

White Paper: Ambient ultrafine particles: evidence for policy makers

Flemming R. Cassee, Lidia Morawska, Annette Peters

Angaben zur Veröffentlichung / Publication details:

Cassee, Flemming R., Lidia Morawska, and Annette Peters. 2019. "White Paper: Ambient ultrafine particles: evidence for policy makers." München: Institute of Epidemiology, Helmholtz Center Munich, German Research Center for Environmental Health Munich.

<https://portal.research.lu.se/en/publications/white-paper-ambient-ultrafine-particles-evidence-for-policy-maker/>.

Nutzungsbedingungen / Terms of use:

CC BY 4.0

Dieses Dokument wird unter folgenden Bedingungen zur Verfügung gestellt: / This document is made available under these conditions:

CC-BY 4.0: Creative Commons: Namensnennung

Weitere Informationen finden Sie unter: / For more information see:

<https://creativecommons.org/licenses/by/4.0/deed.de>



WHITE PAPER

Ambient ultrafine particles: evidence for policy makers

Prepared by the

‘Thinking outside the box’ team

Coordinated by:

Flemming R. Cassee

Lidia Morawska

Annette Peters

Exposure Team (paper contributions)

Lidia Morawska^{1*}, Aneta Wierzbicka², Giorgio Buonanno³, Josef Cyrus⁴, Jüergen Schnelle-Kreis⁵, Michal Kowalski⁴, Michael Riediker⁶, Wolfram Birmili⁷, Xavier Querol⁸

Exposure Team (workshop/correspondence contributions): Nino Künzli⁹

Affiliations:

1. International Laboratory for Air Quality and Health, Queensland University of Technology, 2 George Street, Brisbane QLD 4001, Australia
2. Ergonomics and Aerosol Technology, Lund University, Lund, Sweden
3. University of Cassino / Naples "Parthenope", Italy
4. Institute of Epidemiology (EPI), Helmholtz Center Munich – German Research Center for Environmental Health, Germany
5. Comprehensive Molecular Analytics (CMA), Helmholtz Center Munich – German Research Center for Environmental Health, Germany
6. Swiss Centre for Occupational and Environmental Health (SCOEH)
7. German Federal Environment Agency (UBA)
8. Institute of Environmental Assessment and Water Research (IDÆA-CSIC), Barcelona, Spain
9. Swiss TPH



***Send correspondence to:**
Professor Lidia Morawska
International Laboratory for Air Quality
and Health, Queensland University of
Technology
2 George Street
Brisbane QLD 4001 Australia

phone
email
website

+61 7 3138 2616
l.morawska@qut.edu.au
<https://research.qut.edu.au/ilaqh/>

Toxicology Team (paper contributions)

Flemming R. Cassee^{1,2*}, Ali Önder Yildirim³, Alison Elder⁴, Il Je Yu⁵, Johan Øvrevik^{6,7}, Karin Sørig Hougaard⁸, Steffen Loft⁵, Otmar Schmid⁹, Per Schwarze^{6,7}, Tobias Stöger⁹

Affiliations:

1. National Institute for Public Health and the Environment, Bilthoven, The Netherlands
2. Institute of Risk Assessment Sciences, Utrecht University, Utrecht, The Netherlands
3. HMGU, Helmholtz Center Munich – German Research Center for Environmental Health and Member of German Center for Lung research (DZL), Germany
4. University of Rochester Medical Centre, Rochester, USA
5. HCTm, Co, LTD, Icheon, Korea
6. Department of Air Pollution and Noise, Division of Infection Control and Environmental Health, Norwegian Institute of Public Health (NIPH), Norway
7. Department of Biosciences, Faculty of Mathematics and Natural Sciences, University of Oslo, Norway
8. Danish NanoSafety Centre, National Research Centre for the Working Environment, Copenhagen, Denmark
9. HMGU, Helmholtz Center Munich – German Research Center for Environmental Health, Germany



***Send correspondence to:**
Professor Flemming R. Cassee
National Institute for Public Health and
the Environment, RIVM, Bilthoven, The
Netherlands
Institute for Risk Assessment Sciences,
Utrecht University, The Netherlands

phone
email
website

+31 30 274 2406
flemming.cassee@rivm.nl
www.rivm.nl



Epidemiology Team (paper contributions):

Annette Peters^{1,2,3*}, Alexandra Schneider¹, Enembe Okokon^{4,5}, Evangelia Samoli⁶, Massimo Stafoggia⁷, Regina Pickford¹, Siqi Zhang¹, Susanne Breitner^{1,2}, Tamara Schikowski⁸, Timo Lanki⁴, Tobias Aurelio⁹

Epidemiology Team (workshop/correspondence contributions): Barbara Hoffmann¹⁰, Hicran Altug⁸, Kathrin Wolf¹, Ron Kappeler¹¹, Sarah Lucht¹⁰

Affiliations:

1. Institute of Epidemiology (EPI), Helmholtz Center Munich – German Research Center for Environmental Health, Germany
2. Ludwig-Maximilian University, Institute of Biometry, Medical Informatics and Epidemiology, Munich, Germany
3. Harvard T.H. Chan School of Public Health, Department of Environmental Health, Boston, USA
4. Department of Health Security, National Institute for Health and Welfare, Kuopio, Finland
5. Department of Community Medicine, Faculty of Medicine, University of Calabar, Calabar, Nigeria
6. Department of Hygiene, Epidemiology and Medical Statistics, Medical School, National and Kapodistrian University of Athens, Athens, Greece
7. Department of Epidemiology, Lazio Region Health Service / ASL Roma 1, Rome, Italy
8. IUF – Leibniz Research Institute for Environmental Medicine, Düsseldorf, Germany
9. Centre for Research in Environmental Epidemiology (CREAL)
10. University Hospital Düsseldorf, Germany
11. Swiss TPH



***Send correspondence to:**
Professor Annette Peters
Institute of Epidemiology, Helmholtz
Center Munich, German Research Center
for Environmental Health
Munich, Germany

phone +49 89 3187-4566
email peters@helmholtz-muenchen.de
website <https://www.helmholtz-muenchen.de/epi>



List of Abbreviations

CPC:	Condensation particle counter
DEP:	Diesel exhaust particles
DMPS:	Differential mobility particle sizer
EC:	Elemental carbon
MAAP:	Multi-angle absorption photometer
NPF:	New particle formation
OC:	Organic carbon
PNC:	Particle number concentration
SMPS:	Scanning mobility particle sizer
UFP:	Ultrafine particles
ROS:	Reactive oxygen species

Overall co-ordination:

Lidia Morawska

Version 1

25th October 2019

Acknowledgment

This work was supported by the Helmholtz Zentrum München, German Research Center for Environmental Health (GmbH) in Munich, Germany, and the International Laboratory for Air Quality and Health (ILAQH), Queensland University of Technology, in Brisbane, Australia.

© The Author(s). 2019 Open Access. This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

Table of Contents

List of Abbreviations	iii
Executive Summary	1
<i>What are ultrafine particles?</i>	1
<i>Why are ultrafine particles important?</i>	1
<i>Why are ultrafine particles a special challenge?</i>	1
The current state of knowledge: Exposure	2
The current state of knowledge: Toxicology	3
The current state of knowledge: Epidemiology	4
The current state of knowledge: Regulations	4
1. Exposure	5
1.1. Sources of emission	5
<i>Outdoor air</i>	5
<i>Indoor air</i>	5
1.2 UFP concentrations and their spatial and temporal variation in cities	6
<i>Spatial variation in concentrations</i>	6
<i>Temporal variation</i>	6
1.3 UFP measurement methods	6
1.4 Relationship between UFP, other particle metrics and gaseous pollutants	8
1.5 Indoor versus outdoor UFP	9
1.6 Exposure assessment to UFP for epidemiological studies	9
<i>Temporal variability (short-term studies)</i>	10
<i>Spatial variability (long-term studies)</i>	10
1.7 Total number UFP concentrations or source contributions for health studies?	10
1.8 Personal exposure assessment	11
2. Toxicology	12
2.1 What reasons do we have to consider UFP separately from PM_{2.5}/PM₁₀?	12
2.2 From exposure to internal dose	12
2.3 Which metric should be used to describe UFP concentration-effect relationships?	13
2.4 Does the toxicity of UFP depends on source?	13
2.5 Short term effects on mechanistic biomarker and physiological functions in controlled human exposure studies involving UFP	14
2.6 Do acute (peak) exposures have more impact on human health than long term UFP exposures?	16
3. Epidemiology	16
3.1 Health Effects of UFP – Epidemiological evidence	16
4. Regulations	17
References	18

Executive Summary

What are ultrafine particles?

Ultrafine particle, as defined by ISO/TC 146/SC 2/WG1 N 320 is “A particle sized about 100 nm in diameter or less”. The same definition applies to a **nanoparticle** as “A particle with a nominal diameter smaller than about 100 nm”. Ultrafine particles (UFP) is a term used in atmospheric sciences, while “nanoparticles” applies to material engineering.

UFP constitutes a somewhat arbitrary classification of particles in terms of their size because there is no objective (natural) division between UFP and larger particles. As UFP have little mass, their concentrations are most commonly measured and expressed in terms of particle number concentration (PNC) per unit volume of air (number of particles per cubic cm – particles.cm⁻³). In contrast, larger particles are measured in terms of mass concentration (µg.m⁻³); PM_{2.5}, PM₁₀ – mass concentration of particles with aerodynamic diameter < 2.5 µm and 10 µm, respectively.

The term “UFP” is often used loosely, meaning various ranges of PNC in the submicrometer range, dictated by the instrument used and its settings. The terms quasi-ultrafine, therefore, refers to particles substantially smaller than 1 µm but still also larger than 100 nm. In this report, “UFP” refers to number concentration of quasi-ultrafine particles.

Why are ultrafine particles important?

Adverse health and environmental effects of the particles are strongly linked to their size, which is a determinant (in a probabilistic sense) of their fate in the air and their potential of toxicity.

UFP constitute: (i) a health risk, due to their specific properties such as high number concentration and surface area, high deposition efficiency in the pulmonary region where they can cause inflammation, and a high propensity to penetrate the epithelium and translocate to the blood system, causing a variety of diseases; and (ii) an environmental risk, due to their propensity to for example, act in the air as seeds for cloud nucleation, affecting rainfall patterns, and therefore climate. The impacts of UFP may increase in the future due to changes in the use of fuel as well as in the increase in the number of emissions sources (e.g. aircrafts) and due to the decrease of large particles (which in turn can favour UFP formation) through the control and abatement measures. Emissions of larger and smaller particles are not always correlated, thus there are studies showing that compressed natural gas (CGN) have much lower PM_{2.5} but higher UFP emissions compared with other fuels. However, concentrations of UFP may also decrease due to the trends towards cleaner emissions from ground traffic, in particular by substitution of fossil fuel driven cars and trucks with electric vehicles. Human

exposure will be affected by the change in building construction leading to tighter buildings with less penetration of outdoor particulate matter reducing indoor concentrations of outdoor generated UFP. On the other hand, reduced ventilation may increase exposure to UFP originating from indoor sources such as cooking, candle and wood burning. It is also important to highlight that under relatively high insolation climates (high solar irradiation), new particle formation (NPF) from gaseous precursors might dominate the source contribution to urban UFP. These newly formed particles might or might not be independent of road traffic emissions of UFP precursors. The size and composition of these newly formed UFP considerably differ from those arising from primary combustion sources, and accordingly their health effects might also be very different.

