- Cattaneo, A.; **Spinazzè, A.**; Rovelli, S.; Limonta, S.; Nuzzi, C.P.; Cavallo, D.M. *Importanza della calibrazione sull'accuratezza delle misure di particolato atmosferico mediante analizzatori ottici.*
- Rovelli, S.; Cattaneo, A.; **Spinazzè, A**.; Limonta, A.; Nuzzi, C.P.; Cavallo, D.M. *Particolato atmosferico e co-inquinanti gassosi all'interno di edifici scolastici.*