UMWELT & GESUNDHEIT
05/2018

Health Effects of Ultrafine Particles

Systematic literature search and the potential transferability of the results to the German setting

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Umwelt Bundesamt

UMWELT & GESUNDHEIT 05/2018

Project No. 003777 7205 UKAGEP

Health Effects of Ultrafine Particles

Systematic literature search and the potential transferability of the results to the German setting

by

Simone Ohlwein MPH, Prof. Barbara Hoffmann MD et MPH
Institute for Occupational, Social and Environmental Medicine,
University hospital Düsseldorf

Ron Kappeler MSc Med. Sci. Techn., Meltem Kutlar Joss MSc ETH Environmental Sc. et MPH, Prof. Nino Künzli MD et PhD
Swiss Tropical and Public Health Institute, Switzerland

On behalf of the German Environmental Agency and
the Swiss Federal Office for the Environment

Financial support was provided by the Federal Ministry of Education and Research (Grant 01KX1460).

Imprint

Publisher:
Umweltbundesamt
Wörlitzer Platz 1
06844 Dessau-Roßlau
Tel: +49 340-2103-0
Fax: +49 340-2103-2285
info@umweltbundesamt.de
Internet: www.umweltbundesamt.de

/umweltbundesamt.de
/umweltbundesamt

Study performed by:
Institute for Occupational, Social and Environmental Medicine.
Universitätsklinikum Düsseldorf | AG Umweltepidemiologie |
PO box 101007
40001 Düsseldorf

Study completed in:
February 2018

Edited by:
Section II 1.6 Exposure Assessment and Environmental Health Indicators
Myriam Tobollik

Publication as pdf:
http://www.umweltbundesamt.de/publikationen

ISSN 1862-4340

Dessau-Roßlau, October 2018

The responsibility for the content of this publication lies with the author(s).

Abstract

Background/Aim: Due to their small size, the specific health effects of UFPs are related to their physical capacity to penetrate through the blood system, thenervous system, brain and diverse organs. Five years ago scientific evidence pointed towards adverse effects of UFPs on health. Since then, numerous studies have been published. Therefore, the aims of this project were to review the literature on the effects of UFPs on health, to evaluate the selected studies and to assess the transferability of the results to the situation in Germany.

Methods: We systematically searched MEDLINE (Medical Literature Analysis and Retrieval System Online) for eligible studies published between 01.01.2011 until 11.5.2017 investigating health effects of AP related UFPs. In addition, we searched the LUDOK (Dokumentationsstelle Luftverschmutzung und Gesundheit)-database, provided by the Swiss Tropical and Public Health institute. We included epidemiologic studies with adequate study designs, containing an UFP measure, quantifiable measures of associations and a health outcome.

Results: Upon application of our search strategy, 85 references of original articles were identified for further evaluation. Most of included studies were conducted in North America (n=37) or Western Europe (n=27), investigating short-term effects (n=75). The short-term studies are dominated by panel studies (n=32), scripted exposure studies (n=16), and time-series studies (n=11). Ten studies investigated long-term associations using exposure estimates averaged over a period of months to years. Long-term studies most frequently applied cohort (n=4) and cross-sectional (n=4) study designs.

Conclusion: The evidence on health effects remains inconclusive or insufficient for most of the studied outcomes. Specifically, while a number of studies have investigated mortality and emergency department/hospital admission outcomes, the relatively few studies with co-pollutant adjustment reveal mixed and, up to now, inconclusive evidence. In terms of number of studies, most evidence is available from studies investigating subclinical outcomes. Within this group of studies, cardiovascular outcomes and outcomes of pulmonary and systemic inflammation show the most consistent patterns with associations generally pointing into the direction of the adverse health outcome. A future challenge is the development of enhanced spatiotemporal models which can contribute to a more precise exposure assessment across larger areas as well as incorporating multipollutant models to become clear of independent effects.

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List of Abbreviations

8-OHdG	8-hydroxy-2' –deoxyguanosine (biomarker of oxidative stress)
AccMP	Accumulation mode particles
AitMP	Aitken-mode particles
AAP	Ambient Air Pollution
BAFU	Schweizerisches Bundesamt für Umwelt
BC	Black Carbon
BDNF	brain-derieved neurotropic factor
BP	Blood Pressure
BREATHE	Brain Development and Air Pollution Ultrafine Particles in School Children
CAFEH	eine Kohorte, finde aber nichts im Internet (im Text auf Seite 53 oben)
CO	Carbon monoxide
COPD	Chronic obstructive pulmonary disease
CRP	C reactive protein
CTM	Chemistry-Transport-Model
CV	Cardiovascular
DBP	Diastolic Blood Pressure
DBP	diastolic blood pressure
DOI	Digital Object Identifier
EBC	Exhaled breath condensate
EC	Elemental Carbon
EHP	Environmental Health Perspectives
ETH	Eidgenössische Technische Hochschule Zürich
EURAD	EURopean Air Pollution Dispersion
FEF	Forced expiratory flow
FeNO	fractional exhaled nitric oxide
FEV_1	Forced Expiratory Volume in 1 second
FVC	Forced vital capacity
GUAN	German Ultrafine Aerosol Network
h	hour
HDL	High-Density-Lipoprotein
HEI	Health Effects Institute
HNR	Heinz Nixdorf Recall Study
HR	Heart Rate
HRAPIE	Health risks of air pollution in Europe

HRV	heart rate variability
ICAM-1	Intercellular Adhesion Molecule 1
ICD	International Classification of Diseases
IGT	Impaired glucose tolerance
ijerph	International Journal of Environmental Research and Public Health
IL	Interleukin
IQR	Interquartile range
ISA	Integrated Science Assessment
LBW	low birth weight
LDSA	Lung-deposited surface area
LUDOK	Dokumentationsstelle Luftverschmutzung und Gesundheit
ma	moving averages
MDA	malondialdehyde
MEDLINE	Medical Literature Analysis and Retrieval System Online
MI	Myocardial Infarction
NANOAPP	Nanomaterials & Applications
NIH	National Heart, Lung and Blood Institute of the National Institute of Health
nm	nucleation mode
NO_x	Nitrogen oxides
NO_2	Nitrogen dioxide
NO_x	nitrogen oxide
NSTEMI	Non-ST-Elevated Myocardial Infarction
NucMP	Nucleation mode particles
O_3	Ozone
OR	Odds Ratio
PAC	Particle Area Concentrations
PM	Particulate Matter
PNC	Particle Number Concentrations
PP	Pulse Pressure
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PVC	Particle volume concentrations
REVIHAAP	Review of evidence on health aspects of air pollution
RHI	Reactive Hyperemia Index
RUPIOH	Relationship between Ultrafine and fine Particulate matter in Indoor and Outdoor air and respiratory Health
SAPALDIA	Swiss study on Air Pollution And Lung Disease in Adults

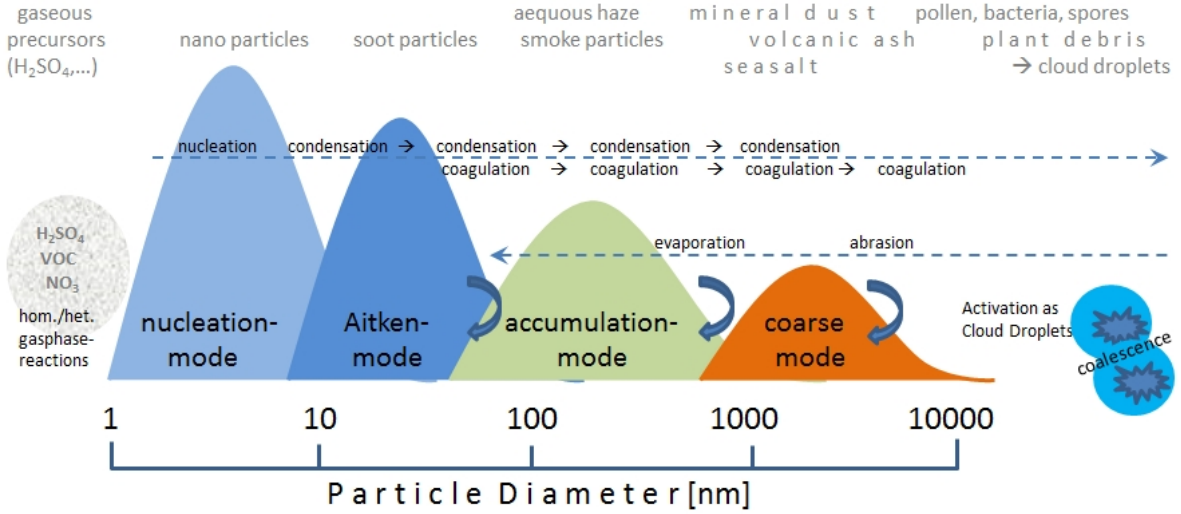
SBP	Systolic Blood Pressure
SO_2	Sulphur Dioxide
SOA	Secondary Organic Aerosols
STEMI	ST-elevation myocardial infarction
Swiss TPH	Schweizerische Tropen- und Public Health-Institut
T2DM	Type 2 Diabetes Mellitus
TNFRII	Tumour Necrosis Factor Type II
TNR	Gene that encodes the protein Tenascin-R
TU	Technische Universität
UFIPOLNET	Ultrafine particle size distributions in air pollution monitoring networks
UFIREG	Ultrafine Particles - an evidence based contribution to the development of regional and European environmental and health policy
UFPs	Ultrafine Particles
UKD	Universitätsklinikum Düsseldorf
US EPA	United States Environmental Protection Agency
VCAM	Vascular Cell Adhesion Molecule
VOCs	Volatile Organic Compounds
VT	Ventricular Tachycardia
WHO	World Health Organization
WP	Work package

Summary

Background

Ultrafine particles (UFPs) represent the smallest size fractions of air pollutants measured from a nanometer to few micrometers. By convention, UFPs are defined as particles not exceeding an aerodynamic diameter of 100 nm. Measurement procedures mostly assess particle number per ml since UFPs contribute only little to the particle mass of ambient air. Further size fractions used in epidemiological research are nucleation mode particles (precursor substances sized up to 20 nm), Aitken-mode particles (condensation particles sized 10 - 80 nm), and accumulation mode particles (condensation and coagulation particles sized 50 - 1,000 nm) covering different particle fractions.

Figure I: Size-fractions of airborne particles (Source: Deutscher Wetterdienst, 2018)



UFPs vary with regard to their chemical composition and physical reactivity. They are emitted directly or are formed from precursors in atmospherical processes. In urban areas, a great proportion of UFPs originate from combustion processes of motorized vehicles (Health Effects Insitute, 2013; Kelly & Fussell, 2012).

The specific health effects of UFPs are related to their physical capacity to penetrate through diverse organ systems (i.e., blood system, nervous system, brain, organs) due to their small size. Hypothesized health effects of UFP include cardiovascular and respiratory morbidity and mortality, the elicitation of local pulmonary and systemic inflammation and oxidative stress, and adverse actions on the brain and the metabolism.

In contrast to other air pollutants, there are no regulations on UFP exposure concentrations. The expert commission of the HEI and the WHO concluded five years ago that scientific studies point towards adverse effects of UFPs on health. However, the evidence base on epidemiologic studies was not sufficient to recommend regulations on UFP exposure concentrations. This report aims to reevaluate the evidence base on the health effects of UFPs.

Aims of the project

The aims of this project were to systematically review the literature systematically on the effects of UFPs on health, to evaluate the selected studies and to assess the transferability of the results to the situation in Germany. For this purpose, we focus on the following objectives:

1. Conducting a systematic literature review

   - Focus on health effects associated with ultrafine particles
   - Emphasis on epidemiologic studies and quantitative effect measures (e.g., relative risks, dose-response relationships)
   - Documentation of the literature search results and storage of all considered articles using a literature management database (EndNote).

2. Evaluation of the identified literature

   - Evaluation of individual study quality based on defined criteria
   - Evaluation of the transferability of the identified findings to the present conditions in Germany

3. Evaluation of the health relevance of ultrafine particles, specifically:

   - Within the context of other AAP exposures (e.g., PM_{10}, $PM_{2.5}$, ozone, nitrogen dioxide)
   - With regard to the current German situation
   - When considering the projected trajectory of ultrafine particle exposure in Germany.

Methods

We systematically searched MEDLINE (Medical Literature Analysis and Retrieval System Online) for eligible studies investigating health effects of AAP related UFPs. The period included in the search was 1.1.2011 until 11.5.2017. In addition, we searched the LUDOK (Dokumentationsstelle Luftverschmutzung und Gesundheit)-database, which is provided by the Swiss Tropical and Public Health institute (Swiss TPH). This database contains scientific literature on the effects of AAP on human health.

The focus of the systematic search was on epidemiologic studies that explore health effects of UFPs including quantitative effect measures *(work package 1 (a) research literature systematically in terms of health effects of UFP and (b) focusing epidemiologic studies and quantitative effect measures (e.g., relative risk, dose-response-functions))*.

Another selection critera was the use of one UFP-measure (particle numbers (PNC) for particles with a diameter of less than 100nm, $PM_{0.1}$, nucleation mode particles, Aitken-mode particles as well as quasi-UFPs-measures: PNC for particles with a maximum diameter of > 100 nm, $PM_{0.25}$, surface area concentrations and accumulation mode particles. Health outcomes were required to include mortality, morbidity, emergency/hospital admissions or subclinical outcomes.

Toxicological studies were assessed only with regard to supporting evidence of the evaluation of UFP-related health relevance as stated in work package 3. Studies which investigate population related exposure to UFPs were assessed in order to evaluate the transferability of the reviewed results to the situation in Germany (work package 2b) and to evaluate the health related relevance of UFPs with regard to the situation in Germany (work package 3b) and in consideration of the potential trends of UFP exposure in Germany (work package 3c).

Search Strategy

The last comprehensive review was performed by the HEI including a systematic literature research in MEDLINE and Web of science up to Mai 2011 (Annex 1, part 1). Within our project, we replicated their search strategy and discussed specific issues on the search strategy. We set the starting time of our

search half a year earlier than the end poind of the search period of HEI in order to assess publications which may not have been indexed yet during the search period of the HEI.

The search strategy of the LUDOK database includes epidemiological and experimental original works studying the effects of „classical"/traditional ambient air particles on humans, as well as effects of further air pollutants (Annex 1, part 2). The search is conducted monthly using a constant, very broad search strategy in PubMed. The LUDOK search is complemented by hand search in more than 20 relevant journals, reference lists of publications and other sources. The search strategy within this project consisted of a modified HEI search strategy, completed by a search in LUDOK and hand searches. The keywords were extended in comparison to the HEI search keywords, following the very general search strategy of the LUDOK database. An alternative search strategy was applied using specific disease related keywords instead of the general keywords "health" and "epidemiology/ic/ical".