Why are ultrafine particles a special challenge?

The challenges constituted by UFP include:

- ▶ Scientific challenges: complex processes involved; sophisticated and costly instrumentation as well as extensive quality control and quality assurance needed to understand underlying processes; complete characterization, including all the parameters potentially of significance is hardly possible on routine basis (e.g. measurements of particle surface area or composition); detection limits of particles with low size in commercial instruments vary widely and this may markedly affect comparability of absolute concentrations; UFP have a relatively short half-life and need to be generated directly from the source for meaningful toxicological studies (this, however, does not suggest that aged aerosolized UFP are less harmful); toxicology mainly focusses on mass as the dose metric, whereas for UFP PNC and surface area may be more relevant.
- ▶ Legislative challenges: there is still little in the way of standardization of measurements (albeit there are ISO standards for measurements using some specific instruments); control is required not only of the direct emissions, but also of their gaseous precursors, however, the understanding of which precursors and from which sources is limited; contrary to PM_{2.5} and PM₁₀ the UFP spatial distribution is very inhomogeneous, with strong local variation; measures to control other pollutants, e.g. larger particles, can have an opposite effect on UFP; there is typically little quantitative understanding of local UFP concentrations; evidence from epidemiological studies suggests (albeit not consistently so) that there are independent effects of UFP, compared to regulated pollutants such as PM mass or nitrogen dioxides, indicating needs for specific regulation of UFP.

Sources of emission

In urban areas, roads and other forms of transportation, including aviation and shipping, are usually the main sources of UFP. These particles are emitted directly by the sources or formed in the air from gaseous precursors that are usually also emitted by the sources. Also emissions from industrial sources, power plants, residential heating and biomass burning are sources of UFP, contributing to various extend to the concentrations of these particles in urban air.

The theories underpinning UFP emission and formation processes are generally well developed.

However, local understanding of the origin of UFP (primary/secondary, specific sources), and their chemical composition (solid/liquid, organic carbon/elemental carbon, metals, etc.) is generally very limited, but tools are available to characterize them.

UFP and precursor emission inventories hardly exist.

Ultrafine particle concentrations and their spatial and temporal variation in cities

The mechanisms and conditions affecting particle concentrations and their trends are in general well understood.

There is a general agreement what constitutes low versus high concentrations (clean versus polluted), and the following daily (24 hours) mean concentrations can be considered as *typical*, based on the recent scientific literature:

- ▶ Clean environments (not affected by anthropogenic emissions) < 1,000 particles.cm⁻³.
- ▶ Urban background < 10,000 particles.cm⁻³.

There is large, often short term, spatial and temporal variation in UFP concentrations. It can be considered that in *typical* clean urban microenvironments hourly mean concentrations < 20,000 particles.cm⁻³.

There is typically limited local data on UFP spatial and temporal concentrations, but modelling tools have already been implemented operationally at urban scale.

UFP measurement methods

Particle number/size distribution are most commonly measured, with relatively well established methods; however, there is no standard methods selected, which makes it difficult to compare results from different

exposure/epidemiological studies or use the data for large population based epidemiological studies:

To overcome this it is proposed to use instruments, which measure at least down to 10 nm, with no restriction on the upper limit. The measured uncertainty accompanying any UFP number concentration measurements should critically reflect the experimental uncertainty associated with different lower size limits.

The uncertainty in the calibration of instruments measuring PNC is based on standardized methodology and varies between 30% for lower concentrations (less than 1,000 particles.cm⁻³) to 10% for typical urban background concentrations (about 10,000 particles.cm⁻³).

There is still an open question how to transform the inter-quantitative data, or a factor converting to, say, 10,000 particles.cm⁻³ based on the measurement device.

Due to the lack of instrumental methods we cannot recommend UFP mass or surface area, concentration measurements as routine approaches.

As strongly suggested by some experts, the common air quality monitoring strategy (at least in the EU) should be extended by set up of so called “supersites”. At such “supersites” parameters, such as size-segregated UFP concentrations, online BC and PM speciation measurements, surface area, or oxidative potential should be measured by standardized methods. Such data will allow to investigate the spatial and temporal variation of UFP at many locations and will provide a good base for future epidemiological studies on health effects of UFP.

Relationship between UFP, other particle metrics and gaseous pollutants, and sources

Generally, there is very little or no relationship between UFP number concentrations and PM_{2.5}, which is due to different sources of these two particle metrics (local combustion process generating mainly UFP and mechanical process as well as production of secondary aerosols at regional scale mainly PM_{2.5}), and their different behaviour in the ambient air. Therefore, UFP and PM_{2.5} measurements are not representative of each other.

There is often a better relationship between concentrations of UFP and traffic emitted gaseous pollutants (CO and NO_x) as well as BC; however, the existence and the degree of correlation vary, and are specific to different urban environments, from those where levels of UFP variability is directly associated with traffic emissions to those having highly effected by photochemical NPF.

There are tools that can allow obtaining source contributions to UFP concentrations and that can increase the robustness of meta-analysis of multi-city data for epidemiological studies.

Indoor versus outdoor UFP

There is a general understanding of the sources and processes leading to indoor UFP.

There is some level of understanding of typical UFP concentrations in *typical* indoor environments. The meaning of *typical* is restricted to the countries and setting where such studies were conducted.

However, there could be large differences in UFP concentrations between specific and *typical* indoor environments (e.g. between a *specific* and a *typical* school).

In general, it is more logistically complicated to investigate UFP in indoor environments, however, since in general their sources are understood, recommendations can be provided regarding source control.

Exposure assessment of UFP for epidemiological studies

The estimation of the population exposure to UFP in epidemiological short- and long-term studies is significantly more complex than the assessment to PM_{2.5} and PM₁₀.

Source contributions to UFP may differ greatly among cities, especially proportions of traffic/NPF/biomass combustion contributions. Then trying to evidence health effects with total number concentrations of UFP in the multicity analysis might be a difficult task. As stated above, there are some tools that can allow obtaining source contributions to UFP concentrations and enabling more robust meta-analysis of multi-city data for epidemiological studies.

The spatial variation of the absolute concentration level of UFP across a single city area is substantially larger compared to the spatial variation of PM_{2.5} or PM₁₀. It means that epidemiological long-term studies cannot adopt the approach of the PM_{2.5} studies that relied on single or a few central monitoring sites to characterize the city-average concentration of UFP. Future long-term studies might consider modelling or increasing the number of monitors in order to cover the spatial variability in cities.

The difficulties in obtaining spatially resolved estimates of long-term exposure (high cost of particle number monitoring equipment prohibits large-scale monitoring; almost no successful modelling approaches for UFP) hamper the conducting of long-term epidemiological studies on UFP.

For some urban areas it has been shown that although the temporal correlation among different monitoring sites was higher for PM_{2.5} than for UFP, the difference was not substantial. It means that using a central monitoring site to characterize outdoor exposure in epidemiological time series studies does not result in substantial more

measurement error for UFP than for PM_{2.5}. However, as in other areas the temporal correlations of UFP were substantially lower than for PM_{2.5}, such correlations need to be carefully evaluated when designing short-term studies (panel studies) in a specific study area.

Scientific progress on many fronts makes personal exposure assessment possible.

Considering exposure to traffic generated UFP, it should be kept in mind that other traffic related exposures (such as to gases, black carbon or noise), should be simultaneously assessed. While they are usually treated as co-variables (or co-pollutants), they are not necessarily co-variables as they have different pathways in the body and their effects are independent. It will be very important to think how to do this well, so in the end we are left without neither evidence for NO₂, nor UFP, nor BC.

There is a need to develop an optimal way of exposure assessment for epidemiological studies, utilising the emerging science and technology.

The current state of knowledge: Toxicology

The differences in size (distribution) between UFP and larger sized particles result in regional differences in the deposited dose, potentially leading to differential biological responses. Focusing only on PM_{2.5} may result in overlooking the impact of UFP.

The toxic potency of UFP when using mass as a dose descriptor differs from the PM_{2.5} potency, often (but not always) showing that UFP cause greater effect. Moreover, the lung shows a different response to UFP compared to larger particles.

For practical reasons, using particle number as a predictor may be preferred above mass and surface area, especially if the particle size distribution is known. Increased understanding of the importance of chemical composition for toxicological effects of UFP and the use of surface area rather than mass as dose metric may shed more light on the issue.

There are considerable differences in the toxic potency of UFP released from various sources when using mass as unifying metric. Soluble components, including organic chemicals, appear to contribute to the effects of UFP, but the key drivers of (differences in) toxic effects remain to be determined.

While shorter averaging times than 24 hours seem relevant to determine the health impact of UFP, there is a lack of data on long-term exposure to UFP from experimental studies. At present, it is unknown whether (repeated) peak

exposures are more relevant than continuous exposure to lower PNC though with the same mean dose. This issue may be especially relevant relative to developmental issues (pregnancy, foetal and child development), where a single high exposure during a sensitive period may have permanent effects, whereas slight, acute perturbations in adults may be without long-term consequences.

The current state of knowledge: Epidemiology

While the health effects of UFP have been substantiated based on toxicological, there is a need to systematically and quantitatively assess the existing evidence based on epidemiological research. These analyses should consider the heterogeneity of source contribution patterns for UFP and PNC in different regions with climatic and emission patterns, the differences of populations or patient groups studied, the differences in UFP measurements, the differences in exposure-response times typically operationalized by lag-periods, different years of investigation and related underlying time-trends altering the sources and composition of UFP. While these quantitative meta-analyses are challenging, they will provide novel insights, impact on regulatory evaluations and generate hypotheses to be tested in epidemiological studies, controlled human exposure studies and toxicological studies.

The current state of knowledge: Regulations

There is no evidence that mitigating only particle mass (PM_{10} , $PM_{2.5}$) as the existing air quality measures do, will ultimately lead to reduction in UFP.

There is some tentative evidence that mitigating particle mass (PM_{10} , $PM_{2.5}$) from combustion sources could lead to a reduction in UFP.

There have been suggestions for mitigation of black carbon in the future, but this would not remove all UFP, in particular, the organic ones.

This highlights the need to establish regulatory approaches and control measures to address the impacts of elevated UFP concentrations, especially in urban areas, considering their potential health risks.

Issues to resolve include: (i) whether the regulations should be set around the base line concentrations without the peak concentrations, or whether they should include the peaks in PNC due to NPF; and (ii) how to define the peaks.

In order to properly control UFP, long-term monitoring is essential. Ideally all the UFP metrics, which have been identified as of relevance based on toxicological evidence, should be monitored. This however, is not feasible with large spatial coverage, but if anything at a limited number of supersites. Therefore there is a need to find the balance between what is feasible and what scientifically essential. At the moment measurements of PNC and to some extent particle size distribution are feasible on a larger scale.

Of particular importance is source control of UFP and there is a body of literature pointing out to the existing methods and opportunities to do so.

Developing a much better picture on a local scale of particle formation dynamics in different environments, including those which are influenced by traffic, would greatly assist such regulation formulation.

It would be highly beneficial to develop and utilize standardised measurement procedures, enabling meaningful comparison between the results from different studies, which is of particular significance for human exposure and epidemiological studies. However, considering the complexity of the measurements, variety of instruments available and difference in the aims of the measurement/monitoring, it is not likely that standard methods to measure UFP will be accepted/established in a foreseeable future. But a way around it would be to provide estimates of variation between the different results, based on the differences in instruments being used, or their settings.



1. Exposure

1.1. Sources of emission

UFP are present in the air due to natural sources and processes, as well as due to anthropogenic activities. UFP are emitted directly by all combustion sources as primary particles (with elemental carbon often, but not always, being the main constituent). UFP are also formed in the air as secondary particles, through complex physio-chemical NPF processes involving inorganic as well as organic gaseous precursors. The latter originate from combustion sources and other anthropogenic processes, but also from natural processes, such as biogenic emissions.

Outdoor air

In natural outdoor environments the main source of UFP is NPF, which involves nucleation of gas-phase compounds with low volatility, such as NH_3 , H_2SO_4 and some VOC, and subsequently growing into small particles by coagulation and condensation. These processes contribute to the natural background concentration of particles in ambient air, although other sources, such as volcanoes and wild fires, might also feed into the total number concentration.

The most significant anthropogenic contributions from outdoor combustion sources, including vehicles and other forms of transportation (aviation and shipping), as well as from industrial and power plants and residential heating. These all utilise fossil and bio-fuels as well as biomass. The emissions of UFP and precursors for NPF depend significantly on the nature of the fuel, the combustion technique and after treatment of emissions (catalytic converters and particle traps). Also brake abrasion and road–tire interaction can be a source of UFP, originating from several components of the tires, and in the future may become the main source of road traffic emissions of UFP. Another significant combustion source is biomass burning, including controlled and uncontrolled forest and savannah fires and agricultural waste burning. In general, in populated urban areas vehicles and solid fuel combustion are the dominant sources of UFP. Industrial and power plant emissions have a significant impact on environment and climate, but as they often (but not always) occur outside the cities or the facilities are equipped with tall chimneys, their direct impact on human exposure is lower than the impact of vehicle emissions.

The main climatic condition favoring NPF is high solar irradiation, experienced by cities of warmer climates for extended periods during the year. In this sense it is important to highlight that: (i) NPF is favoured by insolation, but also by low levels of ambient air particle concentrations (high levels of particles favour

condensation rather than NPF); (ii) a decrease in the mass of particles resulting from the control measures may result in higher frequency of conditions to favour NPF; (iii) at spring-summer midday hours of high insolation in urban areas the NPF might prevail over the direct emissions of particles from combustion; (iv) the sizes and chemical characteristics of the particles formed in the air are very different from primary UFP; accordingly their health effects might also differ; and (v) bursts of NPF often coincide with high levels of other photochemical pollutants, such as O_3 . To quantify UFP emissions and potential for NPF, knowledge of source emission factors and rates are needed (amounts of a substance emitted per unit mass of fuel burned or per a defined task performed, or per unit time). There is general knowledge on the range of emission factors/rates from different types of sources. However, there are large uncertainties in relation to emission factors of specific (given) sources. There has been very little work done on UFP source emission inventories.

Indoor air

There are indoor sources of UFP, the most important being combustion, including stoves and heaters utilising fossil fuels or biomass, tobacco smoking, and the burning of candles or incense. Electric appliances such as stoves and toasters have also been documented to emit UFP. Cooking, regardless of the type of stove used, generates large amounts of UFP, mainly of organic content. Human activities such as vaping, cleaning with cleaning agents, printing or interior decoration (e.g. painting) lead to emissions of intermediate to semi low-volatile gas-phase compounds and in turn to indoor NPF. The outdoor infiltration of O_3 into indoor environments and its interaction with indoor VOC, especially those arising from odour and detergent usage, has been shown to increase indoor UFP concentrations by several orders of magnitude.

The current state of knowledge:

- ▶ In urban areas, roads and other forms of transportation, including aviation and shipping, are usually the main sources of UFP. These particles are emitted directly by the sources or formed in the air from gaseous precursors that are usually also emitted by the sources. Also emissions from industrial sources, power plants, residential heating and biomass burning are sources of UFP, contributing to various extend to the concentrations of these particles in urban air.
- ▶ The theories underpinning UFP emission and formation processes are generally well developed.

- ▶ However, local understanding of the origin of UFP (primary/secondary, specific sources), and their chemical composition (solid/liquid, organic carbon/elemental carbon, metals, etc.) is generally very limited, but tools are available to characterize them.
- ▶ In addition, UFP and precursor emission inventories hardly exist.

1.2 Ultrafine particle concentrations and their spatial and temporal variation in cities

In general, UFP concentrations are of the order: 10^2 , 10^3 , 10^4 and 10^5 to 10^6 particles. cm^{-3} in, respectively: *remote background*, *rural/urban background*, *on-road*, and *street canyons/tunnel* (Morawska, et al., 2008; De Jesus et al., 2019).

Spatial variation in concentrations

There is a decrease (typically exponential) in UFP concentrations with distance from busy roads of up to about 300 m, beyond which the concentrations and size distributions approach the local urban background. This is very different from particle mass, which typically decreases less than 30%, if at all, compared to urban background. Even in smaller cities, with fewer emission sources, high concentrations of UFP in the immediate proximity to the sources may compare to those in large cities, in similar types of environments. At roads, particle concentrations are dominated by the smallest particles, with the peak in the size distribution shifting towards larger sizes at greater distances. Particle size distribution is much more stable at background urban sites, than close to traffic sources, where it changes rapidly.

NPF events (rapid burst of particles) often result in increases in UFP concentration by one to two orders of magnitude, within a larger spatial scale of a region, or the city, and not in immediate proximity to the sources. NPF events are most commonly observed during: (i) morning rush hours, when increased emissions of condensable species from vehicles combined with lower temperatures result in conditions enabling particle nucleation, and (ii) around midday, when increased solar irradiation, presence of precursors in the air, and relatively low concentrations of pre-existing particles lead to NPF through photochemical reactions.

Temporal variation

In urban environments, a strong **diurnal variation** of UFP concentration typically follows closely the temporal variation in traffic density, with the highest levels observed on weekdays during rush hours. In addition, NPF events during midday periods increase UFP concentration beyond that which would be expected due

to the traffic related temporal variation. Hence, in cities highly influenced by NPF, UFP averaged daily patterns might have the highest concentration peak at midday. **Seasonal variation** in UFP concentration are: (i) increases in concentrations due to lower mixing layer height and greater atmospheric stability in winter (due to less dispersion), lower winter temperature (increased NPF of combustion exhaust emitted precursors from motor vehicles particularly during morning rush), increased photochemical particle formation during spring-summer (due to higher irradiation), and biomass combustion particularly during afternoon to night hours (due to cooking); and (ii) decreases, due to: atmospheric precipitations, increased wind speed, and lower traffic flow rate during summer holiday periods. The number of studies investigating **long term trends** in particle concentrations is limited, however there are some tentative results suggesting that measures that have led to reduction of PM_{10} and/or $\text{PM}_{2.5}$ might also have led to an analogous decrease in UFP concentrations.

The current state of knowledge:

- ▶ The mechanisms and conditions affecting particle concentrations and their trends are overall, well understood.
- ▶ There is a general agreement what constitutes low versus high concentrations (clean versus polluted), and the following daily (24 hours) mean concentrations can be considered as *typical*, based on the recent scientific literature:
 - Clean environments (not affected by anthropogenic emissions) $< 1,000$ particles. cm^{-3} .
 - Urban background $< 10,000$ particles. cm^{-3} .
- ▶ There is large, often short term, spatial and temporal variation in PNC. It can be considered that in *typical* clean urban microenvironments hourly mean concentrations $< 20,000$ particles. cm^{-3} .
- ▶ There is typically limited local data on UFP spatial and temporal concentrations, but modelling tools have already been implemented operationally at urban scale.

1.3 UFP measurement methods

There are many parameters by which UFP can be characterized. A small number of them are measured semi-routinely, others only in specialized research investigations.

Number concentration/size distribution – measured commonly. Real time total particle number concentrations (PNC) are measured by condensation particle counters (CPC) or electrometers, while size distributions are measured by differential/scanning mobility particle sizers (DMPS or SMPS, respectively), which require two instruments: a particle classifier (typically electrostatic)

operating in combination with a particle counter. These techniques are well advanced, however relatively costly. When referring to UFP, an unspoken assumption often made is, that the instrumental methods used, provide information on particles in this specific size range (< 100 nm). This is, however, not the case. Firstly, the problem is that the lower detection size limit of all these instruments varies, typically ranges from 2 to 20 nm, and is determined both by instrumental factors and operator's decisions. The lower size detection limit is a key parameter for monitoring sources or processes. For example, if we use a lower size limit close to 30 nm, we will not detect most particles from NPF and most of vehicle emitted UFP will be not detected. Secondly, the upper size limit also varies, and in the case of CPC it could even be up to 3,000 nm. Hence, the choice of the lower window of measurement is usually critical, since the majority of UFP are often within a smaller, lower size range, particularly in environments affected by fresh combustion emissions or NPF. The upper range is less critical; however, while it is true that in most typical environments PNC is dominated by UFP, it is important to keep in mind that the total and the UFP concentrations are not the same. These differences have to be considered when attempting to establish quantitative understanding of variation in particle concentrations between different environments, cities and studies, which is of significance for human exposure and epidemiological assessments. Also the error in the lower size range of particles should be kept in mind: some studies have estimated it to be negligible in respect to the uncertainty for lower size limit less than 5-6 nm. It should be calculated and corrected for lower size limit up to 10 nm. In the case of lower size limit greater than 10 nm the corresponding error in the measurement of background aerosol could be much higher in respect to the uncertainty: consequently, the use is not recommended. A few simpler portable instruments are available that measure number concentration and mean size, however their accuracy (+/-30%) and need for pre- and post-measurement validation does not allow collection of accurate information.

Mass concentration/size distribution – *not commonly measured*. Due to low mass concentration of UFP, gravimetric methods lack accuracy. If anything, impactors are used to collect UFP for post sampling gravimetric analysis. Some studies report “quasi UFP”, also including particles bigger than 100 nm if an impactor stage does not provide a cut-off at 100 nm.

Surface area – *not commonly measured*. Most often, surface area is estimated from particle number and size distribution data and based on the assumptions about particle shape and density. While the number concentration of particles does not take the particle diameter into account, particle surface area concentration

equals the PNC times the squared diameter of the particle (assuming their spherical shape) within a certain size range. In addition, instruments to measure UFP surface area directly have been developed. The epiphaniometer estimates the Fuchs surface area as a function of radioactive decay of ^{211}Pb atoms attached to the measured particles. Other surface area measurements are using diffusion charging technology. Diffusion charging is a process in which particles are exposed to a unipolar ion atmosphere, in which ions undergoing Brownian motion attach to particle surface, transfer an electrical charge to the particle (DiSCmini; Nanoparticle Surface Area Monitor; AeroTrak). However, the existing techniques are not sufficiently accurate. Of importance is that potentially it is not only the surface area, which is of interest, but also functionalisation of the surface, for example, the compounds that are attached to the particle surface, as they get in contact with the environment or human lungs. It has to be noted that some studies report very strong correlation between toxicity and surface area of the particles, thus this metric requires careful consideration.

Liquid/solid – *not commonly measured*. Such measurements require at least a thermodesorber or a more complex system if the composition is to be inferred.