Further hand searches considered reviews of the last six years as well as reviews identified by our search. Finally, published abstract bands from the relevant conferences and symposia were searched as well es publications by authors identified by our search.

Study Selection

Two reviewers screened title, abstracts and – if needed – full texts of the studies with regard to the inclusion and exclusion criteria (see below). 10 % of the studies were screened by both reviewers. In case of uncertainties concerning the selection of a study the case was discussed by the whole team. If necessary, inclusion and exclusion criteria were clarified and extended. The process of the study selection is illustrated in a Flowchart (Annex I, part 4) and documented in a chart adapted to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) method (Figure 4).

All references were organized within a library of a reference management program "Endnote" providing access for all project members (Figure 3).

Inclusion criteria

- Epidemiologic studies with an adequate study design, i.e.: cohort, case-control, cross-sectional, case-crossover, panel-studies, scripted exposures, time-series studies.
- Quantifiable measures of association containing at least one UFP measure/metric: Number (PNC) or size-fractioned PNC for particles < 100 nm, $PM_{0.1,}$ nucleation-mode particles (NucMP) and Aitken-mode particles (AitMP) or containing at least one quasi-UFP effect measures: PNC < 3000, $PM_{0.25}$, $PM_{0.1}$, surface-area concentration or accumulation mode particles (AccMP).
- Quantifiable measures of association including at least one measure: Odds ratio, relative risk, hazard ratio, β-estimates of percent change or exposure-response functions.
- Health outcomes including mortality or ICD-coded diseases, symptoms, emergency/hospital admissions/visits, preclinical outcomes.
- Languages: English, German.
- Year: Studies published from 2011 onward until 11.5.2017 which were not included in the HEI review; studies published after the deadline are listed in the appendix (annex I, part 5)

Exclusion criteria

- Toxicological studies, controlled exposure studies, animal experiments, in-vitro studies,
- Exposure to industrially engineered nanoparticles,
- Exposure to nanoparticles/ UFPs in occupational settings,
- Exposure to source-related indoor nanoparticles/ UFPs,
- Exposure to diesel particles, BC or EC only,
- Distance measures in substitution of exposure measurements
- Health outcomes of unclear health relevance, e.g. epigenetics, metabolomics, methylation.

Data extraction

The identified articles were evaluated concerning their quality of report, significance and contents as well as their transferability to the German context. The established quality criteria (Annex II, Tables A5a-f) are adapted from the Quality Assessment Tools of the National Heart, Lung and Blood Institute of the National Institute of Health (2014). When developing the different criteria, special attention was paid to exposure assessment. In particular, criteria to evaluate the applied measurement devices, the representativeness of the measurement sites for the exposure of the target population, the validity of potentially used exposure models and for the assessment/modeling of several air pollutants.

Results

Literature search

The application of the main search strategy in MEDLINE yielded 1,114 references, the application of the alternative outcome-specific MEDLINE search strategy yielded 992 references, of which 332 were not included in the main search strategy (Figure 4). Together, the MEDLINE search yielded 1,446 references. The search in the LUDOK database yielded 106 references, of which 30 were additional to the MEDLINE search. Another 8 additional references were identified through hand search in other sources, yielding an overall total of 1,484 unique references that were examined for in- and exclusion criteria.

The final number of 85 original references included in this systematic review was achieved from the following sources: Of the 1,114 unique references identified by the main MEDLINE search strategy, 70 references were included in the analysis. Of the 332 unique references identified by the alternative outcome-specific MEDLINE search strategy, 3 additional references were identified for the review.

Of the 106 LUDOK references, 8 relevant studies were identified additionally. Of the 8 studies identified through hand search, 4 studies were added to the final analysis database.

In a repeated search on 23.02.2018, limited to articles published or accepted after the closing date of the full search, we identified another 13 articles, which are listed in the appendix (annex I, part 5).

Evidence base from previous reviews

Our literature research and knowledge draws upon some relevant reviews published recently. At first, the HEI provides the most thorough and complete information on a possible relationship between UFPs and various health effects. The body of research was rated as suggestive but not definitive on the adverse health effects of UFPs on respiratory and cardiovascular outcomes. Reasons for the lack of clarity were (1) inconsistencies of outcomes and methodological aspects of the study designs, (2) inconsistent and possibly biased exposure assessments and (3) a lack of studies adjusting for co-pollutants. On top of those issues, HEI couldn't find any studies on long-term exposure effects of UFPs. Therefore the evidence base in 2013 on epidemiologic studies was not sufficient to recommend regulations on UFP exposure concentrations.

In February 2015 the United States Environmental Protection Agency invited experts from around the world to discuss and present evidence of health effects associated with UFP exposure, which has been summarized in 2016 (Baldauf et al., 2016). According to that workshop, short-term epidemiological studies provided evidence that exposure to traffic pollution (rich in UFPs) was associated with adverse cardiovascular outcomes, however, the effects still couldn't be reliably disentangled from other PM fractions or other gaseous pollutants. Similar to HEI's conclusion, epidemiological studies did not provide enough evidence that UFPs are more potent than other PM size fractions. Nevertheless,

toxicological concerns about health effects of UFPs suggested that particle size may need to be considered in assessing potential adverse effects of exposures to PM.

Chen et al. (2016) thoroughly reviewed articles on composition of UFPs, their sources, typical characters, oxidative effects and potential exposure routes with a main focus on toxicology. Furthermore they also considered evidences emerging from nanotoxicology, as this research field contributes to the understanding of toxicity mechanisms of airborne UFPs in AAP. They concluded that UFPs play a major role in adverse impacts on human health.

An American working group (Li et al. 2016) reevaluated the conclusions made by the HEI report by assessing experimental, epidemiological and clinical trial studies published in 2014 and 2015. The authors mentioned a critical knowledge gap in clearly identifying the impact of exposure to the nano-scale pollutants on human health. However, due to new evidence, especially from experimental and toxicological studies, they questioned the validity of HEI's conclusion that there is no evidence that the adverse health effects of UFP were dramatically different from those of $PM_{2.5}$. Nevertheless, the issues of epidemiological studies assessing health effects of UFPs reported by the HEI Panel still remain.

Heinzerling et al. (2016), examining respiratory health effects of UFPs in children, identified 12 relevant articles from which 4 are not included in HEI. In single pollutant models, exposure to UFPs were associated with incident wheezing, current asthma, lung function and emergency department visits due to exacerbation of asthma. Only one study that reported significant association between asthma emergency department visits and UFPs, also adjusted for co-pollutants (Halonen et al., 2008). In this study, the association was no longer significant after adjusting for NO_2 exposure. Even though the evidence between UFPs and children's respiratory health is accumulating, the authors concluded for the same reasons stated by the HEI Panel that the evidence remains inconclusive.

In addition, Clark et al. published in 2016 a study focusing on biological mechanisms of cardiovascular effects beyond the alveolar barrier within the body or in vitro tissues exposed to UFPs and quasi-UFPs of up to 500 nm size. They concluded that there is some (e.g. altered autonomic modulation with increases of heart rate in animal models) up to strong evidence (e.g. vasoconstriction induced by endothelium-dependent and independent pathways mediated through UFPs) for various cardiovascular outcomes (heart rate, vasoactivity, atherosclerotic advancement, oxidative stress, coagulability, inflammatory changes).

Study characteristics

Most of included studies (n=85) were conducted in North America (n=37) or Western Europe (n=27). Further 12 studies took place in the Western-Pacific region. Only very few studies were conducted in Middle/ South America (n=1), Eastern Europe (n=2) and South-East-Asia (n=1). Three out of five multi-center studies included studies conducted in several Western Europe countries (Karakatsani et al., 2012; Manney et al., 2012; Samoli, Andersen, et al., 2016), two multi-center studies included study sites located both in Western and Eastern Europe countries (Lanzinger et al., 2016a, 2016b).

The majority of the studies were related to the investigation of short-term effects (n=75) measuring outcomes during hours to weeks after exposure. Ten studies investigated long-term associations using exposure estimates averaged over a period of months to years. The studies with a long-term study design consisted of cohort studies (n=4), cross-sectional studies (n=4), one case-cohort and case-control study, respectively (Table I). Short-term studies are dominated by panel studies - 31 as repeated measures and one in a cross-sectional design, scripted exposure studies (n=16), and time-series studies (n=11). Further studies investigating short-term associations were case-crossover (n=8), cohort (n=4) and cross-sectional studies (n=4).

Table I: Study design by long-term/ short-term studies

Design	Number of studies	%
Long-term	all=10	
Case-cohort study	1	1.2%
Case-control study	1	1.2%
Cohort study	4	4.7%
Cross-sectional study	4	4.7%
Short-Term	all=75	
Cohort study	4	4.7%
Cross-Sectional study	4	4.7%
Panel (cross-sectional)	1	1.2%
Panel (repeated measure)	31	36.5%
Case-crossover	8	9.4%
Scripted exposure	16	18.8%
Time-series	11	12.9%
Total	**85**	**100.0%**

Overall, most studies used measurement-based **exposure assessments** (87.1%). Model based exposures were used in 10.6% of the studies. In long-term studies, mostly model-based exposure were used (9 out of 10), whereas the majority of short-term studies used measurement-based exposures (71 out of 75). This pattern is attributable to the fact, that model-based exposures are necessary to capture the spatial variation in exposure, which is the required exposure contrast for the assessment of long-term effects in different study design.

The majority of the studies applied central-site measurements (n=45), followed by mobile measurement techniques (n=17) and combination of different modeling/ measurements (n=10), e.g., central-site measurements in combination with spatio-temporal LUR models, residential measurements or microscale personal exposure models (Table II).

Table II: Type of exposure models/ measurements used in the studies

Exposure model/measurement	Number	N (%)
Chemical-transport model	3	3.5%
Land-use regression model	1	1.2%
Dispersion model	1	1.2%
Measurement: Central site	45	52.,9%
Measurement: Residential	2	2.4%
Measurement: Mobile	17	20.0%
Microscale personal exposure model	2	2.4%
Other	4	4.7%
Combination of different types	10	11.8%

Total	**85**	**100.0%**

In most studies, UFPs were assessed as particle number concentrations (PNCs) per volume. In about one third on the studies, PNCs sized up to 100 nm were used (29 out of 95[1]). In 66 studies, quasi-UFPs sized PNC fractions up to 3,000 nm were used. In relation to different size modes, only few studies used nucleation mode particles (n=1), representing particles with a diameter of less than 10 nm or Aitken-mode particles (n=1), representing particles with a diameter of 10-100 nm. In 14 studies, Accumulation mode particles were used, representing particles with a diameter of 100-1,000 nm[2] (see figure 1, p.13). Particles measured as mass per m^3 are used in 11 studies: In six studies, submicron $PM_{0.1}$ particles were assessed, in seven studies, quasi-UFP $PM_{0.25}$ or $PM_{0.1}$ particles were assessed. LDSA was only used in two studies.

Table III: Health outcome types of long-term and short-term-studies

	Number of studies	%
Long-term	All=10	
Mortality	1	1.1%
Morbidity	4	4.5%
Emergency/hospital call/admission	0	0.0%
Subclinical	5	5.7%
Short-term	All=78	
Mortality	7	8.0%
Morbidity	5	5.7%
Emergency/hospital call/admission	11	12.5%
Subclinical	55	62.5%
Total	88	100.0%

Eight studies assessing mortality analyzed the effects of UFPs on total, cardiovascular or respiratory mortality. Nine studies analyzed the effects on cardiovascular, respiratory, or other morbidity outcomes. Eleven studies investigated UFP effects on cardiovascular or respiratory disease-related emergency calls/ hospital admissions. The vast majority of used various subclinical measures as health outcomes. Three studies investigated several different types of main outcome types. Most studies measured cardiovascular organ system-related outcomes, followed by inflammatory and respiratory/atopy health outcomes. Few studies investigated total mortality, oxidative stress and other outcomes.

Quality indicators

In more than half of the studies (n=49), convenience samples were used. Six studies used random samples. Further seven studies used a combination of random and convenience samples. In 13 studies, study participants represented the general population terms of sociodemographic aspects. In most of

[1] As many studies used various size-fractioned PNCs, the number of analyses using PNCs with a size up to 100 nm (n=29) and/or up to 3,000 nm (n=66) exceed the number of 75 included studies that assessed PNCs.

[2] In literature, different cutpoints are used to divide particles in the different modi.

the included studies (n=62, 72.9%), the study population was a selected group, not representative for the general population. A sample size justification was rarely provided (n=3). Most of the study participants were recruited from the same populations and the same time period (n=71 and n=82).

The majority of the studies (n=66, 77.6%) reported the size-ranges of the measured UFPs. Almost all studies (n=79, 92.9%) reported the technical device used to measure the particles. Less than half (n=34) of the studies assessing other air pollutants (n=78) adjusted for co-pollutants within multi-pollutant-models. Studies without adjustment for co-pollutant were considered as "high risk of bias". 66 studies adjusted for meteorology, from which the majority (n=64) were short-term studies.

In all but one study (n=84) assigned exposure values were measured or modeled for time periods prior or parallel to the assessment of the outcome or for the time period of follow-up. In 5 of the included long-term studies, this was achieved by the use of chemical transport modeling, which allows the estimation of daily air pollutant concentrations for specific time periods. Furthermore, all but one study (n=84) defined and described the outcome measures clearly. In 68 of the studies, a blinding of the outcome assessors could be presumed. In 15 studies, no blinding was ensured.

Short-term health effects

In comparison to the prior evidence, seven additional studies have been conducted with overall mixed results. For all-cause mortality, only two out of four studies found positive estimates in analyses not adjusted for co-pollutants. Of these, only one study showed positive associations for quasi-ultrafine particles after adjustment for other pollutants, while in the other study, elevated point estimates decreased towards the null upon adjustment.

The evidence of respiratory mortality is also scarce and inconsistent. Out of the five studies on respiratory mortality, four studies found positive, though mostly non-significant associations for UFPs or quasi-UFPs. Three studies adjusted for co-pollutants, with opposite effects after NO_2 adjustment, leading either to an enhancement or to an attenuation of effect estimates after adjustment for NO_2. The studies presented two-pollutant associations only for those models/ lags/ size fractions showing the strongest associations. Thus, the specific effect estimates are difficult to compare and consistency of the results can't be fully assessed.

Similar to the overall results for respiratory mortality, associations of UFP/quasi-UFP with CV mortality are inconsistent. The six single exposure studies observe positive (three studies) as well as inverse associations (three studies) with CV mortality. In the two multi-pollutant studies, adjustment for NO_2 led to a decrease in effect estimates, causing the loss of significance in one study and a decrease to a significantly inverse relationship in the other study. Adjustment for $PM_{2.5}$ only caused small or no changes in the UFP estimate.