Elemental/organic carbon – *not commonly measured*. The bulk of UFP mass is typically composed of carbonaceous material with smaller contributions from inorganic ions and metals, reflecting that combustion sources are most dominant for these particles. The carbonaceous material can be broadly defined as compounds containing elemental carbon (EC) and organic carbon (OC). For carbonaceous particles, there are several measurement methods which exploit different physical or physicochemical properties of the particles: optical absorption measurement (BC - Black Carbon) and thermo-optical transmission (EC). High time resolution measurements are currently possible by using of absorption methods, for example, multi-angle absorption photometer (MAAP) or aethalometers (single or multi-wavelength spectrometers). Occasionally it is somewhat confusing that the two terms: UFP and soot-containing particles (combustion particles with a core of graphitised carbon and a coating of organic carbon, whose variability are usually monitored as BC or EC), partly overlap. In fact, many particles <100 nm (UFP) in urban areas consist almost exclusively of soot or contain large amounts of soot. However, BC or EC itself is rarely measured exclusively as $\text{PM}_{0.1}$, which would roughly correspond to the size range of UFP, but in practice mostly as PM_{10} , $\text{PM}_{2.5}$ or PM_{10} , which in turn does not include only UFP.

Metals – *not commonly measured*. There are impactors and nano-impactors that are used to measure elemental concentrations in the UFP range, but long time series

providing data for short and long term effects of UFP are very scarce.

Other composition – *not commonly measured*. As reported for metals.

Morphology – *examples exist, but systematic information/images across different types of UFP are not available*.

The current state of knowledge:

- ▶ Particle number/size distributions are most commonly measured, with relatively well established methods; however, there is no standard methods selected, which makes it difficult to compare results from different exposure/epidemiological studies or use the data for large population based epidemiological studies:
 - To overcome this it is proposed to use instruments, which measure at least down to 10 nm, with no restriction on the upper limit. The measured uncertainty accompanying any UFP number concentration measurements should critically reflect the experimental uncertainty associated with different lower size limits.
 - The uncertainty in the calibration of instruments measuring PNC is based on standardized methodology and varies between 30% for lower concentrations (less than 1,000 particles.cm⁻³) to 10% for typical urban background concentrations (about 10,000 particles.cm⁻³).
 - There is still an open question how to transform the inter-quantitative data, or a factor converting to, this is to say 10,000 #cm⁻³ based on the measurement device.
- ▶ Due to the lack of instrumental methods we cannot recommend UFP mass or surface area measurements as routine approaches.
- ▶ As strongly suggested by some experts the common air quality monitoring strategy (at least in the EU) should be extended by set up of so called “supersites”. At such “supersites” parameters, such as size-segregated UFP, online BC and PM speciation measurements, surface area, oxidative potential should be measured by standardized methods. Such data will allow to investigate the spatial and temporal variation of UFP at many locations and will provide a good base for future epidemiological studies on health effects of UFP.

1.4 Relationship between UFP, other particle metrics and gaseous pollutants, and sources

Many experimental studies in addition to UFP (PNC), measured concentrations of particle mass (e.g. PM_{2.5}, PM₁₀, or mass surrogate and of gaseous pollutants. The

relationship between the measured pollutants was analysed with an aim to gain a better insight into pollution sources or pollution dynamics. In general, the total and UFP number concentrations were poorly correlated with PM_{2.5} levels: while there was some level of correlation between some particle metrics (some particle number or mass fractions) reported by some studies, other studies did not find any correlation. This was explained by different sources of coarser and finer particles in different environments. A general conclusion was made that without local measurements, the degree, if any, of local correlations cannot be predicted from studies conducted elsewhere.

By contrast, studies often showed a reasonably good correlation between concentrations of UFP and traffic emitted gaseous pollutants, in particular CO and to lesser extend NO_x, as well as BC; however, again, the existence and the degree of correlation varied. In high insolation environments the correlation with traffic related pollutants, including BC, and UFP is lost due to the large impact of NPF on number concentration of UFP, however at a seasonal scale, levels of UFP (enhanced by NPF) might show a correlation with other photochemical pollutants such as O₃. There were multicity studies showing that using the same approach evidenced a very clear BC/UFP correlation for long term measurements in cities from Central and Western Europe, and very low for those from Southern Europe. While correlation between UFP and other pollutants have been studied to some degree in outdoor air, such correlations in indoor air, where we spend the majority of our time and where powerful indoor sources contribute to elevated UFP levels, remain unknown. As stated above, there is an important body of evidence of the impact of outdoor O₃ infiltration on indoor UFP concentrations due to interaction of this outdoor pollutant with indoor VOC.

It was argued that the existence of a quantifiable relationship between UFP and other pollutants or other particle metrics would provide justification for using some of the pollutants as surrogates of UFP, thus lowering the overall costs of monitoring. Since associations of UFP with other pollutants differ between different cities, any models developed would have to be city-specific and based on measurements conducted in that city.

Clustering and receptor models are being applied to long term measurements of particle size number concentrations and to UFP concentrations to apportion source contributions to total number or size fractions of UFP, as it is done for source apportionment of PM₁₀ or PM_{2.5}. This might facilitate comparison of health effects of traffic-related, NPF, and regional UFP in studies focusing on meta-analysis of short and long term effects of UFP. However, if this is not done, the meta-analysis might evaluate similar number concentrations from cities while their source contribution might greatly differ.

1.5 Indoor versus outdoor UFP

Indoor particles consist of a mix of ambient particles that have infiltrated indoors, particles emitted indoors, and particles formed indoors through reactions of gas-phase precursors originating from both indoor and outdoor sources. Therefore, in addition to identification of the indoor sources operating in an indoor environment, of significance is the understanding of the indoor versus outdoor origin of indoor particles. While each individual indoor environment (building) is different, leading to differences in exposure and ideally necessitating its own assessment (which is very rarely done), the existence of some generalizable trends for the main types of indoor environments where people spend time have been reported.

It has been shown that in homes UFP originate mainly from indoor sources (contrary to PM_{10} and $PM_{2.5}$, of which the outdoors is the main source); for schools and day cares, outdoor air is the source of indoor UFP (while PM_{10} and $PM_{2.5}$ have indoor sources); and for offices, outdoor air is the source of UFP (as well as of PM_{10} and $PM_{2.5}$).

As stated above, the infiltration of outdoor photo-oxidants into indoor environments, and the subsequent interaction with indoor VOC, might be also a relevant source of UFP.

The current state of knowledge:

- ▶ There is general understanding of the sources and processes leading to indoor UFP.
- ▶ There is some level of understanding of typical UFP concentrations in *typical* indoor environments. The meaning of *typical* is restricted to the countries and setting where such studies were conducted.
- ▶ However, there could be large differences in UFP concentration between specific and *typical* indoor environments (e.g. between a *specific* and a *typical* school).
- ▶ In general it is more logistically complicated to investigate UFP in indoor environments, however, since in general their sources are understood, recommendations can be provided regarding source control.

1.6 Exposure assessment to UFP for epidemiological studies

Assessment of exposure to UFP is significantly more complex than assessment to particle mass ($PM_{2.5}$ or PM_{10}) due to considerable spatial and temporal variability in UFP concentrations, of up to an order of magnitude above background, within a few seconds and over a few metres as people move closer to, or away from pollution sources, or between different microenvironments. This

large variation in UFP concentration across different environments may be of significance in relation to human exposure assessment and epidemiological studies. The influence of time-activity and movement can be easily missed by using averaged results, and thus mean and median concentrations over a time-averaged period may not reflect all aspects of population exposure patterns.

Epidemiological long-term and short-term studies investigating the association between outdoor particle exposure and health effects are largely based on a single monitoring site located somewhere to measure urban background concentrations. Hence, in such studies the central question is to know how well particle concentrations measured at one single measurement station are representative of a wider urban area. Studies in urban areas have shown that spatial variability of $PM_{2.5}$ and PM_{10} is generally small and temporal correlation measured at different sites is high. Hence, there is a consensus in the scientific community that a background station measuring $PM_{2.5}$ and PM_{10} mass concentrations could be regarded as representative of larger urban areas. In contrast, exposure assessment of UFP is still in its initial stage compared to exposure assessment to $PM_{2.5}$ and PM_{10} , but the fact that cause-effect evidences were found in a number of studies based on urban background single site monitoring implies that this approach might also be applicable. A very relevant limitation is the fact that in the same city, a day affected by intensive photochemical pollution causing high rates of NPF, might contribute to UFP concentrations higher than on days when most UFP are attributable to traffic related pollution. This limitation is also present for $PM_{2.5}$ and PM_{10} (e.g. desert dust days compared with local pollution days), however the differences between city-regions concerning the proportion of UFP derived from NPF/traffic/domestic burning/others larger impacts on the daily and annual levels and size of UFP than are these for particle mass.

It should be kept in mind that a concept design for assessment of exposure for epidemiologic studies should be such that it would be easy to measure, universally applicable, shrinking the uncertainties, but still be able to relate this to entities, that we can then measure, model and also regulate.



Temporal variability (short-term studies)

The temporal correlations among different monitoring sites within one urban area are influenced by common diurnal patterns and meteorological factors and consequently, measurements at those locations may be temporally highly correlated, even if there are pronounced differences in absolute levels among sites (high spatial variation). High temporal correlations among monitoring sites indicate that one (or several) fixed monitoring site may be adequate for estimation of the entire outdoor population exposure in short-term studies.

Some studies on the temporal correlation between sites reported high correlation coefficients for site-to-site hourly average measurements, other studies found lower correlations between different monitoring sites. Because of this variability, measurements at one fixed monitoring site within the city (the central site) may or may not be representative of other locations. There is no general agreement that there is a temporal correlation between UFP concentrations measured at different monitoring sites. Given that the temporal correlations of UFP between different monitoring sites are so city-specific, such correlations need to be carefully evaluated when designing studies in a specific study area and when reporting and interpreting study results. Differences between concentration levels and size of UFP at traffic hotspots and urban/suburban areas are known and can be assessed for specific cities. Also source receptor modelling might allow to obtain levels of UFP attributable to different source contributions.

Spatial variability (long-term studies)

Given the pronounced gradients in UFP concentrations near sources such as major roads, substantial spatial variation in UFP can exist across a single city. In fact, there are some studies reporting pronounced differences in the absolute concentrations of UFP measured across the city area. The spatial variability of the absolute concentrations within cities was considerably higher for UFP than for PM_{2.5} and PM₁₀. Therefore, using data from a central site for cross-sectional studies (long term effects studies) may not be appropriate, and the applicability should be preliminarily demonstrated, or should be improved in order to be able to attribute more accurate exposure levels to the study subjects. The greater measurement error can limit the statistical power of epidemiological studies on long-term effects of UFP to detect the true associations that might exist. Future long-term studies might consider different approaches to exposure assessment, for example, modelling, to better characterize outdoor population exposure, or increasing the number of monitors in order to cover the spatial variability in cities.

Using other, or additional, approaches to exposure assessment than ground base (central site) monitoring is another aspect of complexity. While land use regression models (LUR) perform well for PM_{2.5} and some gaseous pollutants, they have been shown to perform significantly worse for UFP. Satellite data cannot be used at all for UFP: the particles are too small to be measured by optical means, and this is how larger particles are detected by satellite instruments (using Aerosol Optical Depth). Therefore, high resolution maps of UFP are currently rare. However, it is becoming possible to build exposure distribution maps using other relevant inputs. There are traffic models providing real traffic patterns down to individual streets. The measured activity level (from wearable devices, e.g. the heart rate) can help estimating quite well the air intake; which in turn can help to estimate the lung deficit. All these inputs can be used for exposure simulation, which would have to be done with a clear objective of application for epidemiological study.