Evidence from this as well as from prior reviews suggests that effects may be larger in the warm season; therefore possible effect modification by season is an important factor to consider in future short-term effect studies. Moreover, the observed effects at least partially overlap with other air pollutant effects, most clearly seen for NO_2. Due to differences in investigated size fractions, no conclusions can be made about the most important fractions.

Table IV: Summary table of conducted analyses in the seven mortality studies

Study	All-Cause	Single pollutant associations	Multipollutant associations	Respiratory	Single pollutant associations	Multipollutant associations	Cardiovascular	Single pollutant associations	Multipollutant associations
Lanzinger et al. 2016a	✓	0	0	✓	(+)	+	✓	(-)	-

Leitte et al. 2012				✓	UFP: (+), quasi-UFP: +	UFP: 0 quasi-UFP: (+)			
Meng et al. 2013, (only quasi-UFP)	✓	+	+	✓	(+)	nc	✓	+	nc
Samoli et al. 2016	✓	0	0	✓	-	-	✓	(-)	nc
Stafoggia et al.,2017	✓	(+)	(-)	✓	+	nc	✓	(-)/(+)*	nc
Su et al. 2015							✓	+	(+)
Wolf et al. 2015							✓	(+)	nc

0 indicates no association. (+) and (-) indicates primarily non-significant associations, + and - indicate significant associations. Nc: not conducted. *varying across lags

Of the few studies investigating short-term effects of UFPs/quasi-UFPs on **morbidity** outcomes, only two studies observed significantly elevated estimates with a marker of perceived stress and with various symptoms. Since none of the above mentioned studies adjusted for co-pollutants or were by design able to disentangle the independent effects of different constituents of the air pollution mixture, we cannot conclude an independent effect of UFPs on morbidity outcomes. The evidence base for CV morbidity outcomes is scarce with only two studies available on different outcomes. This evidence suggests that participants with preexisting cardiovascular disease might be more susceptible to adverse associations with elevated UFP/quasi-UFP concentrations.

However, while both studies show generally positive associations, no inference on the independence on the reported UFPs effect can be made. The evidence for associations with short-term changes in mental health symptoms is insufficient.

The evidence base for UFP-related effects on utilization of the healthcare system due to respiratory symptoms is scarce (Tables A1c, A3c). Possible associations seem to be most probable for children as a susceptible subgroup. While single-pollutant associations were observed in few studies, multi-pollutant models of the studies could not verify independent associations of UFPs/quasi-UFPs with respiratory hospital admissions/emergency department visits. Specifically adjustment for NO_2 led to a decrease in estimates, which mostly reached the null in co-pollutant models.

Most studies investigating cardiovascular disease-related use of the healthcare system indicate weak associations being stronger for shorter time lags of up to 24 hours. These associations decreased upon adjustment for co-pollutants with no clear evidence for independent associations of UFPs/quasi-UFPs with cardiovascular emergency department visits/hospital admission.

Table V: Summary table of conducted analyses in the 11 studies on emergency department visits/hospital admissions

Study	Respiratory	Single pollutant associations	Multipollutant associations	Cardiovascular	Single pollutant associations	Multipollutant associations
Evans et al., 2014	✓	(+)	(+) (no NO_2 adjustment)			
Gardner et al., 2015				✓	(+)/0	nc
Iskandar et al., 2012	✓	(+)	0			
Rosenthal et al., 2013				✓	(+)/+	0
Wichmann et al., 2013				✓	(+)/0	nc
Delfino et al., 2014	✓	nr	nr			

Diaz-Robles et al., 2014	✓	+				
Lanzinger et al., 2016	✓	(+)	0	✓	(+)/0	0
Samoli UK, 2016	✓	(+)/(-)	(+)	✓	(+)	(-)/(+)
Samoli EU, 2016	✓	(+)/(-)	(-)/-			
Liu et al., 2013				✓	+/(+)	nc

0 indicates no association. (+) and (-) indicates primarily non-significant associations, + and - indicate significant associations. nc: not conducted, nr: not reported

An overview over **subclinical outcomes** is provided in table VI.

Most of the studies on **subclinical respiratory endpoints** have only limited sample sizes (15-84 participants). Moreover, study samples were frequently selective, either representing healthy young adults or persons suffering from atopy and/or asthma. The investigated lags and averaging periods differ across studies, but generally, most associations were found in a time range of 0-48 hours after increased exposure. Finally, results of the studies are mostly inconsistent in relation to the specific respiratory endpoints. With regard to peak-flow endpoints, measurement error could be an issue in this self-monitored endpoint, especially in the study by Cole-Hunter et al. (2013) which could not be blinded. Due to the lack of adjustment for co-pollutants, little can be concluded regarding the independence of effects. The scarce evidence on studies with co-pollutant adjustment suggests an at least partial overlap of UFP, respectively PNC effects, with NO_2-effects.

The majority of studies found adverse associations between exposure to UFP/quasi-UFP and **blood pressure indices**, indicating increases in BP. These results differed across different endpoints (SBP, DBP, PP), different size fractions and lag periods. Apart from one study with more than 1,000 participants, the studies consisted of smaller study populations. In addition, all study samples represented selected group, impeding a transfer to the general population. Apart from these limitations, the evidence from two-pollutant studies is too scarce to draw conclusions on independent UFP effects on blood pressure indices.

A relatively large body of evidence (16 studies) is available for **heart-rate variability** (HRV) **indices**, of which 12 showed UFP-related associations on at least on one HRV outcome. Upon adjustment for co-pollutant, associations changed in both directions. Across studies, different time-windows and different co-pollutants were examined, so that no clear pattern can be observed.

Considering the limited number of studies on **arrhythmia** outcomeswith only one study, the evidence base is still insufficient.

The majority of the seven studies examining associations between UFP/quasi-UFP and **vascular function** indicate a possible association. However, a lack of consistency regarding the study design, specifically the outcome parameters, as well as missing co-pollutant models do not allow overall conclusions.

All 12 studies which have been investigated UFP-effects on pulmonary inflammations suggest positive associations between UFP and adverse changes in the pulmonary inflammation marker, in particular immediately after exposure. Nevertheless, the evidence base for pulmonary inflammation in response to UFP is still limited as the studies used different subgroups, exposure metrics, outcome measures and time frames. The two studies that conducted two-pollutant models observed overall robust effect estimates.

The majority of the 18 studies investigating UFP effects on systemic inflammation markers indicate inconsistent associations. Effects of UFP on indices for hs-CRP, fibrinogen, blood cell counts, myeloperoxidase varied, which may originate from different compositions of participants, assessed PNC fractions and exposure assessment types. In most studies, effects seem to be most pronounced for

shorter lag periods. Only few multi-pollutant models do not allow statements on independent effects of UFPs/ quasi-UFPs, as only two of the five conducted studies with multi-pollutant models showed robust results.

Table VI: Summary table of conducted analyses in the 55 studies on subclinical outcomes

Outcome	Number of studies	Number of studies with single-pollutant--associations in expected direction	Number of studies with multi-pollutant associations in expected direction	Comments (i.e. studies with significant results in the non-expected direction)
Respiratory indices	11	4/11	3/3	Li et al. (2016) found significantly positive associations between UFP and FEV_1 & FVC
Blood pressure	13	9/13	2/4[3]	Two of the nine studies with associ. showed inconsistent results across lags
HRV	16	12/16	3/5	In Zhang et al. (2013), effect estimates decreased upon adj. for NO_2 and increased upon adj. for O_3
Arrhythmia	1	1/1	nc	Strong associations with $PM_{0.25}$, nearly protective associations between PN and hourly nighttime measured tachycardia
Vascular function	7	4/7	1/2	
Pulmonary inflammation	12	12/12	2/2	Most studies investigated effects on FeNO
Systemic inflammation (incl. fibrinogen)	18	7/18[4]	2/5	Significant inverse associations between fibrinogen & PNC upon adjustment for NO_2 (Strak et al., 2013)
Neurocognitive outcomes	2	1	nc	-

HRV: Heart rate variability, Nc: not conducted.

Long-term health effects

A limited number of studies, varying outcomes and exposure assessment methods as well as lacking co-pollutant adjustment do not allow to draw final conclusions. The summarized results are presented in table VII.

Table VII: Summary table of the 10 long-term studies in single and multipollutant associations.

Outcome type/ study		Outcome	Single pollutant associations	Multipollutant associations
Mortality/	Ostro et al. 2015	- all-cause - cardiovascular/ IHD - pulmonary	0 (+)/0 0	nc nc nc

[3] One of the four studies did not show assoc. in single-pollutant models, either. A further study (Rich et al., 2012) did not show all results, therefore rated as non-associated here

[4] Most positive associations relate to fibrinogen

Morbidity /	Li et al. 2017	- cardiometabolic	(+)	nc
	Laurent et al. 2014/2016b	- low birth weight	+/(+)	nc
	Laurent 2016a	- preterm birth	-/+	nc
Subclinical/				
	Aguilera et al. 2016	- carotid-intima-media thickness (PNC/LDSA)	+/+	-/(+)
	Viehmann et al. 2015	- hs-CRP/ fibrinogen/ WBC	(+)/+/(+)	nc
	Lane et al. 2015	- hs-CRP/ IL-6	(+)/(+)	nc
	Lane et al. 2016	- hs-CRP/ IL-6/ TNRFIII/ fibrinogen	(+)/(+)/(+)/(-)	nc
	Sunyer et al. 2016	- working memory,	(+)	nc
		- superior working memory	+	
		- inattentiveness	+	

IHD: Ischemic heart disease, 0 indicates no association. (+) and (-) indicates primarily non-significant associations, + and - indicate significant associations. Nc: not conducted.

Summary of short-term and long-term health effects

An overview on all included short-term and long-term studies reflects the inconsistency of the results (Table VIII). More than half (n=49) of the studies on short-term effects (n=79) reported at least one significant effect in the single pollutant model, especially those studying mortality or subclinical outcomes. For less than half of the single-pollutant associations (21 of 49), the general pattern of the association was consistent regardless of the significance level. 18 out of 32 studies found at least one significant association in multi-pollutant models. The associations in multi-pollutant studies remained consistent in about half of the studies (n=7).

Associations between health outcomes and long-term exposure with ultrafines were more consistent in the single pollutant models even though there were considerably fewer studies. Nevertheless, long-term studies adjusting for other pollutants are still lacking with only one study, which did not show effects in the multipollutant model.

Table VIII: Summary table of associations for all included studies.

Outcome	Single pollutant effect	Consistency of general pattern	Multipollutant effect	Consistency of general pattern
Short-term	49/79*	21/49	18/32	7/18
Mortality	5/7	2/5	4/6	1/4
Morbidity	3/7	0/3	-	-
Hospital admission	4/10	2/4	0/5	-
Subclinical	37/55	17/37	14/21	6/14
Long-term	8/10	1/1	0/1	-
Mortality	1/1	1/1	-	-
Morbidity	3/4	-	-	-
Hospital admission	-	-	-	-
Subclinical	4/5	-	0/1	-

*the number of short-term studies exceed 75, as three studies used different outcome types. NC: not conducted

Discussion

Literature search

We conducted a systematic comprehensive search of relevant epidemiological studies on ultrafine and quasi-ultrafine particles for the period from 01.01.2011 until 11.05.2017. The different strategies of our search consisted of a MEDLINE search, using two alternative strategies, a search in the specialized data base LUDOK, and a hand search in review articles and reference lists of identified publications. Overall, the additional yield of the alternative MEDLINE search strategy, and of the complementary search strategies (LUDOK and hand search), and of the repeated search was substantial, with altogether 15 additional references added to the final analysis data base and an additional 13 articles identified per MEDLINE and hand search in February 2018. This relatively high yield reflects the lag in indexing newly published studies in large literature data bases as well as the fast development of an emerging scientific field. More specialized data bases such as the dedicated LUDOK literature data base are therefore very useful for targeted and timely research.

Evaluation of health relevance of ultrafine particles

Our evaluation of the health relevance of ultrafine particles is based on the above described epidemiologic studies and how they add to the available the evidence since the comprehensive review conducted by the HEI, published in 2013. Overall, the epidemiological evidence is quickly increasing and it can be expected, that the next few years will bring a substantial increase in relevant studies. Currently, we are still in the beginnings of health-related research of UFPs, which is in part due to the still developing methods (see sections below on exposure assessment).

The HEI concluded in its review that "the current database of experimental and epidemiologic studies does not support strong and consistent conclusions about the independent effects of UFPs on human health" (Health Effects Institute, 2013). Major reasons for this lack of evidence, specifically for epidemiologic studies, lie in the difficulty of assessing population-based exposure to UFPs for short-term as well as for long-term studies. Due to the specific properties of UFPs with a high temporal and spatial variability, common exposure assessment strategies, which have been developed for the more homogeneously distributed larger particle fractions, will lead to larger exposure misclassification when applied to UFPs. Nevertheless, HEI does not conclude that independent effects of UFPs can be ruled out, but rather recommends the exploration of alternative exposure metrics, spatial modeling techniques, and statistical methods.

In this review, we use similar design- and outcome-specific categories as in the HEI review to be able to integrate our findings with the prior evidence. Since independence of effects is the key question regarding the health relevance of UFPs, we specifically focus on studies with co-pollutant adjustment.

Inconcistency of results by endpoint

Previous evaluations have concluded, that the combined results for respiratory as well as for cardiovascular endpoints are still inconsistent (Health Effects Institute, 2013). When considering the newly acquired evidence during the years from 2011 to 2017, this picture has not changed substantially. Even though there is a growing number of specifically designed studies to investigate health effects of UFP/quasi-UFP, we cannot identify a consistent pattern of health effects on either respiratory or cardiovascular disease across the different endpoints including mortality, morbidity, emergency department visits/hospital admissions or subclinical endpoints. For other outcomes such as mental disorders, neurocognitive function or birth outcomes, the evidence base is still too small to derive firm conclusions.

Even though results are not consistent across different outcomes types, the majority of the 11 studies investigating short-term effects on BP, the major risk factor for cardiovascular disease, indicate an association with increased blood pressure. Once again, evidence from the three co-pollutant-adjusted studies is mixed, which underscores the necessity of further studies with co-pollutant adjustments.

The lack of consistent findings can be explained by a number of factors. These include differences in exposure assessment (see below), endpoint assessment, study design and size, and different confounder control, specifically differences in the adjustment for co-pollutants (see below).

Long-term exposure and health effects

In contrast to the last prior comprehensive review by HEI (2013), ten studies have been published investigating long-term effects of UFPs on various health outcomes. While most of these studies found elevated point estimates for associations of UFPs with adverse health outcomes, only one study adjusted for co-pollutants, including NO_2. Adjustment with NO_2 led to a decrease in the effect estimate to an inverse association.

While the current evidence base does not support an independent effect of UFPs on health outcomes, this should by no means be mistaken for a proof of the absence of such an effect. As will be discussed below, current exposure assessment techniques are not well suited to describe and investigate long-term exposure to UFPs. More studies applying novel methods for individual-level exposure to UFPs are therefore urgently needed. Important applications are next to road traffic-related exposures also the emerging problem regarding exposure to UFPs in the vicinity of airports, which has only recently been described (N. Hudda, Simon, Zamore, Brugge, & Durant, 2016).

Exposure assessment

Overall, the number of studies including the assessment of exposure to and the investigation of health effects of UFPs is rapidly increasing. One important factor contributing to this rapid increase is the development of new instrumentation, which enables a less expensive assessment of UFP/quasi-UFP for example with condensation particle counters. However, research is still at the beginning and new exposure assessment methods need to be defined and employed in epidemiological studies.

Challenges of exposure assessment for UFPs include the high spatial and temporal variability of UFP/quasi-UFP, which necessitate different exposure assessment designs than the “classical” air pollutants like $PM_{2.5}$ and PM_{10} with a much more homogeneous spatial distribution. This high spatial variability is of concern not only for long-term health effects studies, which are based on long-term spatial differences in exposure, but also for short-term studies with a central-site measurement. These studies assume that the temporal changes from day to day are evenly distributed across the sometimes very large study areas; an assumption that might not hold true for UFPs. Given the possibility of a larger exposure estimation error for UFPs compared to other pollutants, a systematic bias towards the null in single-pollutant studies and in multi-pollutant studies is probable (Dionisio, Baxter, & Chang, 2014).

In the future, the development of enhanced spatiotemporal models can contribute to a more precise exposure assessment across larger areas. Current models such as the German EURopean Air Pollution Dispersion (EURAD) model need to be adapted to incorporate specific sources, validation measurements and increase the spatial resolution.

A further challenge of UFP/quasi-UFP exposure assessment is the non-standardized equipment and the non-standardized use of size fractions in the studies. The commonly used measurement devices have different lower cutpoints for the particle size. Since the majority of particles are located in the nucleation mode (< 20 nm) of the particle size distribution, even small differences in the lower cutpoint between 1 and 20 nm can lead to substantial differences in particle number concentration. Futhermore, the reporting of the exposure assessment often does not include the exact size range of particles, which prevents direct comparisons of exposure between studies.

Independence of effects

Even though several studies across the investigated endpoints have observed positive associations of UFP/quasi-UFP with various health effects, the overall evidence for independent effects is still insufficient. We noticed, that specifically the newer studies conduct multi-pollutant models with a higher frequency than the older studies, which is a positive development (e.g., Aguilera et al., 2016; Croft et al., 2017; Lanzinger et al., 2016a; Samoli et al., 2016; Stafoggia et al., 2017). However, the type of adjustment still varies substantially between studies and there is no standard strategy for co-pollutant adjustment yet. At the moment, adjustment for NO_2 generally seems to exert a greater effect on the point estimate than other co-pollutants (e.g., Lanzinger et al., 2016a&b; Su et al., 2015; Samoli, Andersen et al., 2016, Zhang et al., 2013). One reason for this is the overlap in sources and spatial/temporal distribution of UFP/quasi-UFP and NO_2, which can lead to instability in the models and biased effect estimates in two-exposure models.

Transferability of results to the situation in Germany

The transferability of the above reported results to the situation in Germany will be judged according to the following criteria: Localizations of identified studies and level of exposure to ultrafine particles, level of exposure to airborne co-pollutants, baseline prevalence of investigated diseases and selection of study populations.

Exposure

The vast majority of the identified studies are located in North America (n=37, 43.5%) or Western Europe (n=27, 31.8%) and 5 studies (6%) located in more than one world region (Table 2). When examining the study sites of studies with multiple study centers, we can observe that the majority of study sites are located in Western and Southern Europe (n=44 of 101 study sites, 43.6%)(Table 3). The concentrations of ultrafine particles vary considerably in time and space and direct comparisons of single center measurements are subject to large variation depending on hour, day and season of measurement as well as exact placement of the measurement site (traffic, urban background, regional background site)(Birmili et al., 2016; UFIPOLNET, 2008). In the German Ultrafine Aerosol Network (GUAN), long-term measurements of ultrafine and fine particles have been conducted at 17 sites across Germany, including alpine sites (Zugspiptze), rural sites, urban background and roadside measurement sites (Birmili et al. 2016). Of note, the size of the measured particles ranges from 20 to 800 nm, thereby not encompassing the nucleation mode of particles and including the accumulation mode particles. Preliminary results of GUAN measurements indicate a range of hourly median concentrations of particle number (sized 20-800 nm) between 900/ml (Zugspitze) and 9000/ml at the roadside in Leipzig. Hourly mean concentrations are higher with 1120/ml at the Zugspitze and 10.500/ml in Leipzig. The 95 percentile of the distribution of hourly values reaches 22.400/ml in Leipzig-Mitte. All three roadside measurement sites had P95 values above 19.900/ml, while the urban background sites ranged between 10.000 and 20.000/ml. GUAN also demonstrates the substantial variation in particle size distribution during the course of a week at six mainly urban sites.

The identified studies conducted in Western Europe typically have similar or higher mean total particle counts. A direct comparison is not possible with the available information, since instruments for measurements differ and have different lower cutpoints. 16 out of the 27 studies in Western Europe report the lower cutpoint of their measurement device as 10 nm or lower. Some devices go down as far as 3 nm as their lower cutpoint. Since the majority of particles is sized below 20 nm (nucleation mode) (HEI perspectives, 2013), small differences in the lower cutpoint leads to substantial differences in mean exposures. In addition, the upper cutpoint also varies considerably, with only few studies examining ultrafine particles in the more strict sense (<100 nm), but rather use the surrogate of total particle number concentration as the exposure of interest. This, however,

presents a minor problem as total particle number is dominated by the size fraction below 100 nm (HEI perspectives, 2013).

For the benefit of this review, GUAN primarily demonstrates the large variability of exposures within Germany, but it is not well suited to compare absolute values with other studies, which used different measurement devices. The 5 studies from Germany included in the review are based on central-site or personal measurements (n=4) with lower cutpoints ranging between 3 and 10 nm. These studies yield mean exposures between 10.000/ml and 20.000/ml, which is comparable to other studies in this review. In comparison, the 13 studies located in the Western Pacific region or in South-East-Asia, in the metropolitan areas of China, South Korea or Taiwan, report measured mean particle number concentrations in similar or slightly higher ranges. The only German study based on modelled exposures applying the EURAD CTM yielded substantially higher mean exposures due to the modelling process, which included the complete nucleation mode and therefore also encompasses short-lived particles sized below 3 nm. We therefore conclude that the level of exposure in the identified studies, while very variable across time and space, is generally comparable to the German situation.

The development of population exposure to ultrafine and quasi-ultrafine particles in Germany in the coming years depends on several factors: (1) the formation and emission of these particles, (2) the spatial distribution of the population, and (3) the concentration of fine particles in ambient air.

According to a size-resolved pan-European anthropogenic particle number inventory, the most important sources of emissions are road traffic in urban areas and alongside highly trafficked roads (Health Effects Insitute, 2013). Traffic-related emitters of primary UFP are direct injection engines in vehicles, which have increased in number during the last decade and will probably increase further (Köllner, 2016). On the other hand, vehicles with Diesel-powered engines, which also emit particles in the ultrafine and quasi-ultrafine size range, have been equipped with particle filters. This has reduced the emission of fine particles substantially (according to EURO5a less than 5 mg/km). For UFP, the EURO5b norm for the first time sets a limit at 6×10^{11} (European Union, 2007). Overall, with increasing traffic and a rising number of city dwellers expected in the future (Vallance & Perkins, 2010), exposure to road traffic-related UFPs is likely to increase in Germany in the next decade.

A further source of mostly ultrafine particles is aircraft traffic. Several exposure studies have documented increased UFP exposure downwind of airports around the world world (Neelakshi Hudda, Gould, Hartin, Larson, & Fruin, 2014; Keuken, Moerman, Zandveld, Henzing, & Hoek, 2015; Masiol et al., 2017; Shirmohammadi et al., 2017; Stafoggia et al., 2016). The increased short-term exposure is correlated with aircraft movements over time and reach concentrations up to 50,000 particles/ml (Keuken et al., 2015) 7 km downwind of the airport in Amsterdam and up to 75,000 particles/ml (Hudda et al., 2014) 8 km downwind in Los Angeles. The same studies show that long-term concentrations are elevated up to 3-fold 7 km downwind with more than 200,000 exposed inhabitants close to Schipohl airport, Amsterdam (Keuken et al., 2015) and up to 4-5-fold in Los Angeles, 8-10 km downwind (Hudda et al., 2014). Similar exposure studies are ongoiong in Germany and will yield first information about the exposure of residents close to German airports. Given the increase in air travel, the exposure due to airdraft emissions is likely to play an increasing role in the future.

Moreover, the concentration of fine particles in ambient air is a determinant of UFP in a way that UFP will collide and coagulate with larger particles. A high concentration of ambient fine particles will therefore support the clearance of UFP in ambient air. With the reduction of fine particles, UFP will likely stay longer airborne than in an environment with high PM concentrations.

Exposure to co-pollutants

The level of airborne co-pollutants are important, as most of these co-pollutants have own effects on the outcomes of interest. 78 of the 85 identified studies (92%) assessed the level of at least one other

air pollutant; however, only 34 studies adjusted for at least one co-pollutants in their analysis (see section 4.3). Assessment of and adjustment for airborne co-pollutants is therefore not conducted in a comparable way across the identified studies.

Analysis of the multi-pollutant models revealed, that $PM_{2.5}$ and NO_2 are the co-pollutants which tend to influence the UFP/quasi-UFP estimate the most. Often, but not always, does the adjustment for NO_2 lead to an attenuation of the association of UFP/quasi-UFP with the health outcome (Leitte et al. 2012; Meng et al. 2012; Stafoggia et a.. 2017; Su et al. 2015; Iskandar et al. 2012; Lanzinger et al. 2016; Rosenthal et al. 2013; Gong et al. 2014; Janssen et al. 2015; Steenhof et al. 2013). Adjustment for PM_{10} and $PM_{2.5}$ also attenuates the UFP/quasi-UFP association in several studies, but in most cases less than the NO_2 adjustment.

The level of co-pollutants, and specifically $PM_{2.5}$ and NO_2, can be compared across Europe using the "Air quality in Europe — 2017 report" by the European Environmental Agency (European Environmental Agency, 2017). According to this report, Germany ranks top among the 28 member states regarding the annual mean of NO_2 at the included monitoring sites (European Environmental Agency, 2017; Fig 6.1). Similar to UFP/quasi-UFP, the annual mean at selected monitoring sites is not able to give a comprehensive overview of the exposures of the study populations in the included studies, as NO_2 concentrations are subject to a high variability across time and space. Of the 34 studies that adjusted for co-pollutants, 15 were conducted in Western Europe. Of those, 3 were conducted in Germany, Augsburg, and all other studies were conducted in mostly major cities in Switzerland, the Netherlands, Sweden, Denmark and Finland with comparable traffic exposures.

We therefore conclude that the findings of an at least partial overlap of effects between UFPs and NO_2, which we observe in the Western European studies included in this review (Iskandar et al. 2012; Janssen et al. 2015; Rosenthal et al. 2013; Stafoggia et al. 2017; Steenhof et al. 2013), hold true for Germany as well.

Disease prevalence

The majority of the studies identified in this review is located in Western/Southern Europe and North America. The cause-specific age-adjusted death rates for all non-communicable diseases and for respiratory diseases for 2015 are similar for the WHO Region of the Americas (including South America, which is not included in this review) and the WHO European Region (World Health Organization, 2016b). On the other hand, the annual cause-specific age-adjusted death rates for cardiovascular diseases differ, with a substantially lower age-specific death rate in the Americas (211/10,000) compared to the European Region (344/10.000). This difference is primarily due to the combination of both Americas in this statistic. Compared to other European countries and the USA included in this review, Germany has a similar distribution of causes of premature deaths as the Netherlands with ischemic heart disease, lung cancer, Alzheimer disease, cerebrovascular disease and COPD ranking 1 to 5 in both countries. This ranking is very similar in the UK, Denmark, Sweden, Spain and the USA.

Moreover, the majority of studies investigate short-term sublinical outcomes (Table 8) and of those, cardiovascular, respiratory and biomarker outcomes present the focus of the included studies (Table 9). The outcome assessment of these studies is not subject to country-specific ICD-coding conventions. Unless baseline differences in physiological markers exist between the populations included in this review and the German population, which we have evidence for, transferability on results for Germany can be inferred.

Study population

Most studies included in this review are based on selected study populations (n=62, 72.9%) and only 10 (11.8%), respectively 13 (15.3%) studies were deemed representative or at least somewhat representative of the general population (Table 14). The studies deemed to be completely representative of the target population are the time-series studies, which are based on general populations of the city of study. One of these time-series studies (Diaz-Robles et al. 2014) targeted selected age-groups within the general population. Of the other studies, 13 (15%) studies include at least one random sample of the source population. Almost all identified articles describe the study population well. The 10 studies investigating long-term effects are mostly analyses based on existing cohorts of several hundreds to thousands of participants, exclusively located in Western Europe or North America. Of these, 6 studies target the adult population of either sex or limited to one sex (Ostro et al. 2015), and 4 studies target children (Laurent et al. 2014, 2016a and 2016b; Sunyer et al. 2015). Among the short-term studies, the study populations are mostly highly selected small groups of either healthy (younger) adults or participants with a respiratory or cardiovascular disorder such as asthma, COPD, coronary artery disease, etc.

Transferability – conclusions

Based on the above descriptions of exposure level, co-pollutant exposure, baseline disease prevalence and included study populations we conclude that the overall results of this review can be transferred with the appropriate caution to the German situation.

Important limitations are (1) the paucity of studies with co-pollutant adjustment, which is specifically important because of the high NO_2 exposures in Germany, and (2) the use of highly selected groups in short-term studies, as these often do not include specifically vulnerable populations such as patients with badly controlled disease, newborns and children.

Overall conclusions

The investigation of health effects in epidemiological studies is a rapidly increasing field of research and substantial developments have been made during the last 7 years, tackling two of the most urgent open questions of research: First, several studies on long-term health effects of UFPs have been conducted and published. Second, specifically the more recent studies have undertaken efforts to control for co-pollutants to identify the independent effect of UFPs.

Despite the obvious development in the field, the overall conclusions have not changed substantially over the time period investigated in this study.

First, the evidence on health effects remains inconclusive or insufficient for most of the studied outcomes. Specifically, while a number of studies have investigated mortality and emergency department/hospital admission outcomes, the relatively few studies with co-pollutant adjustment reveal mixed and, up to now, inconclusive evidence. In terms of number of studies, most evidence is available from studies investigating subclinical outcomes. Within this group of studies, cardiovascular outcomes and outcomes of pulmonary and systemic inflammation show the most consistent patterns with associations generally pointing into the direction of the adverse health outcome. Nevertheless, the evidence for independence of effects remains limited here as well, as only few studies have adjusted for co-pollutants.