1.7 Total number UFP concentrations or source contributions for health studies?

As it is very well known in relation to PM_{2.5} and PM₁₀, the use of bulk or source apportioned concentrations that are considered in epidemiological studies might yield quite different results concerning attributable health outcomes. As an extreme case, in areas with high sea salt loads in PM₁₀, the use of bulk PM₁₀ levels as outdoor exposure input for health studies might hinder the health effect of the anthropogenic load of PM₁₀; whereas if PM speciation and source apportionment analyses are implemented we might better characterise the health effects attributable to specific sources, or even simply eliminating the interference of the seasalt-PM₁₀ contribution. For UFP the situation might be very similar in some cases. There is a study comparing averaged annual daily patterns of particle mass, NO_x, BC and UFP concentrations from urban and industrial background and traffic sites from a selection of Western, Central and Southern Europe. It is shown that for both urban background and traffic locations from Central and Western Europe, UFP are markedly correlated with BC and NO_x, pointing to a clear contribution of traffic UFP to the total number UFP concentrations. However, for Southern Europe, the high insolation accounted for an intensive NPF at midday, and the generation of UFP was so high that the highest levels of UFP were not measured at traffic rush hours, but during the midday hours, when levels of traffic tracers, such as BC and NO_x were at their lowest levels. A subsequent

study focusing on high insolation cities from Southern Europe, Australia and US found the same patterns for the UFP traffic contributions. If we include in epidemiological meta-analysis total number concentrations and health outcomes of the above cities from different climate/emission patterns, finding a relationship between them might be a difficult issue. However, by using receptor modelling, or primary ratios of traffic related BC/UFP concentrations, traffic and secondary or non-traffic related UFP can be apportioned to supply the exposure data for the epidemiological studies. In this sense, although the available results for health outcomes of UFP seem inconclusive, when evaluating the results of the studies taking into account the above possible UFP patterns-origin, an explanation for opposite results might be obtained.

1.8 Personal exposure assessment

In principal *personal exposure* assessment, which could be used for small cohort studies, is becoming possible, albeit, still costly and scientifically challenging. The emergence of small and robust instruments, which can be carried by individuals or placed on taxis, buses, drones, etc., for UFP concentration and even size distribution measurements, is the first step. Also, there is an increasing availability of time activity data, by using tracking devices (GPS), with the data transmitted to the database or downloaded from the devices. Finally, it is becoming increasingly more feasible to use big data approaches to obtain more personalised exposure attribution, by combining modelling with monitoring stations and information about temporal variation of meteorological conditions.

Whether using the 'traditional definition of UFP' (particles < 100 nm) for the purpose of exposure assessment, or considering the whole particle concentration count (in other words, not cutting them through the particle mode), is another open question in exposure assessment to UFP.

For all portable measurements, but specially for UFP, it is greatly recommended to inter-compare measurements with collocated reference instruments, and select the instruments with an appropriate lower size detection limit (close to 10 nm). The latter has to be corroborated by comparing the instrument measurement with a reference instrument (for example, in terms of comparing number concentration) since many commercial instruments are sold with a lower size detection limit but the real efficiency for detecting <30 nm is markedly diminished in many portable instruments.

The current state of knowledge:

- ▶ The estimation of the population exposure to UFP in epidemiological short- and long-term studies is

significantly more complex than assessment to PM_{2.5} and PM₁₀.

- ▶ Source contributions to UFP may differ greatly among cities, especially proportions of traffic/NPF/biomass combustion contributions. Then trying to evidence health effects with total number concentrations of UFP in multicity analysis might be a difficult task. As stated above, there are tools that allow obtaining source contributions to UFP concentrations and enabling robust meta-analysis of multi-city data for epidemiological studies.
- ▶ The spatial variation of the absolute concentration level of UFP across a single city area is substantially larger compared to the spatial variation of PM_{2.5} or PM₁₀. It means that epidemiological long-term studies cannot adopt the approach of the PM_{2.5} studies that relied on single or a few central monitoring sites to characterize the city-average concentration of UFP. Future long-term studies might consider modelling or increasing of the number of monitors in order to cover the spatial variability in cities.
- ▶ The difficulties in obtaining spatially resolved estimates of long-term exposure (high cost of particle number monitoring equipment prohibits large-scale monitoring; almost no successful modelling approaches for UFP) hamper the conducting of long-term epidemiological studies on UFP.
- ▶ For some urban areas it has been shown that although the temporal correlation among different monitoring sites was higher for PM_{2.5} than for UFP, the difference was not substantial. It means that using a central monitoring site to characterize outdoor exposure in epidemiological time series studies does not result in substantial more measurement error for UFP than for PM_{2.5}. However, as in other areas the temporal correlations of UFP were substantially lower than for PM_{2.5}, such correlations need to be carefully evaluated when designing short-term studies (panel studies) in a specific study area.
- ▶ Scientific progress on many fronts makes personal exposure assessment possible.
- ▶ Considering exposure to traffic generated UFP, it should be kept in mind that other traffic related exposures (such as to gases, black carbon or noise), should be simultaneously assessed. While they are usually treated as co-variables (or co-pollutants), they are not necessarily co-variables as they actually have different pathways in the body and their effects are actually independent. It will be very important to think how to do this well, so in the end we are not left without neither evidence for NO₂, nor UFP, nor BC.
- ▶ There is a need to develop an optimal way of exposure assessment for epidemiological studies, utilising the emerging science and technology.

2. Toxicology

2.1 What reasons do we have to consider UFP separately from PM_{2.5}/PM₁₀?

There are two main aspects that separate UFP from larger airborne particles (PM_{2.5}/PM₁₀) in terms of toxicology: (i) differences in the deposition (local dose) upon inhalation, and (ii) differences in intrinsic toxicity as a result of physicochemical properties. Of note, associations have been observed between UFP exposure and health effects independently of other metrics of air pollution such as PM_{2.5} and NO_x. Interestingly, some UFP might translocate out of the lung and then reach the circulatory system and other organs, while this is not the case for larger PM.

2.2 From exposure to internal dose

The site where inhaled particles will deposit in the respiratory tract is highly dependent on their airborne size. As a rule of thumb, the smaller the particles the deeper they penetrate into the lung. Inhalation of airborne particles including UFP will lead to deposition onto the regional epithelia of the respiratory tract. The actual deposition depends on: (i) the aerodynamic, diffusional and thermodynamic properties of the particles and only on the latter two for UFP; (ii) the geometric branching pattern of the individual airways and alveoli, and (iii) on the breathing patterns of the human subject (Oberdörster et al. 2005; Kreyling et al. 2006; Möller et al. 2008, 2009). This deposition occurs primarily by diffusion and secondarily by thermophoretic effects in the first few branches of the airways of the lung during exhalation. While impaction and interception are factors that are more dominant for μm sized particles, UFP deposition is more driven by diffusion (particularly at low air flow) and surface charge. Particles can translocate to other organs via the blood, where they can also lead to adverse health outcomes.

Once deposited, the chemical composition, including surface reactivity and dissolution rates, are the driving forces for toxicity. Oxidative stress is a common mechanism of response that leads to inflammatory responses and tissue damage. A few studies have compared the relative toxicities of UFP sampled at different locations. For example, (Li et al., 2003) showed that differences in the size and composition of coarse (2.5-10 μm), fine (< 2.5 μm), and ultrafine (< 0.1 μm) particulate matter are related to their uptake in macrophages and epithelial cells and their ability to induce oxidative stress. Jalava et al. (2007) exposed RAW264.7 macrophages to coarse PM, fine PM and quasi-UFP (PM_{0.2}) from six European cities with different air pollution conditions. Site-dependent variabilities in

toxicity were observed for all size fractions. Most notably, wintertime PM_{0.2} from Prague, a city with high impact from local coal and biomass combustion, induced considerably more cytotoxicity and apoptosis and cell cycle arrest, compared to PM_{0.2} from the other cities in the study (Jalava et al. 2007). A similar approach was taken in the RAPTES project, also reporting site-dependent variability in toxicity to macrophages among all PM size fractions (Steenhof et al. 2011). What has not been well studied is interactions between different particle sizes, including the extent to which UFP will stick to larger particles possibly making them more toxic or changing the site of deposition after inhalation.

A good example of the differences in toxic potency was presented by Plummer et al. (1994) who demonstrated the differences in biological responses in an *in vivo* study. These results not only suggested that there can be differences in toxic potency due to sampling from various sources, but also due to the meteorological conditions, where summer UFP are often more potent than the winter UFP, but the reverse was true for the submicron fraction. Gilmour and colleagues used size fractionated ashes and concluded that on an equal-mass basis, the UFP from combusted Montana coal induced a higher degree of neutrophil inflammation and cytokine levels than did the fine or coarse PM in the lungs of mice (Gilmour et al, 2004).

The current state of knowledge:

- The differences in size (distribution) between UFP and larger sized PM result in regional differences in the deposited dose, potentially leading to differential biological responses. Focusing only on PM_{2.5} may result in overlooking the impact of UFP.
- The toxic potency of UFP when using mass as a dose descriptor differs from PM_{2.5}, often (but not always) showing that UFP cause greater effect. Moreover, the lung shows different response to UFP compared to larger particles.



2.3 Which metric should be used to describe UFP concentration-effect relationships?

Most of the time, UFP get defined by their size only, though this is too simplistic a view, as UFP have also inherent characteristics that renders them toxic. The potential to produce reactive oxygen species (ROS) or to otherwise induce oxidative stress has been recognized as one of their major driver of toxicity. It has been shown in a study of bus maintenance staff exposed to diesel engine exhaust that fast reacting functional chemical groups (e.g. aldehydes) are much more important than just having functional groups that can eventually produce ROS (Setyan et al. 2010). When looking at a large variety of combustion generated carbon nanoparticles, ROS production was quite well correlated with surface area rather than total particle number concentration or mass. However, for metal UFP, mass was better-correlated, as long as the metal species could be oxidized and subsequently dissolved (Sauvain et al., 2013).

At least some of the observed source-specific differences in particle toxicity are due to the use of mass rather than surface area as the dose metric. Stöger and colleagues have investigated acute pulmonary inflammation in mice (neutrophil influx into lung) 24h after intratracheal instillation of six types of carbonaceous UFP including flame soot with low/high organic content (7/17%), carbon black particles with small/large primary diameter ($d = 10/51$ nm), non-combustion derived carbon particles with extremely high specific surface area ($800 \text{ m}^2/\text{g}$) and diesel exhaust particles (DEP) (SRM 1650a) (Stöger et al., 2006). They found that acute pulmonary inflammation scaled very well with mass-specific Brunauer-Emmett-Teller (BET) surface area, but not with particle number or mass (Stöger et al., 2006). Independent of particle type, the onset of inflammation occurred at about 20 cm^2 BET surface area (per mouse). In a follow-up study with the same panel of UFP they furthermore showed that the observed effects could be explained by the combined effects of oxidative potency (assessed *in vitro*) and the degree of Cyp1a1 in the lung. BET surface area remained the best predictor of acute pulmonary inflammation (Stöger et al., 2009). Chronic health effects and disease prevalence also scale better with surface area dose rather than with mass. Enhanced tumour prevalence was observed in a 6 month inhalation study with rats using various types of biopersistent particles (toner/coal dust, diesel exhaust/carbon black particles as well as talc and TiO_2 particles) (Maynard & Kuempel, 2005). Surface area was a better predictor of these effects than mass. When different carbonaceous particles (graphene oxide, the carbon blacks Flamruss 101 and Printex 90, and the diesel exhaust particle SRM1650) were instilled at $0.1 \text{ mg}/\text{mouse}$ once weekly for 7 weeks (0.018 mg for graphene oxide), the deposited surface area showed a correlation with neutrophil cell influx in the lungs

(Skovmand et al., 2019). In studies with mass as the dose metric, UFP (primary particles $< 100 \text{ nm}$) appear to be about 2.5-fold more toxic than particles larger than 100 nm , while no size-dependent difference in toxicity was observed for surface area as dose metric (Gebel 2012). Hence, at least some of the observed source-specific differences may be eliminated, if surface area and not mass is used as the dose metric.