Second, exposure assessment in the population remains difficult, due to the specific characteristics of UFPs. Studies using central-site exposure assessment probably miss a large part of the variability. Studies using classical spatial modeling techniques need to incorporate the very high spatial and temporal variability. Null findings or reductions in UFP/quasi-UFP effect estimates upon co-pollutant adjustment can at least in part be explained by exposure misclassification and measurement error.

Exposure assessment has to devote special attention to measurement techniques, size-fractions and localisations of monitor placement. Reporting needs to be standardized to make studies more easily comparable.

Third, the independence of UFPs cannot be evaluated at the moment, due to the low number of studies with adjustment and the above mentioned limitations to exposure assessment for UFPs. A positive development is the increase in studies paying attention to this issue.

Fourth, there is still an urgent need for long-term studies on health effects of UFPs. This will require the development of modeling techniques. Furthermore, specific high-exposure situations need to be identified and described in more detail to be able to assess long-term health effects. Specifically, while near road exposures have already been recognized as important factors, airport-related exposures, which have recently been shown to be substantially above background concentrations, have not been included in health effects studies yet.

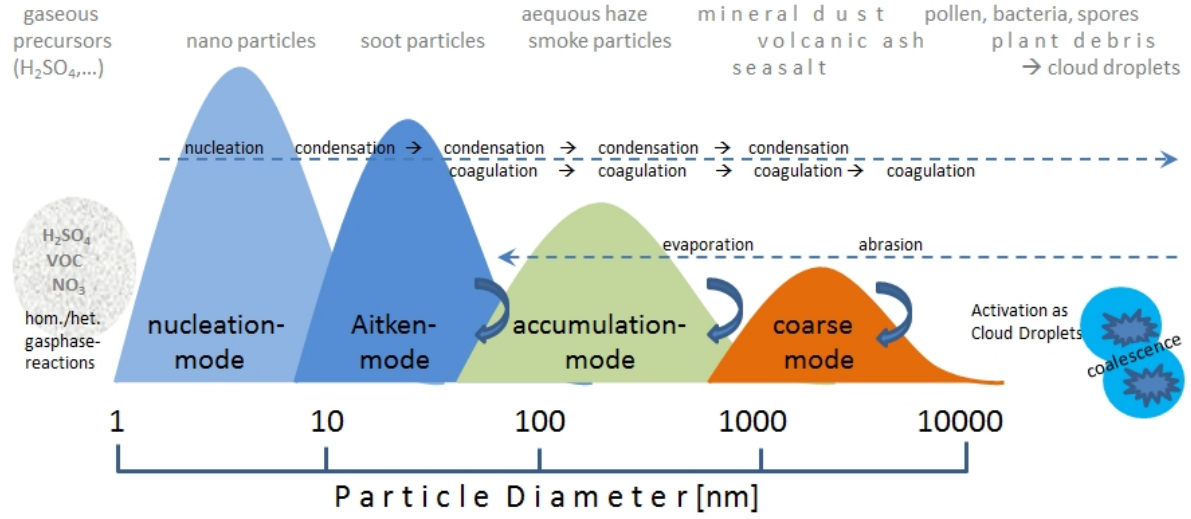
In addition to these general conclusions, we conclude that the overall results of this review can be transferred with the appropriate caution to the German situation. Important limitations are (1) the paucity of studies with co-pollutant adjustment, which is specifically important because of the high NO_2 exposures in Germany, and (2) the use of highly selected groups in short-term studies.

Zusammenfassung

Hintergrund

Ultrafeine Partikel (UFP) bzw. Ultrafeinstäube sind Partikel, welche einen aerodynamischen Durchmesser von maximal 100 Nanometer haben. Da UFP nur einen geringen Anteil zur Partikelmasse der Umgebungsluft beitragen, werden UFP meist als Partikelanzahl pro ml erfasst. Darüber hinaus werden in der epidemiologischen Forschung Partikelfraktionen unterschiedlicher Größenfraktionen genutzt. Dazu zählen nucleation-mode Partikel (Durchmesser von bis zu ca. 20 nm), Aitken-mode Partikel (Kondensationspartikel mit einem Durchmesser von ca. 10 bis 80 nm) sowie accumulation-mode Partikel (Partikel aus Kondensation und Koagulation mit einem Durchmesser von ca. 50 bis 1.000 nm). UFP unterscheiden sich aufgrund physikalischer und chemischer Eigenschaften von größeren Partikeln und werden direkt emittiert oder aus Vorläufersubstanzen im Rahmen sekundärer atmosphärischer Prozesse gebildet. In städtischen Gebieten stammen die UFP vor allem aus Verbrennungsprozessen durch motorisierte Fahrzeuge, insbesondere in Straßennähe (Health Effects Insitute, 2013; Kelly & Fussell, 2012).

Abbildung I: Größenfraktionen luftgetragener Partikel (Quelle: Deutscher Wetterdienst, 2018)



Aufgrund ihrer geringen Größe können UFP nach Inhalation bis in den Alveolarbereich eindringen und sogar Zellmembranen durchdringen. Hierdurch können sie in die Blutbahn übergehen sowie letztlich in alle Körperorgane inklusive des Gehirns und des Nervensystems gelangen. Experimentelle Studien deuten auf einen Zusammenhang ultrafeiner Partikel mit kardiovaskulärer und respiratorischer Morbidität und Morbidität sowie der Entstehung von lokalen und systemischen Entzündungsprozessen sowie adverse Effekte auf Gehirn und Stoffwechsel hin (Health Effects Insitute, 2013). Mehrere Expertenkommissionen haben in den vergangenen Jahren eine kritische Interpretation unter anderem der epidemiologischen Evidenz der zu UFP vorliegenden Erkenntnisse vorgenommen (Health Effects Insitute, 2013; World Health Organization, 2013). Die vom Health Effects Institute, Boston (HEI) und der WHO eingesetzten Kommissionen stellten im Jahr 2013 fest, dass es zwar wissenschaftliche Hinweise auf gesundheitsschädigende Wirkungen von UFPs gibt, wobei aber speziell für epidemiologische Studien die Evidenz insgesamt noch nicht ausreicht, um eine gesetzliche Regulierung von UFPs zu empfehlen. Die in den letzten Jahren deutlich zugenommene Anzahl an wissenschaftlichen Publikationen macht nun eine Neubewertung der Evidenzlage notwendig.

Hypothesen und Ziele der Studie

Die Ziele dieses Projekts sind die Durchführung einer systematischen Literaturrecherche zu den gesundheitlichen Effekten von Ultrafeinstaub, eine Bewertung der identifizierten Literatur und eine Bewertung der Übertragbarkeit der Ergebnisse auf die Situation in Deutschland. Zu diesem Zweck sollen folgende Fragen beantwortet werden:

a. Systematische Literaturrecherche
    a. Zu gesundheitlichen Effekten von Ultrafeinstaub
    b. Fokus auf epidemiologischen Studien und quantitativen Effektmaßen (z. B. Relative Risiken, Konzentrations-Wirkungsfunktionen)
    c. Dokumentation der Suche und Archivierung der berücksichtigten Artikel in einem Literaturverwaltungsprogramm (vorzugsweise Endnote)
b. Bewertung der identifizierten Literatur
    a. Bewertung der Studienqualität anhand festzulegender Kriterien
    b. Bewertung der Übertragbarkeit der identifizierten Erkenntnisse auf die Verhältnisse in Deutschland
c. Bewertung der gesundheitlichen Relevanz von Ultrafeinstaub
    a. Mit Bezugnahme auf weitere Luftschadstoffe (z. B. PM_{10}, $PM_{2,5}$, Ozon, Stickstoffdioxid)
    b. Im Hinblick auf die Situation in Deutschland
    c. Unter Berücksichtigung der wahrscheinlichen Entwicklung von Ultrafeinstäuben in Deutschland

Methoden

Es wurde eine systematische Literaturrecherche nach Studien zu Gesundheitseffekten von Außenluft-bezogenen UFPs der MEDLINE-Datenbank (Medical Literature Analysis and Retrieval System Online)durchgeführt. Die Suche umfasste alle im Zeitraum vom 1.1.2011 bis zum 11.5.2017 veröffentlichten Studien. Zusätzlich suchten wir in der vom Schweizer Tropen- und Public Health Institut (Swiss TPH) zur Verfügung gestellten LUDOK (Dokumentationsstelle Luftverschmutzung und Gesundheit)-Datenbank. Diese Datenbank umfasst Fachliteratur zu den Effekten von Luftverschmutzung auf die menschliche Gesundheit.

Der Schwerpunkt der Recherche lag auf epidemiologischen Studien zu gesundheitlichen Effekten von Ultrafeinstäuben mit quantitativen Effektmaßen (Arbeitspaket 1 (a) Systematische Literaturrecherche zu gesundheitlichen Effekten von Ultrafeinstaub, (b) mit dem Fokus auf epidemiologischen Studien und quantitativen Effektmaßen (z. B. Relative Risiken, Konzentrations-Wirkungsfunktionen). Die Studien sollten zudem mindestens eines der folgenden UFP-Maße enthalten: Anzahl (PNC) für Partikel mit einer Größe von maximal 100 nm, $PM_{0,1}$, nucleation-mode, Aitken-mode sowie quasi-UFPs: PNC für Partikel mit einer maximalen Größe von über 100 nm, $PM_{0.25}$, surface area concentrations und accumulation mode. Als gesundheitliche Endpunkte werden neben Mortalität, Morbidität und Krankenhauseinweisungen/ Ambulanzbesuchen auch präklinische Endpunkte berücksichtigt.

Toxikologische Studien wurden lediglich im Hinblick auf unterstützende Evidenz für die im Arbeitspaket 3 (Bewertung der gesundheitlichen Relevanz von Ultrafeinstaub) durchzuführende Bewertung gesichtet. Ebenso wurden Studien gesichtet, welche die Erfassung von bevölkerungsbezogenen Expositionen zum Thema haben, da diese für die Bearbeitung der Teilaspekte 2b) (Bewertung der Übertragbarkeit der identifizierten Erkenntnisse auf die Verhältnisse in Deutschland) und der Teilaspekte 3b) und 3c) (Bewertung der gesundheitlichen Relevanz von Ultrafeinstäuben im Hinblick auf die Situation in Deutschland und unter Berücksichtigung der wahrscheinlichen Entwicklung von Ultrafeinstäuben in Deutschland) notwendig sind.

Suchstrategie

Wir führten eine kombinierte Suche basierend auf MEDLINE, LUDOK und einer Handrecherche durch. Die MEDLINE-Suche basierte auf dem letzten umfassenden Review zu Gesundheitseffekten ambienter UFPs, welcher das HEI im Jahr 2013 veröffentlicht hat. Der Review umfasste Suchergebnisse der Literaturdatenbanken MEDLINE und Web of Science bis Mai 2011 (Annex I, Teil 1). Innerhalb des hier vorliegenden Projekts wurde die für den HEI-Review gewählte Suchstrategie in MEDLINE repliziert und einzelne Aspekte angepasst (Verweis auf die Suchstrategie im Anhang). Der Startpunkt unserer Suche wurde ein halbes Jahr vor dem Endpunkt der HEI-Suche gesetzt, um auch Publikationen zu erfassen, die zum Zeitpunkt der HEI-Suche noch nicht indiziert waren.

Daneben wurde eine alternative Suchstrategie von MEDLINE mit spezifischen Gesundheitsendpunkten eingesetzt. Statt der allgemeinen Gesundheitsendpunkte enthielt diese statt der allgemeinen Suchtermini „health“ und „epidemiology/ic/ical“ spezifische Krankheitsendpunkte.

Die LUDOK-Datenbank umfasst epidemiologische und experimentelle Originalarbeiten über die Auswirkungen der „klassischen“ Aussenluftschadstoffe auf Menschen, sowie von weiteren Schadstoffen, die via Luft auf die Allgemeinbevölkerung einwirken (d. h. keine alleinig arbeitsmedizinisch relevanten Stoffe) (Annex I, Teil 2) In LUDOK wird monatlich eine Recherche über PubMed mit gleich bleibender, sehr breiter Formulierung durchgeführt. Zusätzlich zur regelmäßigen Suche wird eine intensive Handsuche in über 20 relevanten Fachzeitschriften, allgemein wichtigen Journals sowie den Referenzlisten aus Publikationen durchgeführt. Die Suchstrategie innerhalb des hier vorliegenden Projekts bestand aus einer modifizierten HEI Suchstrategie, ergänzt um eine Suche innerhalb der LUDOK-Datenbank sowie Handssuchen. Die Suchtermini wurden im Vergleich zu den HEI-Termini in Anlehung an die breite LUDOK-Suchstrategie erweitert (Annex I, Teil III).

Weitere Zugangswege zu Publikationen bot die Handrecherche in den vorhandenen Übersichtsarbeiten der letzten sechs Jahre sowie Übersichtsarbeiten, die im Rahmen unserer Literaturrecherche identifiziert wurden.

Studienselektion

Zwei Reviewer prüften Titel, Abstracts, sowie nach Bedarf Volltexte der Studien auf die Ein- und Ausschlusskriterien (s. u.) hin. 10 % der Studien wurden doppelt bewertet. Falls Unsicherheiten bzgl. der Selektion einer Studie bestanden, wurden diese im Team besprochen, evaluiert und bei Bedarf die Ein- und Ausschlusskriterien überarbeitet. Der Prozess der Studienselektion wurde in einem angepassten PRISMA-Diagramm dokumentiert (Abbildung 4).

Einschlusskriterien

- Epidemiologische Studien mit einem geeignete Studiendesigns: Querschnitt-, Fall-Kontroll-, Kohorten, Zeitreihen-, Panel-, und Case-crossover-Studien, scripted-exposure Studien
- Quantifizierbare Assoziationsmaße mit mindestens einem UFP-Maß: Anzahl (PNC) oder größenfraktionierte PNC für Partikel <100 nm, PM0,1, nucleation-mode, Aitken-mode oder einem quasi-UFP-Maß: PM 0.25, surface area concentrations, accumulation-mode
- Gesundheitliche Endpunkte: Mortalität, Morbidität, Krankenhauseinweisungen/ Notfalleinweisungen, präklinische Endpunkte
- Quantifizierbare Effekte mit mindestens einem der folgenden Maße: Odds ratio, Relatives Risiko, Hazard ratio, β-Schätzer, prozentuale Veränderung oder Expositions-Wirkungsfunktionen.
- Sprachen: Englisch, Deutsch

- Zeitrahmen: Studien, die zwischen dem 1.1.2011 bis zum 11.05.2017 publiziert wurden und nicht im HEI-Review enthalten sind. Studien, die nach diesem Zeitraum publiziert wurden, sind im Anhang gelistet (Annex I, Teil 5)

Ausschlusskriterien

- Toxikologische Studien, kontrollierte Expositionsstudien, (Tier-)Experimente, in-vitro Studien
- Exposition ggü. Nanopartikeln, die über industriell gefertigte Produkte in die Umwelt gelangen.
- Exposition ggü. UFP oder Nanopartikeln am Arbeitsplatz.
- Exposition ggü. Innenraum generierte UFP mit Quellenbezug
- Expositionen beschränkt auf Dieselpartikel, BC, EC
- Expositionen beschränkt auf Entfernungsmessungen
- Gesundheitliche Endpunkte unklarer gesundheitlicher Bedeutung wie Epigenetik, Metabolomics, Methylierung

Alle Referenzen werden in einer Bibliothek des Literaturverwaltungsprogramms Endnote verwaltet (Abbildung 3).

Datenextraktion

Die identifizierten Studien wurden hinsichtlich ihrer Qualität in Bezug auf die Berichterstattung, der Rigorosität/Aussagekraft und der inhaltlichen Aussage, und bezüglich ihrer Übertragbarkeit auf die Verhältnisse in Deutschland bewertet. Die entwickelten Qualitätskriterien (Annex I, Teil 6) sind angelehnt an Quality Assessment Tools des National Heart, Lung and Blood Institute des National Institute of Health (2014). Besondere Aufmerksamkeit bei der Entwicklung des Erhebungsinstrumentes zur Datenextraktion wurden der Erfassung der Expositionserhebung gewidmet. Insbesondere wurden Kriterien für die Beurteilung der angewandten Messtechnik, der Repräsentativität der Messorte für die Exposition der Zielbevölkerung, die Modellgüte von genutzten Expositionsmodellen sowie für die Erfassung/ Modellierung mehrerer Luftschadstoffe entwickelt.

Ergebnisse

Literatursuche

Die Anwendung der Haupt-Suchstrategie in MEDLINE ergab 1.114 Referenzen, die alternative Suchstrategie ergab 992 Referenzen, von welchen 332 Referenzen noch nicht in der Haup-Suchstrategie enthalten waren (Abbildung II). Insgesamt erzeugte die MEDLINE Suche 1.446 Treffer. Die Suche in in der LUDOK-Datenbank ergab 106 Treffer, von welchen 30 nicht in der MEDLINE-Suche enthalten waren. Weitere 8 Referenzen wurden durch Handsuchen und andere Quellen generiert. Insgesamt ergab die kombinierte Suchstrategie 1.484 Referenzen.

Von der finalen Anzahl von 85 Studien wurden 70 über die Haupt-Suchstrategie und 3 Studien über die alternative Suchstrategie in MEDLINE generiert. Hinzu kamen acht weitere Studien aus der LUDOK-Datenbank und vier Studien aus zusätzlichen Quellen.

Eine Replikation der MEDLINE Suchstrategie am 23.02.1018 für den Zeitraum nach der initialen Suche ergab weitere 13 Studien, die im Anhang gelistet sind (Annex I, Teil 5).

Evidenz aus vorangegangenen Übersichtsarbeiten

Unsere Literaturrecherche und das bisherige Wissen basieren auf einigen relevanten Übersichtsarbeiten, die in den letzten Jahren veröffentlicht wurden. Als erstes ist der HEI-Review zu nennen, welcher die bis dahin umfangreichste und vollständigste Datenbasis zu einer möglichen

Assoziation zwischen UFP und verschiedensten Gesundheitsendpunkten liefert. Adverse Gesundheitseffekte durch UFP werden als möglich, jedoch nicht eindeutig erwiesen bewertet. Gründe für diese uneindeutige Lage sind unterschiedliche Gesundheitsendpunkte und Methoden des Studiendesigns, die einen direkten Vergleich von Studienergebnissen verhindern, unterschiedliche und möglicherweise verzerrte Expositionserfassungen sowie fehlende Studien, welche für weitere Luftschadstoffe adjustiert haben. Darüber hinaus hat die HEI-Suche keine Langzeitstudie identifiziert, so dass die Evidenzlage insgesamt nicht ausreichend war, um Regulierungsmaßnahmen bezüglich UFP zu empfehlen.

Eine weitere Übersichtssarbeit dokumentiert Ergebnisse einer Expertenkonferenz zu Gesundheitseffekten durch Exposition gegenüber UFP (Baldauf et al., 2016). Die Teilnehmer resümieren, dass epidemiologische Kurzzeit-Studien auf einen Zusammenhang zwischen verkehrsinduzierten Feinstaub (welcher reich an UFP ist) und adversen kardiovaskulären Gesundheitsendpunkten hinweisen. Jedoch können beobachtete adverse Gesundheitseffekte durch UFP nicht zuverlässig von einer potentiellen Mitwirkung weiterer Luftschadstoffe separiert werden. Ähnlich wie das HEI fassen Baldauf et al. (2016) zusammen, dass der aktuelle Forschungsstand keine ausreichende Evidenz liefert, dass UFP toxischer sind als andere Partikelfraktionen. Nichtsdestotrotz liefern toxikologische Erkenntnisse Bedenken bezüglich potentieller Gesundheitseffekte durch UFP, was es nötig macht, die Partikelgröße bei der Erfassung adverser Effekte durch Feinstaubexpositionen zu berücksichtigen.

Chen et al. (2016) betrachten umfassend Artikel zur Zusammensetzung von UFP, deren Quellen, typische Eigenschaften, oxidative Effekte und potentielle Expositionswege mit einem Hauptfokus auf toxikologischen Studien. Des Weiteren berücksichtigen sie die Evidenz aus dem Bereich der Nanotechnologie, was das Verständnis bezüglich toxischer Mechanismen luftgetragener UFP erweitert. Die Autoren resumieren, dass UFP einen bedeutenden Einfluss auf die menschliche Gesundheit haben.

Eine amerikanische Arbeitsgruppe (Li et al., 2016) nehmen eine Neubewertung die Schlussfolgerungen des HEI-Berichts vor und untersuchen experimentelle, epidemiologische und klinische Studien, welche 2014 und 2015 publiziert wurden. Die Autoren benennen eine kritische Wissenslücke in Bezug auf Effekte von UFP auf die menschliche Gesundheit. Neuere Studien, insbesondere experimenteller und toxikologischer Art, stellen die Validität der HEI-Schlussfolgerungen in Frage, dass die Evidenz in Bezug auf Gesundheitseffekte durch UFP im Vergleich zu $PM_{2.5}$ keine radikalen Unterschiede belegt. In Bezug auf epidemiologische Studien sehen Li et al. (2016) keine neuen Erkenntnisse.

Heinzerling et al. (2016) untersuchen UFP-bedingte respiratorische Gesundheitheitseffekte bei Kindern anhand 12 relevanter Artikel. In Ein-Schadstoff-Modellen waren UFP mit inzidenter keuchender Atmung („wheezing“), bestehendem Asthma, eingeschränkter Lungenfunktion und durch Asthmaanfälle ausgelösten Besuchen von Notfallambulanzen assoziiert. Nur eine der Studien (Halonen et al., 2008) adjustierte für weitere Luftschadstoffe, woraufhin die Effektschätzer nicht länger signifikant waren. Die Autoren schlussfolgern, dass trotz einer Zunahme der Evidenz bezüglich UFP und der respiratorischen Gesundheit bei Kindern, die Evidenzlage uneindeutig bleibt.

Zusätzlich publizierten Clark et al. (2016) eine Studie, die auf biologische Mechanismen kardiovaskulärer Effekte über die alveoläre Barriere hinaus im Körper oder Gewebeproben, welche UFP und quasi-UFP mit einer Größe bis 500 nm exponiert waren. Die Autoren schlussfolgeren, dass eine mögliche bis hin zu starker Evidenz für verschiedene kardiovaskuläre Gesundheitsendpunkte besteht.

Studiencharakteristika

Die meisten eingeschlossenen Studien wurden in Nordamerika (n=37) oder Westeuropa (n=27) durchgeführt. Weitere 12 Studien fanden in der West-Pazifik-Region statt. Nur sehr wenige Studien wurden in Mittel-/Südamerika (n=1), Osteuropa (n=2) und Südostasien (n=1) durchgeführt. Drei von fünf multizentrische Studien schlossen Studien ein, die in verschiedenen westeuropäischen Ländern (Karakatsani et al., 2012; Manney et al., 2012; Samoli, Andersen, et al., 2016) durchgeführt wurden, zwei multizentrische Studien beinhalteten Studienstandorte in West- und Osteuropa (Lanzinger et al., 2016a, 2016b).

Die Mehrzahl der eingeschlossenen Studien bezogen sich auf die Untersuchung von Kurzzeit-Effekten (n=75) mit Gesundheitsendpunkten, die innerhalb von Stunden bis hin zu Wochen nach der Exposition gemessen wurden. Die Kurzzeitstudien waren dominiert von Panelstudien (31 mit wiederholten Messungen in eine in einem Querschnitts-Design), „Scripted exposure"-Studien (n=16) und Zeitreihenstudien (n=11). Weitere Studienarten der Kurzzeit-Studien waren „case-crossover"-Studien (n=8), Kohortenstudien (n=4) und Querschnittsstudien (n=4). Zehn Studien untersuchten Langzeit-Assoziationen und nutzten hierbei Expositionsschätzungen für Zeiträume von Monaten bis Jahren. Die Studien mit einem Langzeit-Studiendesign bestanden aus Kohortenstudien (n=4), Querschnittsstudien (n=4), jeweils einer Fall-Kohorten und Fall-Kontroll Studie (Tabelle I).

Tabelle I: Studiendesigns unterschieden nach Langzeit-/Kurzzeitstudien

Design	Studienanzahl	%
Langzeit	gesamt=10	
Fall-Kohortenstudie	1	1.2%
Fall-Kontrollstudie	1	1.2%
Kohortenstudie	4	4.7%
Querschnittsstudie	4	4.7%
Short-Term	gesamt =75	
Kohortenstudie	4	4.7%
Querschnittsstudie	4	4.7%
Panelstudie (Querschnitt)	1	1.2%
Panelstudie (wiederholte Messungen)	31	36.5%
Case-crossover	8	9.4%
Scripted exposure	16	18.8%
Zeitreihenstudien	11	12.9%
Gesamt	**85**	**100.0%**

Insgesamt nutzten die meisten Studien messbasierte Expositionserfassungen (87.1%). Modellbasierte Expositionen wurden in 10.6 % der Studien genutzt. In Langzeit-Studien wurden zumeist modellbasierte Expositionen genutzt (9 von 10 Studien), wohingegen die Mehrzahl der Kurzzeitstudien messbasierte Expositionen nutzte (71 out of 75). Dieses Muster ist darauf zurückzuführen, dass modellbsierte Expositionen notwenig sind, um die räumliche Variation der Exposition zu erfassen, welche den notwendigen Expositionskontrast für die Erfassung von Langzeiteffekten widergibt.

Die Mehrheit der Studien verwendete zentrale Messstationen zur Messung der Expositionen (n=45), gefolgt von mobilen Messtechniken (n=17) sowie Kombinationen verschiedener Modelle bzw. Messtechniken (n=10), z.B. zentrale Messstationen in Kombination mit Landnutzungsmodellen, Messungen im Wohngebiet oder kleinräumige individuelle Expositionsmodellen (Tabelle II).

Tabelle II: Art der Expositionsmodelle bzw. Messungen in den Studien

Expositionsmodell/Messung	Studienanzahl	%
Chemie-Transport-Modell	3	3.5%
Landnutzungsmodell	1	1.2%
Dispersionsmodell	1	1.2%
Messung: zentrale Station	45	52.,9%
Messung: Wohngebiet	2	2.4%
Messung: Mobil	17	20.0%
Kleinräumiges personales Expositionsmodell	2	2.4%
Weitere	4	4.7%
Kombination verschiedener Modelle	10	11.8%

Expositionsmodell/Messung	Studienanzahl	%
Gesamt	**85**	**100.0%**

In den meisten Studien wurden UFP als Partikelanzahlkonzentrationen (PNC) pro Volumen bestimmt. In etwa einem Drittel der Studien wurden PNC mit einer Größe von bis zu 100 nm verwendet (29 von 95[5] Studien) genutzt. In 66 Sudien wurden quasi-UFP mit PNC-Fraktionen von bis zu 3.000 nm Größe genutzt. In Bezug auf die verschiedenen Größenmodi werwendeten nur wenige Studien nucleation-mode Partikel (n=1), Aitken-mode Partikel (n=1), oder accumulation-mode Partikel (siehe 1.1). Elf Studien nutzten Partikelmassen-Konzentrationen pro Kubikmeter: Sechs Studien schätzten submikrone $PM_{0.1}$-Partikel, sieben Studien erfassten quasi-UFP $PM_{0.25}$ oder $PM_{0.1}$ Partikel. Die Oberflächenkonzentration, gemessen als „*lung deposited surface area*" (LDSA) wurde nur in zwei Studien verwendet.

Tabelle III: Arten von Gesundheitsendpunkten in Langzeit- und Kurzzeitstudien

	Studienanzahl	%
Langzeit	**All=10**	
Mortalität	1	1.1%
Morbidität	4	4.5%
Krankenhauseinweisung	0	0.0%
Subklinisch	5	5.7%
Kurzzeit	All=78	
Mortalität	7	8.0%
Morbidität	5	5.7%
Krankenhauseinweisung	11	12.5%
Subklinisch	55	62.5%
Total	**88**	**100.0%**

Acht Studien analysierten UFP in Zusammenhang mit Gesamtmortalität, kardiovaskulärer oder respiratorischer Mortalität. Neun Studien analysierten Effekte auf kardiovaskuläre, respiratorische oder weitere Morbidität-bezogene Gesundheitsendpunkte. Elf Studien untersuchten Effekte von UFP auf Krankenhauseinweisungen/Ambulanzkontakte aufgrund von kardiovaskulärer oder respiratorischer Erkrankungen. Die große Mehrheit der Studien untersuchte zahlreiche subklinische Messungen als Gesundheitsendpunkte. Unterteilt nach Organsystemen, untersuchte die Mehrheit der Studien kardiovaskuläre Gesundheitsendpunkte, gefolgt von Entzündungsmarkern und respiratorischen/atopischen Gesundheitsendpunkten. Insgesamt nur wenige Studien untersuchten Gesamtmortalität und oxidativen Stress.