The current state of knowledge:

- For practical reasons, using particle number as a predictor may be preferred over mass and surface area, especially if the particles size distribution is known. However, increased understanding of the importance of chemical composition for toxicological effects of UFP and the use of surface area rather than mass as dose metric may possibly shed more light on the issue.

2.4 Does the toxicity of UFP depend on the source?

First we need to know that ambient UFP in urban areas might vary widely, but we might simplify these patterns as follows: primary combustion-related UFP are mostly made by condensed organic compounds (e.g. PAH, alkenes, alkanes, hopanes) on an elemental carbon-rich core, with minor proportions of sulfate, nitrate, and metals. By contrast, brake wear may produce metal rich UFP (Gasser et al., 2009), and tire-wear may produce UFP with a composition presumably reflecting the composition of the tires (Dahl et al., 2006) and may contain both zinc and PAHs. Secondary UFP will be more water soluble, with more oxygenated organic compounds, nitrate, sulphate, ammonium, and lower levels of metals.

It is well known that particles and fibers of different source and origin, elicit different pathological effects. This has been exemplified by Donaldson et al. (2009) who pointed out that different conventional pathogenic particle types – e.g. PM_{10} , asbestos and quartz – cause diverse pathological effects. Different particle properties also appear to be involved in different biological effects *in vitro* (Schwarze et al 2007). Between-city and seasonal differences in toxicity from urban air coarse and fine PM ($\text{PM}_{10-2.5}$ and $\text{PM}_{2.5}$) have been reported from both *in vitro* and *in vivo* studies, further suggesting that sources may affect the toxicity of ambient PM (Hetland et al. 2005; Steerenberg et al 2006). In line with this, a study from Denmark assessing personal exposure showed that outdoor UFP exposure away from home was associated with a decline in microvascular function and pulse amplitude, and increase in blood leukocytes and neutrophils, while UFP from indoor sources was not associated with adverse vascular effects (Olsen et al. 2015). Thus, it can be anticipated that the source of UFP

will affect their toxicity, given sufficient difference in physicochemical properties. However, is the variation in sources of outdoor ambient UFP considerable enough to affect toxicity of UFP to such an extent that it could have regulatory implications?

Combustion processes are among the major sources of outdoor air UFP. A number of studies have compared effects from DEP from different engines and fuel types. Studies from US EPA compared pulmonary toxicity and mutagenicity of an automobile DEP (A-DEP) with the National Institute of Standards Technology standard reference material (SRM2975) from a forklift engine. A-DEP caused increased interleukin (IL)-5, IL-6, and macrophage inflammatory protein (MIP)-2, as well as an increase in macrophages in lungs of exposed mice. By comparison, SRM2975 only induced IL-6, and increased polymorphonuclear cells (Singh et al. 2004). A-DEP were also more mutagenic than SRM2975 per mass, in *Salmonella* (DeMarini et al. 2004). Furthermore, studies in human bronchial epithelial BEAS-2B cells showed that A-DEP induced IL-8 through AP-1-dependent and NF- κ B-independent mechanisms, while SRM2975 induced IL-8 through NF- κ B-independent mechanisms (Tal et al. 2010). A-DEP is rich in organic chemicals (26%), containing 10 times more extractable organic material (EOM) than SRM2975 (2%), and 227 times more PAH. These studies show a striking difference in pulmonary toxicity and mutagenicity between two different DEP of different physical and chemical characteristics. Different pro-inflammatory potential was also reported in a comparison of two DEPs with contrasting PAH and metal content (Totlandsdal et al. 2013). In contrast, when UFP from a non-commercial airfield, the apron of a commercial airport, the NIST diesel exhaust particle NIST2975, and carbon black Printex 90 were instilled in mice and toxicity assessed 1, 3 and 28 days later, all particles induced acute phase response, inflammation, and genotoxicity, with no apparent differences in the dose-effect relationships between particle types and origin. Of note, airport particle contents of polycyclic aromatic hydrocarbons and metals were similar to the content in NIST2975 (Bendtsen et al., 2019).

It appears difficult to conclude on the relative toxicity of UFP from diesel engine emission and biomass burning. However, it seems clear that chemical composition affects the toxicity of combustion-derived particles, and that organic chemicals attached to the particle surface is of importance for effects. A number of studies showing that much of the biological effects from DEP could be attributed to extractable organic matter (reviewed in Øvreik et al. 2015) support this notion.

The overall evidence suggests that the source of UFP affect their toxicity, at least to some extent, as has been demonstrated for larger particles. However, the currently

available evidence does not allow for conclusions as to whether some sources give rise to UFP of higher toxicity than others. However, it is clear that UFP collected from different locations (and sources) possess different toxic potencies. An inherent problem is exemplified by DEP and biomass particles (wood smoke particles – WSP). The variability in chemical composition and toxicity of DEP from different diesel engines, different engine load/driving cycles and fuel types is so considerable that DEP cannot be considered as an “homogenous” particle group. The same can be argued for biomass particles, which also displays large variation depending on fuel type and combustion conditions. This is likely the reason that some studies observe higher toxicity from DEP than for WSPs, while others report the opposite. Until such issues are solved, concluding on the relative toxicity of UFP from different sources would be difficult.

2.5 Short term effects on mechanistic biomarker and physiological functions in controlled human exposure studies involving UFP

Very few studies applied a pure ambient air-relevant UFP exposure to allow direct causal inference (Table 1). Essentially CAPS (concentrated ambient particles) are the only pure exposure in that sense. Many studies have applied exposure to mixtures such as diesel exhaust or prescribed exposure in traffic aerosol lasting 1½ to 5 hours. In some studies, causal inference related to UFP was well supported by using filtration and NO₂ as controls. Otherwise, the evidence is supportive of effect of UFP by, for example, closer correlations with outcome than or with adjustment for concomitant PM_{2.5} or NO₂ exposure levels, although independence of these other components cannot be that well documented. Supportive evidence also originates from controlled exposure to pure carbon-based UFP. The evidence for UFP effects are strongest in relation to vasomotor function, oxidative stress-induced genotoxicity and decreased lung function in patients with obstructive airway disease. As the specific effects of UFP in short-term controlled exposure studies and dose-response relationships are not well documented it is difficult to suggest threshold values.

The current state of knowledge:

- There are considerable differences in the toxic potency of UFP released from various sources when using mass as unifying metric. Soluble components, including organic chemicals, appear to contribute the effects of UFP, but the key drivers of differences in toxic effects remain to be determined.

Table 1: Examples of studies in which effects of ambient UFP have been assessed in humans

Cardiovascular markers	Evidence of effect of UFP	Key references
Heart rate variability	Limited support for UFP effects from exposure to ambient UFP and traffic-derived PM but no sign of effect from pure diesel exhaust	Gong et al. 2008 Samet et al. 2009 Mills et al. 2011 Devlin et al. 2014 Breitner et al. 2019
Blood pressure	Very limited support for UFP effects	Mills et al. 2011 Devlin et al. 2014 Magalhaes et al. 2018 Sinharay et al 2018
Vasomotor function	Independent effect of diesel UFP evidenced by null effect of NO ₂ and filter controls as well as pure carbon UFP Supportive evidence from traffic derived exposure in elderly and exercising women although contribution from PM _{2.5} and for some NO ₂ cannot be excluded. Outdoor UFP and indoor PM _{2.5} were more related to decrease in vasomotor function than indoor UFP in filtration studies in homes of elderly and observational studies with personal monitoring	Mills et al. 2011 Weichenthal et al. 2014 Bräuner et al. 2008 Karotki et al. 2014 Karotki et al. 2015 Olsen et al. 2014
Arrhythmia	No support for effects from multiple air pollution exposure studies	Langrish et al. 2014
Systemic inflammatory markers	Very limited evidence of effects of UFP in terms of IL8 increase after UF CAP in only one study and IL6 increase associated with airport UFP, whereas the majority of studies showed no effects related to UFP	Gong et al. 2008 Samet et al. 2009 Habre et al. 2018 Mills et al. 2011 Devlin et al. 2014
Oxidative stress	Some evidence of oxidative stress to DNA from UF CAP and UF fraction of traffic derived PM	Vinzents et al. 2005 Brauner et al. 2007



2.6 Do acute (peak) exposures have more impact on human health than long term UFP exposures at low levels?

While it seems likely that long-term exposures have a higher impact than immediate, short-term peak exposures, very little is known about the influence of exposure duration to UFP, not to mention timing in different phases of life. Yet, people are often confronted with peak exposures within a day, for example, due to transportation during rush hour. There is no systematic comparison available that enables distinction between short and long-term exposure based on toxicological evidence.

Clinical studies suggest that there is an immediate biological response when exposure to UFP was started intentionally (studies by Mills et al., 2011; and Langrish et al., 2014).

The current state of knowledge:

- ▶ While shorter averaging times than 24 hours seem relevant to determine the health impact of UFP, there is a lack of data from long-term exposure to UFP from experimental studies. At present, it is unknown whether (repeated) peak exposures are more relevant than continuous exposure to lower PNC though with the same mean dose. This issue may be especially relevant relative to developmental issues (pregnancy, foetal and child development), where a single high exposure during a sensitive period may have permanent effects, whereas slight, acute perturbations in adults may be without long-term consequences.



3. Epidemiology

3.1 Health Effects UFP – Epidemiological evidence

Health effects of UFP, defined as particles smaller than 100 nm, have been discussed over the past three decades (Stone et al., 2017). The first epidemiological study demonstrating short-term effects of ambient UFP came from a small panel study of asthmatics conducted in Erfurt, Germany in the 1990's (Peters et al., 1997). It showed that UFP number concentrations were associated with decreases in lung function consistent with a cumulative response over several days. These associations were independent of fine particles concentrations and other pollutants. Since then, approximately 200 additional studies have been published.

Two recent systematic reviews assessed published reports from 1997 to 2017 (HEI, 2013; Ohlwein et al., 2019). These reviews documented the rising number of studies and together identified 174 studies (Table 2) as well as discussed the studies qualitatively. Most, namely 164, investigated the short-term impact of UFP or total particle number concentrations (PNC), however, with heterogeneous results (HEI, 2013; Ohlwein et al., 2019). Since 2017, research on short-term health effects of UFP and PNC has continued. We identified, for example, three new studies on mortality, six studies on lung function, one study on cardiac function, and eight on blood biomarkers, all considering UFP exposures within hours or days. Despite the growing literature, there is an absence of quantitative meta-analyses. An underlying reason may be that both exposure assessments and study designs are very heterogeneous across studies. One recent attempt by Li and co-authors (2019), however, shows that quantitative meta-analyses will indeed advance our understanding. Therefore, it seems timely to re-evaluate the overall evidence and consider different designs such as time-series analyses, case-crossover studies, panel studies and quasi-experiments using a systematic approach.