Qualitätsindikatoren

In mehr als der Hälfte der Studien (n=49) wurden „Convenience"-Stichproben genutzt, sechs Studien nutzten zufällige Stichproben und weitere sieben Studien nutzte eine Kombination beider Stichprobenarten. In 13 Studien repräsentierten die Studienteilnehmer die allgemeine Bevölkerung,

[5] Da viele Studien mehrere Größenfraktionen nutzten, übersteigt die Summe der Studien hier 85.

die Mehrheit der Studien (n=62, 72.9%) verwendete ausgewählte Gruppen, welche nicht die Allgemeinbevölkerung repräsentieren. Eine Berechnung der Stichprobengröße war selten angegeben (n=3). Die Mehrheit der Studien (n=66, 77.6%) nannte das Größenspektrum der gemessenen UFP. Nahezu alle Studien (n=79, 92.9%) nannte das Messgerät zur Partikelmessung. Weniger als die Hälfte (n=34) der Studien, welche weitere Luftschadstoffe erfassten (n=79), adjustierte für weitere Schadstoffe in Mehrschadstoff-Modellen, und wurden als Studien mit hohem Risiko für Verzerrung bewertet. Mit Ausnahme von einer Studie wurden die zugewiesenen Expositionswerte vor oder parallel zur Erfassung der Endpunkte gemessen. In fünf der eingeschlossenen Langzeitstudien wurde dies durch die Anwendung von Chemietransport-Modellen erreicht, welche die Abschätzung von täglichen Schadstoffkonzentrationen in spezifischen Zeitperioden ermöglichen. Des Weiteren wurden die Endpunkte in allen bis auf eine Studie (n=84) klar beschrieben und definiert. In 68 Studien konnte eine Verblindung der Endpunkt-Erfasser angenommen werden.

Akute Gesundheitseffekte

Mortalität

Im Vergleich zur bisherigen Evidenzbasis wurden sieben zusätzliche Studien zur **Gesamtmortalität** mit gesamt gemischten Ergebnissen durchgeführt (Tabelle IV). Im Bereich der Gesamtmortalität fanden nur zwei von vier Studien positive Schätzer in Einschadstoff-Modellen. Von diesen zeigte nach Adjustierung für weitere Schadstoffe nur eine Studie positive Assoziationen für quasi-UFP, wohingegen in der anderen Studie die erhöhten Effektschätzer gegen null tendierten.

Die Evidenz bezüglich **respiratorischer Mortalität** ist ebenfalls sehr begrenzt und inconsistent: Von den fünf Studien zu respiratorischer Mortalität beobachteten vier Studien positve, wenn auch zumeist nicht-signifikante Assoziationen für UFP oder quasi-UFP. Drei der Studien adjustierte für weitere Luftschadstoffe, wobei die Adjustierung für NO_2 gegensätzliche Effekte zeigte: Teilweise wurden die Schätzer erhöht, teilweise verringert. Die Studien präsentierten die Mehrschadstoff-Modelle lediglich für die jeweiligen Modelle/ Zeitfenster/ Größenfraktionen, in welchen die stärksten Assoziationen beobachtet wurden. Daher können die unterschiedlichen Effektschätzer nur sehr eingeschränkt verglichen werden bzw. die Konsistenz der Ergebnisse nur sehr eingeschränkt bewertet werden.

Die Evidenz bezüglich **kardiovaskulärer Mortalität** ist ähnlich inkonsistent. Die sechs Studien zu diesem Endpunkt zeigten sowohl positive (drei Studien) als auch entgegengesetzte Assoziationen (drei Studien). In den zwei Studien mit Mehrschadstoffmodellen führte die Adjustierung für NO_2 zu verringerten Effektschätzern, was in einer Studie zu einem Verlust der Signifikanz führte und in einer anderen Studie zu einer signifikant inversen Assoziation. Adjustierungen für $PM_{2.5}$ führte wenn überhaupt nur zu geringen Veränderungen der UFP-Schätzer.

Die Evidenz aus dieser wie auch vorheriger Reviews weist darauf hin, dass die Effekte in der warmen Jahreszeit größer sind; daher sollte in zukünftigen Kurzzeit-Studien eine mögliche Effektmodifikation durch die Jahreszeit unbedingt berücksichtigt werden. Darüberhinaus überlappen die beobachteten Effekte zumindest teilweise mit den Effekten weiterer Luftschadstoffe, was am deutlichsten für NO_2 beobachtet werden kann. Aufgrund von Unterschieden bei den erfassten Partikelfraktionen kann keine Aussage zu den relevantesten Fraktionen gemacht werden.

Table IV: Zusammenfassung der Analysen in sieben Studien zur Mortaliät

Studie	Alle Ursachen	Ein-Schadstoff-Assoziation	Zwei-Schadstoff-Assoziation	Respiratorisch	Ein-Schadstoff-Assoziation	Zwei-Schadstoff-Assoziation	Kardiovaskulär	Ein-Schadstoff-Assoziation	Zwei-Schadstoff-Assoziation

Lanzinger et al. 2016a	✓	0	0	✓	(+)	+	✓	(-)	-
Leitte et al. 2012				✓	UFP: (+), quasi-UFP: +	UFP: 0 quasi-UFP: (+)			
Meng et al. 2013, (only quasi-UFP)	✓	+	+	✓	(+)	nc	✓	+	nc
Samoli et al. 2016	✓	0	0	✓	-	-	✓	(-)	nc
Stafoggia et al.,2017	✓	(+)	(-)	✓	+	nc	✓	(-)/(+)*	nc
Su et al. 2015							✓	+	(+)
Wolf et al. 2015							✓	(+)	nc

0 bezeichnet keine Assoziation. (+) und (-) bezeichnen primär nicht-signifikante Assoziationen, + und - bezeichnen signifikante Assoziationen. Nc: nicht durchgeführt. *variieren je nach Zeitfenster.

Morbidität

Von den wenigen Studien, die Kurzzeit-Effekte von UFP/quasi-UFP in Bezug auf verschiedene Maße der **Morbidität** untersuchten, beobachteten ledigleich zwei Studien erhöhte Schätzer in Zusammenhang mit einem Indikator zu wahrgenommenem Stress und mit unterschiedlichen Symptomen. Da keine dieser Studien für weitere Schadstoffe adjustierten, konnten die Effekte verschiedener Komponente des Luftverschmutzungs-Gemisches nicht separiert werden. Daher ist ein Fazit zu unabhängigen Effekten von UFP/quasi-UFP auf die Morbidität nicht möglich. Die Evidenz für kardiovaskuläre Morbidität ist eingeschränkt mit nur zwei Studien zu unterschiedlichen Endpunkten. Diese Studien weisen darauf hin, dass Teilnehmer mit bestehender kardiovaskulären Erkrankung möglicherweise empfindlicher gegenüber UFP/quasi-UFP sind. Auch wenn beide Studien generell auf positive Assoziationen hinweisenn, kann aufgrund fehlender Mehrschadstoffmodelle keine Aussage zu unabhängigen Effekten von UFP/quasi-UFP gemacht werden. Die Evidenz zu Assoziationen mit akuten Veränderungen von Symptomen psychischer Gesundheit ist unzureichend.

Die Evidenzbasis für UFP-bedingte Effekte auf die **Inanspruchnahme der ambulanten und stationären Gesundheitsversorgung (Krankenhausaufnahmen, Notfallambulanzen)** aufgrund respiratorischer Symptome ist limitiert (Tabellen im Annex: A1c, A3c). Mögliche Assoziationen scheinen am wahrscheinlichsten bei Kindern als vulnerable Subgruppe. Während Einschadstoff-Modell-Assoziationen in einigen Studien beobachtet werden konnten, war dies nicht der Fall für Mehrschadstoff-Modelle. Insbesondere die Adjustierung für NO_2 führte zu verringerten Schätzern bis hin zum Nulleffekt.

Die meisten Studien untersuchten kardiovaskulär-bedingte Krankenhausaufnahmen. Diese weisen auf einen stärkeren Zusammenhang für kürzere Zeitfenster bis zu 24 Stunden hin. Diese Assoziationen wurden nach Adjustierung für weitere Schadstoffe schwächer und zeigten keine klare Evidenz mehr für unabhängie UFP/quasi-UFP-Assoziationen.

Table V: Gesamttabelle zu Analysen in 11 Studien zu Krankenhauseaufnahmen/Ambulanzkontakte

Study	Respiratorisch	Ein-Schadstoff-Assoziation	Zwei-Schadstoff-Assoziation	Kardiovaskulär	Ein-Schadstoff-Assoziation	Zwei-Schadstoff-Assoziation
Evans et al., 2014	✓	(+)	(+) (no NO_2 adjustment)			
Gardner et al., 2015				✓	(+)/0	nc
Iskandar et al., 2012	✓	(+)	0			
Rosenthal et al., 2013				✓	(+)/+	0
Wichmann et al., 2013				✓	(+)/0	nc
Delfino et al., 2014	✓	nr	nr			
Diaz-Robles et al., 2014	✓	+				
Lanzinger et al., 2016	✓	(+)	0	✓	(+)/0	0
Samoli UK, 2016	✓	(+)/(-)	(+)	✓	(+)	(-)/(+)
Samoli EU, 2016	✓	(+)/(-)	(-)/-			
Liu et al., 2013				✓	+/(+)	nc

0 bezeichnet keine Assoziation. (+) und (-) bezeichnen primär nicht-signifikante Assoziationen, + und - bezeichnen signifikante Assoziationen. Nc: nicht durchgeführt. nr: nicht berichtet.

Subklinische Endpunkte

Die Mehrzahl der 11 Studien zu **subklinischen respiratorischen Endpunkten** (Tabelle VI) verfügen nur über eine geringe Stichprobengröße (15 bis 84 Teilnehmer). Darüber hinaus waren die Stichproben zumeist selektiv und repräsentierten entweder junge, gesunde Erwachsene oder Personen die unter Atopie/Asthma leiden. Die untersuchten Zeitfenster und Mittelungsperioden variieren je nach Studie, wobei die meisten Assoziationen in einem Zeitfenster von 0 bis 48 Stunden im Zusammenhang mit erhöhter Exposition beobachtet wurden. Letztendlich waren die Ergebnisse der meisten Studien inkonsistent in Bezug auf die einzelnen respiratorischen Endpunkte. Im Hinblick auf Peak-flow Endpunkte sind Messfehler aufgrund von selbst-erfassten Messwerten möglich, insbesondere in der Studie von Cole-Hunter et al. (2013), die nicht verblindet werden konnte. Trotz beobachteter Assoziationen in Einschadstoffmodelle in vier der 11 Studien kann aufgrund von fehlender Adjustierung für weitere Schadstoffe in Bezug auf unabhängige Effekte in den meisten Studien keine Schlussfolgerung gezogen werden. Studien mit Zwei-Schadstoffmodellen weisen auf eine zumindest teilweise Überlappung von UFP, bzw. PNC Effekten mit NO_2-Effekten hin.

13 Studien beobachtete adverse Assoziationen zwischen UFP/quasi-UFP-Expositionen und **Blutdruck-Indizes**, d.h. sie wiesen auf erhöhte Blutdruckwerte hin. Die Ergebnisse variierten je nach Endpunkt (systolischer, diastolischer, Pulsdruck), nach unterschiedlichen Größenfraktionen und untersuchten Zeitfenstern. Abgesehen von einer Studie mit mehr als 1.000 Teilnehmern, bestanden die Studien aus kleineren, überwiegend selektierten Studienpopulationen. Die Evidenz aus Studien mit Mehrschadstoff-Modellen ist zu gering um Schlussfolgerungen im Hinblick auf unabhängige UFP Effekte auf Blutdruck-Indizes zu ziehen.

Für **Herzratenvariabilität** (HRV) ist eine relativ große Studienanzahl von 16 Studien verfügbar, von denen 12 Studien in mindestens einen HRV-Endpunkt einen Zusammenhang beobachteten. Nach Adjustierung für weitere Luftschadstoffe veränderten sich die Assoziationen je nach Studie in beide Richtungen. Die einzelnen Studien nutzten verschiedene Zeitfenster und unterschiedliche Luftschadstoffe in den Mehrschadstoff-Modellen, so dass keine eindeutigen Muster beobachtet werden konnten.

In Anbetracht der begrenzten Studienanzahl zu **Arrhythmie**-Endpunkten mit nur einer vorliegenden Studie, ist die Evidenz weiterhin unzureichend.

Die Mehrzahl der sieben Studien, welche Assoziationen zwischen UFP/quasi-UFP und **vaskulärer Funktion** untersucht haben, weisen auf eine mögliche Assoziation hin. Auch hier verhindern jedoch fehlende Konsistenz im Studiendesign und insbesondere bei den Parametern des Endpunkts sowie fehlende Mehrschadstoffmodelle Schlussfolgerungen.

Alle der 12 durchgeführten Studien zu **pulmonalen Entzündungsprozessen** weisen auf Assoziationen zwischen UFP und adversen Veränderungen der pulmonalen Inflammationsmarker hin, insbesondere sofort nach der Exposition. Die Evidenzbasis für pulmonale Entzündungsprozesse in Folge von UFP-Exposition ist dennoch weiterhin begrenzt, da unterschiedliche Subgruppen, Expositionsmetriken, Endpunktmessungen und Zeitfenster genutzt wurden. Die zwei Studien, welche Mehrschadstoff-Modelle angewandt haben, beobachteten insgesamt robuste Effektschätzer.

Die Mehrzahl der 18 Studien, welche UFP-Effekte auf **systemische Entzündungsprozesse** untersucht haben, deuten auf inkonsistente Assoziationen hin. Effekte auf hs-CRP, Fibrinogen, Anzahl weißer Blutzellen, Myeloperoxidase variierten, was auf unterschiedliche Zusammensetzungen der Teilnehmer, erfasste PNC fraktionen und Expositionserfassung zurückzuführen sein kann. Die meisten Studien zeigen deutlichere Effekte für kürzere Zeitfenster. Eine begrenzte Anzahl an Mehrschadstoff-Modellen lassen keinen Rückschluss auf unabhängige Effekte von UFP/UFP zu, da nur zwei der durchgeführten 5 Studien mit Mehrschadstoffmodellen robuste Ergebnisse zeigten.

Table VI: Gesamttabelle der durchgeführten Analysen zu subklinischen Endpunkten in 45 Studien.

Endpunkt	Studien anzahl	Studienanzahl mit Ein-Schadstoff-Assoziationen in der erwarteten Richtung	Studienanzahl mit Mehr-Schadstoff-Assoziationen in der erwarteten Richtung	Kommentare (z.B. zu Studien mit signifikanten Schätzern in der nicht erwarteten Richtung)
Respiratory Indizes	11	4/11	3/3	Li et al. (2016) beobachtete signifikant positive Assoziationen zwischen UFP und FEV1‘* FVC*
Blutdruck	13	9/13	2/46	Zwei der neun Studien mit Assoziationen zeigten inkonsistente Ergebnisse je nach Zeitfenster.

[6] Eine von vier Studien zeigte keine Assozioationen in Einschadstoffmodellen. Eine weitere Studie (Rich et al., 2012) zeigte nicht alle Ergebnisse und wurden daher hier als nicht assoziiert dargestellt.