One major challenge is the exposure assessment of UFP or PNC as pointed out previously (exposure section). Dedicated measurement campaigns are needed to conduct epidemiological studies as UFP or PNC are not measured routinely. Only recently, advances have been made in determining the spatial distribution reliably so as to allow the investigation of long-term health effects. As a consequence, additional evidence on long-term health effects of UFP has been published recently (Bai et al., 2019; Downward et al., 2018). These new studies indicate associations between UFP number concentration and cardiovascular morbidity. Importantly, they were able to show that the health impact of UFP was independent of fine particles and nitrogen dioxide concentrations.

Therefore, these studies are starting to fill a major research gap (Downward et al., 2018).

Table 2: Summary of the number of epidemiological studies published between 1997 to 2017 based on two systematic assessments (3, 4).

	1997- 2011 (3)	2011- 2017 (4)	Sum
Long-term Health Effect Studies			
Mortality	0	1	1
Morbidity	0	4	4
Subclinical Outcomes*	0	5	5
All	0	10	10
Short-term Health Effect Studies			
Mortality	11	7	18
Morbidity, Emergency department visits, hospital admissions (Respiratory)	15	5	20
Symptoms	8	11	19
Subclinical Outcomes**	52	55	107
All	86	78	164
Total	86	88	174

* for example blood biomarker of inflammation;

** for example blood pressure, lung function measurements, blood biomarkers of inflammation and coagulation.

4. Regulations

With the advance in understanding of the impact of air pollution on health, the general advance of atmospheric science and the progress in monitoring techniques, there is an increasing focus placed by national and international bodies on UFP. Some jurisdictions, especially in Europe, have implemented monitoring programs in preparation for implementation of likely future new regulations. The World Health Organization is currently reviewing and updating its Air Quality Guidelines and UFP is one of the specific foci of the review; hence the new version of the document is likely to include some recommendations on UFP.

There is no regulatory monitoring of UFP anywhere in the world as yet. However, vehicle emissions regulators have already adopted UFP controls, with EURO 6 vehicle emission standard set at 6×10^{11} particles/km travelled.

While lack of exposure response relationship makes it impossible to propose health guidelines for UFP, it is important to point out that as discussed above, the current concentration of UFP levels in environments affected by vehicle emissions are up to an order of magnitude higher than in the natural environments. Thus, if there is also no threshold level in response to exposure to UFP (or if it is very low), future control and management strategies should target a decrease of these particles in urban environments by more than one order of magnitude. At present there is a long way to go to achieve this.

The current state of knowledge:

- ▶ While the health effects of UFP have been substantiated based on toxicological studies (toxicology section), there is a need to systematically and quantitatively assess the existing evidence based on epidemiological research. These analyses should consider the heterogeneity of source contribution patterns for UFP and PNC in different regions, with climatic and emission patterns, the differences of populations or patient groups studied, the differences in UFP measurements, the differences in exposure-response times typically operationalized by lag-periods, different years of investigation and related underlying time-trends altering the sources and composition of UFP. While these quantitative meta-analyses are challenging, they will provide novel insights, impact on regulatory evaluations and generate hypotheses to be tested in epidemiological studies, controlled human exposure studies and toxicological studies.

The current state of knowledge:

- ▶ There is no evidence that mitigating only particle mass (PM_{10} , $PM_{2.5}$) as the existing air quality measures do, will ultimately lead to reduction in UFP.
- ▶ There is some tentative evidence that mitigating particle mass (PM_{10} , $PM_{2.5}$) from combustion sources could lead to reduction in UFP.
- ▶ There have been suggestions for mitigation of black carbon in the future, but this would not remove all UFP, in particular, the organic ones.
- ▶ This highlights the need to establish regulatory approaches and control measures to address the impacts of elevated UFP concentrations, especially in urban areas, considering their potential health risks.
- ▶ Issues to resolve include: (i) whether the regulations should be set around the base line concentrations without the peak concentrations, or whether they should include the peaks in PNC due to NPF or presence in close proximity to source of primary emissions of UFP; and (ii) how to define the peaks.

- In order to properly control UFP, long-term monitoring is essential. Ideally all the UFP metrics, which have been identified as of relevance based on toxicological evidence, should be monitored. This however, is not feasible with large spatial coverage, but if anything at a limited number of supersites. Therefore there is a need to find the balance between what is feasible and what scientifically essential. At the moment measurements of PNC and to some extent particle size distribution are feasible on a larger scale.
- Of particular importance is source control of UFP and there is a body of literature pointing out to the existing methods and opportunities to do so.
- Developing a much better picture on a local scale of particle formation dynamics in different environments, including those which are influenced by traffic, would greatly assist such regulation formulation.
- It would be highly beneficial to develop and utilize standardised measurement procedures, enabling meaningful comparison between the results from different studies, which is of particular significance for human exposure and epidemiological studies.
- However, considering the complexity of the measurements, variety of instruments available and difference in the aims of the measurement/monitoring, it is not likely that standard methods to measure UFP will be accepted/established in a foreseeable future. But a way around it would be to provide estimates of variation between the different results, based on the differences in instruments being used, or their settings.

References

- AirMonTech, UFIREG Project, 2019. <https://www.interreg-central.eu/Content.Node/projects/projects.html> (accessed 07/08/2019).
- Bai, L., Weichenenthal, S., Kwong, J.C., Burnett, R.T., Hatzopoulou, M., Jerrett, M., van Donkelaar, A., Martin, R.V., Van Ryswyk, K., Lu, H. and Kopp, A., 2018. Associations of Long-Term Exposure to Ultrafine Particles and Nitrogen Dioxide With Increased Incidence of Congestive Heart Failure and Acute Myocardial Infarction. *American Journal of Epidemiology*, 188(1), pp.151-159.
- Bendtsen, K.M., Broström, A., Koivisto, A.J., Koponen, I., Berthing, T., Bertram, N., Kling, K.I., Dal Maso, M., Kangasniemi, O., Poikkimäki, M. and Loeschner, K., 2019. Airport emission particles: exposure characterization and toxicity following intratracheal instillation in mice. *Particle and Fibre Toxicology*, 16(1), p.23.
- Bräuner, E.V., Forchhammer, L., Möller, P., Simonsen, J., Glasius, M., Wählin, P., Raaschou-Nielsen, O. and Loft, S., 2007. Exposure to ultrafine particles from ambient air and oxidative stress-induced DNA damage. *Environmental Health Perspectives*, 115(8), pp.1177-1182.
- Bräuner, E.V., Möller, P., Barregard, L., Dragsted, L.O., Glasius, M., Wählin, P., Vinzents, P., Raaschou-Nielsen, O. and Loft, S., 2008. Exposure to ambient concentrations of particulate air pollution does not influence vascular function or inflammatory pathways in young healthy individuals. *Particle and Fibre Toxicology*, 5(1), p.13.
- Breitner, S., Peters, A., Zareba, W., Hampel, R., Oakes, D., Wiltshire, J., Frampton, M.W., Hopke, P.K., Cyrys, J., Utell, M.J. and Kane, C., 2019. Ambient and controlled exposures to particulate air pollution and acute changes in heart rate variability and repolarization. *Scientific Reports*, 9.
- Dahl, A., Gharibi, A., Swietlicki, E., Gudmundsson, A., Bohgard, M., Ljungman, A., Blomqvist, G. and Gustafsson, M., 2006. Traffic-generated emissions of ultrafine particles from pavement-tire interface. *Atmospheric Environment*, 40(7), pp.1314-1323.
- De Jesus, A.L., Rahman, M.M., Mazaheri, M., Thompson, H., Knibbs, L.D., Jeong, C., Evans, G., Nel, W., Ding, A., Qiao, L., Li, L., Potin, H., Niemi, J.V., Timonen, H., Luoma, K., Petäjä, T., Kulmala, M., Kowalski, M., Peters, A., Cyrys, J., Ferrero, L., Manigrasso, M., Avino, P., Buonanno, G., Reche, C., Querol, X., Beddows, D., Harrison, R.M., Sowlat, M.H., Sioutas, C. and Morawska, L., 2019. Ultrafine particles and PM_{2.5} in the air of cities around the world: are they representative of each other? *Environmental International*, 129: pp. 118-135.
- DeMarini, D.M., Brooks, L.R., Warren, S.H., Kobayashi, T., Gilmour, M.I., Singh, P., 2004. Bioassay-directed fractionation and salmonella mutagenicity of automobile and forklift diesel exhaust particles. *Environmental Health Perspectives*, 112(8): 814-819.
- Devlin, R.B., Smith, C.B., Schmitt, M.T., Rappold, A.G., Hinderliter, A., Graff, D. and Carraway, M.S., 2014. Controlled exposure of humans with metabolic syndrome to concentrated ultrafine ambient particulate matter causes cardiovascular effects. *Toxicological Sciences*, 140(1), pp.61-72.
- Donaldson, K., Borm, P.J., Castranova, V. and Gulumian, M., 2009. The limits of testing particle-mediated oxidative stress in vitro in predicting diverse pathologies; relevance for testing of nanoparticles. *Particle and Fibre Toxicology*, 6(1), p.13.
- Downward, G.S., van Nunen, E.J., Kerckhoffs, J., Vineis, P., Brunekreef, B., Boer, J.M., Messier, K.P., Roy, A., Verschuren, W.M.M., van der Schouw, Y.T. and Sluijs, I., 2018. Long-term exposure to ultrafine particles and incidence of cardiovascular and cerebrovascular disease in a prospective study of a Dutch cohort. *Environmental Health Perspectives*, 126(12), p.127007.
- Gasser, M., Riediker, M., Mueller, L., Perrenoud, A., Blank, F., Gehr, P. and Rothen-Rutishauser, B., 2009. Toxic effects of brake wear particles on epithelial lung cells in vitro. *Particle and Fibre Toxicology*, 6(1), p.30.
- Gebel, T., 2012. Small difference in carcinogenic potency between GBP nanomaterials and GBP micromaterials. *Archives of Toxicology*, 86(7), pp.995-1007.
- Gilmour, M.I., O'Connor, S., Dick, C.A., Miller, C.A. and Linak, W.P., 2004. Differential pulmonary inflammation and in vitro cytotoxicity of size-fractionated fly ash particles from pulverized coal combustion. *Journal of the Air & Waste Management Association*, 54(3), pp.286-295.
- Gong Jr[deceased], H., Linn, W.S., Clark, K.W., Anderson, K.R., Sioutas, C., Alexis, N.E., Cascio, W.E. and Devlin, R.B., 2008. Exposures of healthy and asthmatic volunteers to concentrated ambient ultrafine particles in Los Angeles. *Inhalation Toxicology*, 20(6), pp.533-545.
- Habre, R., Zhou, H., Eckel, S.P., Enebish, T., Fruin, S., Bastain, T., Rappaport, E. and Gilliland, F., 2018. Short-term effects of airport-associated ultrafine particle exposure on lung function and inflammation in adults with asthma. *Environment International*, 118, pp.48-59.
- HEI, 2013. Understanding the Health Effects of Ambient Ultrafine Particles. Health Effects Institute.
- Hettland, R.B., Cassee, F.R., Låg, M., Refsnes, M., Dybing, E. and Schwarze, P.E., 2005. Cytokine release from alveolar macrophages exposed to ambient particulate matter: heterogeneity in relation to size, city and season. *Particle and Fibre Toxicology*, 2(1), p.4.
- Jalava, P.I., Salonen, R.O., Pennanen, A.S., Sillanpää, M., Hälinen, A.I., Happonen, M.S., Hillamo, R., Brunekreef, B., Katsouyanni, K., Sunyer, J. and Hirvonen, M.R., 2007. Heterogeneities in inflammatory and cytotoxic responses of RAW 264.7 macrophage cell line to urban air coarse, fine, and ultrafine particles from six European sampling campaigns. *Inhalation Toxicology*, 19(3), pp.213-225.
- Karotki, D.G., Bekö, G., Clausen, G., Madsen, A.M., Andersen, Z.J., Massling, A., Ketzel, M., Ellermann, T., Lund, R., Sigsgaard, T. and Möller, P., 2014. Cardiovascular and lung function in relation to outdoor and indoor exposure to fine and ultrafine particulate matter in middle-aged subjects. *Environment International*, 73, pp.372-381.
- Karotki, D., Spilak, M., Frederiksen, M., Jovanovic Andersen, Z., Madsen, A., Ketzel, M., Massling, A., Gunnarsen, L., Möller, P. and Loft, S., 2015. Indoor and outdoor exposure to ultrafine, fine and microbiologically derived particulate matter related to cardiovascular and respiratory effects in a panel of elderly urban citizens. *International Journal of Environmental Research and Public Health*, 12(2), pp.1667-1686.

- Kreyling, W.G., Semmler-Behnke, M. and Möller, W., 2006. Ultrafine particle–lung interactions: does size matter? *Journal of Aerosol Medicine*, 19(1), pp.74-83.
- Langrish, J.P., Li, X., Wang, S., Lee, M.M., Barnes, G.D., Miller, M.R., Cassee, F.R., Boon, N.A., Donaldson, K., Li, J. and Li, L., 2012. Reducing personal exposure to particulate air pollution improves cardiovascular health in patients with coronary heart disease. *Environmental Health Perspectives*, 120(3), pp.367-372.
- Li, Q., Yi, Q., Tang, L., Luo, S., Tang, Y., Zhang, G. and Luo, Z., 2019. Influence of Ultrafine Particles Exposure on Asthma Exacerbation in Children: A Meta-Analysis. *Current Drug Targets*, 20(4), pp.412-420.
- Li, N., Sioutas, C., Cho, A., Schmitz, D., Misra, C., Sempf, J., Wang, M., Oberley, T., Froines, J. and Nel, A., 2003. Ultrafine particulate pollutants induce oxidative stress and mitochondrial damage. *Environmental Health Perspectives*, 111(4), pp.455-460.
- Magalhaes, S., Baumgartner, J. and Weichenthal, S., 2018. Impacts of exposure to black carbon, elemental carbon, and ultrafine particles from indoor and outdoor sources on blood pressure in adults: A review of epidemiological evidence. *Environmental Research*, 161, pp.345-353.
- Maynard, A.D. and Kuempel, E.D., 2005. Airborne nanostructured particles and occupational health. *Journal of nanoparticle research*, 7(6), pp.587-614.
- Mills, N.L., Robinson, S.D., Fokkens, P.H., Leseman, D.L., Miller, M.R., Anderson, D., Freney, E.J., Heal, M.R., Donovan, R.J., Blomberg, A. and Sandström, T., 2008. Exposure to concentrated ambient particles does not affect vascular function in patients with coronary heart disease. *Environmental Health Perspectives*, 116(6), pp.709-715.
- Möller, W., Felten, K., Meyer, G., Meyer, P., Seitz, J. and Kreyling, W.G., 2009. Corrections in dose assessment of 99mTc radiolabeled aerosol particles targeted to central human airways using planar gamma camera imaging. *Journal of Aerosol Medicine and Pulmonary Drug Delivery*, 22(1), pp.45-54.
- Möller, W., Felten, K., Sommerer, K., Scheuch, G., Meyer, G., Meyer, P., Haussinger, K. and Kreyling, W.G., 2008. Deposition, retention, and translocation of ultrafine particles from the central airways and lung periphery. *American Journal of Respiratory and Critical Care Medicine*, 177(4), pp.426-432.
- Morawska, L., Ristovski, Z., Jayaratne, E.R., Keogh, D.U. and Ling, X., 2008. Ambient nano and ultrafine particles from motor vehicle emissions: Characteristics, ambient processing and implications on human exposure. *Atmospheric Environment*, 42(35), pp.8113-8138.
- Oberdörster, G., Oberdörster, E. and Oberdörster, J., 2005. Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. *Environmental Health Perspectives*, 113(7), pp.823-839.
- Ohlwein, S., Kappeler, R., Joss, M.K., Künzli, N. and Hoffmann, B., 2019. Health effects of ultrafine particles: a systematic literature review update of epidemiological evidence. *International Journal of Public Health*, 64(4), pp.547-559.
- Olsen, Y., Karotki, D.G., Jensen, D.M., Bekö, G., Kjeldsen, B.U., Clausen, G., Hersoug, L.G., Holst, G.J., Wierzbicka, A., Sigsgaard, T. and Linneberg, A., 2014. Vascular and lung function related to ultrafine and fine particles exposure assessed by personal and indoor monitoring: a cross-sectional study. *Environmental Health*, 13(1), p.112.
- Oberdörster G, Maynard A, Donaldson K, Castranova V, Fitzpatrick J, Ausman K, Carter J, Karn B, Kreyling W, Lai D, Olin S. Principles for characterizing the potential human health effects from exposure to nanomaterials: elements of a screening strategy. *Particle and fibre toxicology*. 2005 Dec;2(1):8.
- Øvreik, J., Refsnes, M., Låg, M., Holme, J. and Schwarze, P., 2015. Activation of proinflammatory responses in cells of the airway mucosa by particulate matter: oxidant- and non-oxidant-mediated triggering mechanisms. *Biomolecules*, 5(3), pp.1399-1440.
- Peters, A., Wichmann, H.E., Tuch, T., Heinrich, J. and Heyder, J., 1997. Respiratory effects are associated with the number of ultrafine particles. *American Journal of Respiratory and Critical Care Medicine*, 155(4), pp.1376-1383.
- Plummer, L.E., Ham, W., Kleeman, M.J., Wexler, A., Pinkerton, K.E., 2012. Influence of season and location on pulmonary response to California's San Joaquin Valley airborne particulate matter. *Journal of Toxically Environmental Health A*, 75 (5): 253-271.
- Samet, J.M., Rappold, A., Graff, D., Cascio, W.E., Berntsen, J.H., Huang, Y.C.T., Herbst, M., Bassett, M., Montilla, T., Hazucha, M.J. and Bromberg, P.A., 2009. Concentrated ambient ultrafine particle exposure induces cardiac changes in young healthy volunteers. *American Journal of Respiratory and Critical Care Medicine*, 179(11), pp.1034-1042.
- Sauvain, J.J., Rossi, M.J. and Riediker, M., 2013. Comparison of three acellular tests for assessing the oxidation potential of nanomaterials. *Aerosol Science and Technology*, 47(2), pp.218-227.
- Setyan, A., Sauvain, J.J., Guillemain, M., Riediker, M., Demirdjian, B. and Rossi, M.J., 2010. Probing functional groups at the gas–aerosol interface using heterogeneous titration reactions: a tool for predicting aerosol health effects?. *ChemPhysChem*, 11(18), pp.3823-3835.
- Singh, P., DeMarini, D.M., Dick, C.A., Tabor, D.G., Ryan, J.V., Linak, W.P., Kobayashi, T. and Gilmour, M.I., 2004. Sample characterization of automobile and forklift diesel exhaust particles and comparative pulmonary toxicity in mice. *Environmental Health Perspectives*, 112(8), pp.820-825.
- Sinharay, R., Gong, J., Barratt, B., Ohman-Strickland, P., Ernst, S., Kelly, F.J., Zhang, J.J., Collins, P., Cullinan, P. and Chung, K.F., 2018. Respiratory and cardiovascular responses to walking down a traffic-polluted road compared with walking in a traffic-free area in participants aged 60 years and older with chronic lung or heart disease and age-matched healthy controls: a randomised, crossover study. *The Lancet*, 391(10118), pp.339-349.
- Skovmand, A., Jensen, A.C., Maurice, C., Marchetti, F., Lauvås, A.J., Koponen, I.K., Jensen, K.A., Goericke-Pesch, S., Vogel, U. and Hougaard, K.S., 2019. Effects of maternal inhalation of carbon black nanoparticles on reproductive and fertility parameters in a four-generation study of male mice. *Particle and Fibre Toxicology*, 16(1), p.13.
- Steenhof, M., Gosens, I., Strak, M., Godri, K.J., Hoek, G., Cassee, F.R., Mudway, I.S., Kelly, F.J., Harrison, R.M., Lebret, E. and Brunekreef, B., 2011. In vitro toxicity of particulate matter (PM) collected at different sites in the Netherlands is associated with PM composition, size fraction and oxidative potential-the RAPTES project. *Particle and Fibre Toxicology*, 8(1), p.26.
- Steenenbergh, P.A., van Amelsvoort, L., Lovik, M., Hetland, R.B., Alberg, T., Halatek, T., Bloemen, H.J., Rydzynski, K., Swaen, G., Schwarze, P. and Dybing, E., 2006. Relation between sources of particulate air pollution and biological effect parameters in samples from four European cities: an exploratory study. *Inhalation Toxicology*, 18(5), pp.333-346.
- Stöeger, T., Reinhard, C., Takenaka, S., Schroeppel, A., Karg, E., Ritter, B., Heyder, J. and Schulz, H., 2005. Instillation of six different ultrafine carbon particles indicates a surface area threshold dose for acute lung inflammation in mice. *Environmental Health Perspectives*, 114(3), pp.328-333.
- Stone, V., Miller, M.R., Clift, M.J., Elder, A., Mills, N.L., Möller, P., Schins, R.P., Vogel, U., Kreyling, W.G., Alstrup Jensen, K. and Kuhlbusch, T.A., 2017. Nanomaterials versus ambient ultrafine particles: an opportunity to exchange toxicology knowledge. *Environmental Health Perspectives*, 125(10), p.106002.
- Schwarze, P.E., Øvreik, J., Hetland, R.B., Becher, R., Cassee, F.R., Låg, M., Løvik, M., Dybing, E. and Refsnes, M., 2007. Importance of size and composition of particles for effects on cells in vitro. *Inhalation Toxicology*, 19(sup1), pp.17-22.
- Tal, T.L., Simmons, S.O., Silbajoris, R., Dailey, L., Cho, S.H., Ramabhadran, R., Linak, W., Reed, W., Bromberg, P.A. and Samet, J.M., 2010. Differential transcriptional regulation of IL-8 expression by human airway epithelial cells exposed to diesel exhaust particles. *Toxicology and Applied Pharmacology*, 243(1), pp.46-54.
- Totlandsdal, A.I., Låg, M., Lilleaas, E., Cassee, F. and Schwarze, P., 2015. Differential proinflammatory responses induced by diesel exhaust particles with contrasting PAH and metal content. *Environmental Toxicology*, 30(2), pp.188-196.
- Vinzents, P.S., Möller, P., Sørensen, M., Knudsen, L.E., Hertel, O., Jensen, F.P., Schibye, B. and Loft, S., 2005. Personal exposure to ultrafine particles and oxidative DNA damage. *Environmental Health Perspectives*, 113(11), pp.1485-1490.
- Weichenthal, S., Hatzopoulou, M. and Goldberg, M.S., 2014. Exposure to traffic-related air pollution during physical activity and acute changes in blood pressure, autonomic and micro-vascular function in women: a cross-over study. *Particle and Fibre Toxicology*, 11(1), p.70.
- WHO, 2013. Review of evidence on health aspects of air pollution – REVIHAAP. Copenhagen.