Endpunkt	Studien anzahl	Studienanzahl mit Ein-Schadstoff-Assoziationen in der erwarteten Richtung	Studienanzahl mit Mehr-Schadstoff-Assoziationen in der erwarteten Richtung	Kommentare (z.B. zu Studien mit signifikanten Schätzern in der nicht erwarteten Richtung)
HRV	16	12/16	3/5	In Zhang et al. (2013), sanken die Effektschätzer nach Adjustierung für NO2 und stiegen nach Adjustierung für O3.
Arrhythmie	1	1/1	nc	Starke Assoziationen mit PM0.25, nahezu protective Assoziationen zwischen Partikelanzahl und stündlich gemessener nächtlicher Tachykardie.
Vaskuläre Funktion	7	4/7	1/2	
Entzündungsprozesse in der Lunge	12	12/12	2/2	Die Mehrzahl der Studien untersuchten den Endpunkt FeNO
Systemische Entzündungsprozesse (inkl. Fibriongen	18	7/187	2/5	Signifikant inverse Assoziationen zwischen Fibrinogen und PNC nach Adjustierung für NO2 (Strak et al., 2013)
Neurokognitive Endpunkte	2	1	nc	-

HRV: Herzratenvariabilität, Nc: nicht durchgeführt. *Forciertes Lungenvolumen in einer Sekunke, ** forcierte Vitalkapazität

Chronische Gesundheitseffekte

Aufgrund einer geringen Anzahl an Studien, unterschiedlichen Endpunkte und Expositionserfassungsmethoden sowie fehlende Mehrschadstoffmodellen ist es nicht möglich, finale Schlussfolgerungen zu ziehen. Eine Übersicht der Studienergebnisse ist in Tabelle VII dargestellt.

Tabelle VII: Gesamttabelle der zehn Langzeitstudien

Endpunkt/ Studie	Endpunkt	Ein-Schadstoff-Assoziationen	Mehr-Schadstoff-Assoziationen
Mortalität/ Ostro et al. 2015	- Gesamtmortalität - kardiovaskular/ IHD - pulmonal	0 (+)/0 0	nc nc nc

[7] Die Mehrzahl der positive Assoziationen beziehen sich auf Fibrinogen.

Morbidität / Li et al. 2017	- kardiometabolisch	(+)	nc
Laurent et al. 2014/2016b	- geringes Geburtsgewicht	+/(+)	nc
Laurent 2016a	- vorzeitige Geburt	-/+	nc
Subklinisch/			
Aguilera et al. 2016	- karotid-intima-media Dicke		
Viehmann et al. 2015	(PNC/LDSA)	+/+	-/(+)
Lane et al. 2015	- hs-CRP/ Fibrinogen/ WBC	(+)/+/(+)	nc
Lane et al. 2016	- hs-CRP/ IL-6	(+)/(+)	nc
Sunyer et al. 2016	- hs-CRP/ IL-6/ TNRFIII/ Fibrinogen	(+)/(+)/(+)/(-)	nc
		(+)	nc
	- Arbeitsgedächtnis,	+	
	- übergeordnetes Arbeitsgedächtnis	+	
	- Unaufmerksamkeit		

0 bezeichnet keine Assoziation. (+) und (-) bezeichnen primär nicht-signifikante Assoziationen, + und - bezeichnen signifikante Assoziationen. Nc: nicht durchgeführt.

Zusammenfassung der akuten und chronischen Gesundheitseffekte

Eine Übersicht über alle eingeschlossenen Kurz- und Langzeitstudien spiegelt die Inkonsistenz der Ergebnisse wider (Tabelle VIII). Mehr als die Hälfte (n=49) der Studien zu Kurzzeiteffekten (n=79) berichtete zumindest einen signifikanten Effekt in Einschadstoff-Modellen, insbesondere Studien zu Mortalität oder subklinischen Endpunkten. Bei mehr als der Hälfte der Einschadstoff-Assoziationen (21 von 49) war das generelle Muster der Assoziationen konsistent - unabhängig vom Signifikanz-Niveau. 18 von 32 Studien in Mehrschadstoff-Modellen beobachteten zumindest einen signifikanten Schätzer. Von diesen war in etwa der Hälfte der Studien (7 von 18) das Muster generell konsistent. Die Assoziationen zwischen Langzeitexpositionen gegenüber UFP mit Gesundheitsendpunkten waren in Einschadstoffmodellen konsistenter (8 von 10), auch wenn es wesentlich weniger Studien waren. Es fehlen jedoch Langzeitstudien, welche für weitere Luftschadstoffe adjustieren - es wurde nur eine Studie identifiziert, welche im Mehrschadstoff-Modell keine Assoziationen zeigte.

Tabelle VIII: Gesamttabelle zu den Resultaten aller eingeschlossenen Studien

Endpunkt	Einschadstoff-Modell-Assoziationen	Konsistenz des generellen Musters	Mehrschadstoff-Modell-Assoziationen	Konsistenz des generellen Musters
Kurzzeit	49/79	21/49	18/32	7/18
Mortalität	5/7	2/5	4/6	1/4
Morbidität	3/7	0/3	nc	nc
Krankenhaus-einweisungen	4/10	2/4	0/5	nc
Subklinisch	37/55	17/37	14/21	6/14
Langzeit	8/10	1/1	0/1	nc
Mortalität	1/1	1/1	nc	nc
Morbidität	3/4	nc	nc	nc
Krankenhaus-einweisungen	nc	nc	nc	nc
Subklinisch	4/5	nc	0/1	nc

NC: nicht durchgeführt

Diskussion

Literatursuche

Wir haben eine umfassende systematische Suche zu relevanten epidemiologischen Studien über UFP und quasi-UFP für den Zeitraum vom 01.01.2011 bis 11.05.2017 durchgeführt. Unsere Suchstrategie setzte sich aus einer MEDLINE-Suche mittels einer Haupt- und einer alternativen Suchstrategie, einer Suche in der spezialisierten LUDOK-Datenbank sowie einer Handsuche in Reviewartikeln und Referenzlisten der identifizierten Publikationen, wobei die alternative Suche in MEDLINE sowie die LUDOK-Suche mit 15 von insgesamt 85 Publikationen einen beträchtlichen Zugewinn an Studien bedeutete. Ebenso ergab die Replikation der Suchstrategie im Februar 2018 mit 13 zusätzlichen Artikeln einen erhebliche Anzahl an Treffern. Diese relativ hohen Zahlen spiegeln die rasante Entwicklung des neu entstehenden Forschungsfeldes wider sowie den Wert einer spezialisierten Datenbank - wie in diesem Fall LUDOK -für eine zielgerichtete und zeitnahe Suche.

Bewertung der Relevanz von UFP für die Gesundheit

Unsere Bewertung der Relevanz von UFP auf die Gesundheit basiert auf den oben beschriebenen epidemiologischen Studien. Dabei wird zusätzlich berücksichtigt, inwiefern diese neu publizierten Studien die Evidenz des letzten umfassenden HEI-Berichts von 2013 erweitern. Insgesamt hat die epidemiologische Evidenz in den letzten Jahren erheblich zugenommen und es ist in den nächsten Jahren ein weiterer deutlicher Zuwachs an relevanten Studien zu erwarten. Derzeit befinden wir uns noch in den Anfängen der gesundheitsbezogenen Forschung zu UFP, was teilweise an den sich noch entwickelnden Methoden liegt (siehe Abschnitt unten zu Expositionserfassung).

Das HEI schlussfolgerte in seiner Übersichtsarbeit, dass die derzeitige Datenbasis an experimentellen und epidemiologischen Studien keine starken und konsistenten Rückschlüsse zu den unabhängigen Effekten von UFP auf die menschliche Gesundheit zulässt. Wesentliche Gründe für diese fehlende Evidenz, insbesondere der epidemiologischen Studien, liegen in der Schwierigkeit, die bevölkerungsbezogene Exposition gegenüber UFP sowohl für Kurz- als auch für Langzeit-Studien zu erfassen. Aufgrund der ausgeprägten zeitlichen und räumlichen Variabilität von UFP führen gängige Expositionserfassungsstrategien, welche für homogener verteilte größere Partikelfraktionen entwickelt worden sind, bei der Anwendung auf UFP zu größeren Fehlern bei der Expositionserfassung. Im Hinblick darauf folgert das HEI, dass unabhängige UFP-Effekte nicht ausgeschlossenen werden können, und empfiehlt die Erforschung alternativer Expositionsmetriken, räumlicher Modellierungstechniken und statistischer Methoden.

In dieser Übersichtsarbeit werden ähnliche Design- und Outcome-spezifische Kategorien verwendet wie im HEI-Review, um neue Erkenntnisse in den bisherigen Wissensstand integrieren zu können. Da die Unabhängigkeit der Effekte von anderen Luftschadstoffen ein Kernthema bezüglich der Relevanz von UFP auf die Gesundheit darstellt, fokussieren wir insbesondere auf Studien mit Mehrschadstoffmodellen.

Inkonsistenz der Ergebnisse nach Endpunkt

Vorherige Bewertungen folgerten, dass die kombinierten Ergebnisse für respiratorische wie auch kardiovaskuläre Endpunkte noch inkonsistent sind (Health Effects Institute, 2013). Wenn man die neu gewonnene Evidenz aus den Jahren 2011 bis 2017 betrachtet, hat sich dieses Bild nicht wesentlich verändert. Trotz einer wachsenden Zahl an Studien können wir kein eindeutiges Muster für respiratorische oder kardiovaskuläre Gesundheitseffekte in Bezug auf die Endpunkte Mortalität,

Morbidität, Krankenhaus-/Notfalleinweisungen oder subklinische Endpunkte identifizieren. Für weitere Endpunkte wie z.B. psychische Erkrankungen, neurokognitive Funktionen oder Geburtsergebnisse ist die Evidenzbasis noch zu gering um sichere Schlüsse zu ziehen.

Auch wenn die Ergebnisse bezogen auf die unterschiedlichen Endpunktarten nicht konsistent sind, weist die Mehrzahl der 11 Studien zu UFP-Kurzzeiteffekten auf Blutdruck, dem Hauptrisikofaktor für kardiovaskuläre Erkrankungen, auf einen Zusammenhang mit erhöhtem Blutdruck. Die Evidenz der drei für weitere Luftschadstoffe adjustierten Studien ist gemischt, was die Notwendigkeit weiterer Studien mit Mehrschadstoffmodelln unterstreicht.

Das Fehlen von konsistenten Ergebnissen kann durch mehrere Faktoren erklärt werden. Diese beinhalten Unterschiede in der Expositionserfassung (s.u.), in der Erfassung des Endpunkts, des Studiendesigns und -größe sowie unterschiedlicher Kontrolle von Störfaktoren, insbesondere unterschielliche Korrektur für weitere Luftschadstoffe (siehe unten).

Expositionserfassung

Insgesamt nimmt die Anzahl der Studien welche die Erfassung der Exposition und die Erforschung von Gesundheiteffekten durch UFP rasant zu. Ein wesentlicher Faktor, der zu dessen rasanten Zuwachs beiträgt, ist die Entwicklung neuer Messinstrumente, welche eine kostengünstigere Erfassung von UFP, z.B. mit Kondensationspartikelzählern, ermöglichen. Die Forschung ist jedoch noch in den Anfängen und neue Expositionserfassungsmethoden in epidemiologischen Studien müssen noch entwickelt und evaluiert werden.

Herausforderungen bei der Erfassung von UFP beinhalten deren hohe Variabilität in Zeit und Raum, was andere Erfassungsdesigns benötigt als die „klassischen“ Methoden, mit welchen die räumlich homogener verteilten größeren Luftschadstoffe wie $PM_{2.5}$ und PM_{10} erfasst werden. Die hohe räumliche Variabilität ist nicht nur in Langzeitstudien zu Gesundheitseffekten, welche auf Langzeit-Unterschieden in der Exposition beruhen von Bedeutung, sondern ebenfalls für Kurzzeitstudien mit zentralen Messstationen. Diese Studien setzen voraus, dass zeitliche Veränderungen von Tag und Tag in relativ großen Studiengebieten gleichmäßig stattfinden, das heißt wenn an der zentralen Messstation die Schadstoffkonzentration um einen bestimmten Wert steigt, so steigt die Schadstoffkonzentration an anderer Stelle um einen ähnlichen Wert. Diese Annahme muss für UFP nicht korrekt sein, da die örltiche UFP-Konzentration stärker von örtlichen Quellen abhängt, als die Feinstaubkonzentration. Wenn man davon ausgeht, dass bei der Erfassung von UFP daher im Vergleich zu anderen Luftschadstoffen größere Messfehler zu erwarten sind, ist eine systematische Verzerrung der Schätzer gegen Null in Zusammenhangsanalysen mit Gesundheitseffekten wahrscheinlich (Dionisio et al., 2014).

In Zukunft kann die Entwicklung von erweiterten Luftschadstoffmodellen, die räumliche und zeitliche Faktoren integrieren, zu einer präziseren Expositionserfassung in größeren Gebieten beitragen. Aktuelle Chemie-Transport-Modelle wie z.B. das deutsche EURAD-Modell, benötigen eine Anpassung der Methodik mit Aufnahme spezifischer UFP-Quellen, eine Validierung von Modelloutput mit Messungen und eine erhöhte räumliche Auflösung.

Eine zukünftige Herausforderung bezüglich der UFP-Expositionserfassung sind die bisher nicht standardisierten Messgeräte und der nicht-standardisierte Gebrauch von Partikelfraktionen in den Studien. Die üblicherweise verwendeten Messgeräte haben unterschiedliche untere Messgrenzen bezüglich der Partikelgröße. Da die Mehrheit der Partikel dem nucleation-mode (< 20 nm) der Partikelgrößenverteilung zugeordnet werden kann, können bereits geringe Unterschiede der unteren Messgrenzezwischen 1 und 20 nm zu erheblichen Unterschieden in der Partikelanzahlkonzentration führen. Des Weiteren beinhaltet die Beschreibung der Expositionserfassung nicht immer das exakte Größenspektrum der Partikel, was einen direkten Vergleich der Exposition der unterschiedlichen Studien behindert.

Langzeitexpositionen und Gesundheitseffekte

Im Gegensatz zur letzten umfassenden Übersichtsarbeit durch das HEI wurden zehn Studien veröffentlicht, die Langzeiteffekte von UFP auf verschiedene gesundheitliche Endpunkte untersuchen. Während die meisten dieser Studien erhöhte Punktschätzer für Assoziationen zwischen UFP und adversen Gesundheits-Endpunkten fanden, adjustierte nur eine Studie für weitere Luftschadstoffe einschließlich NO_2. Adjustierung für andere Luftschadstoffe führte zu verringerten Effektschätzern bis hin zu Effektschätzern in die entgegengesetzte Richtung.

Auch wenn die gegenwärtige Evidenz keine unabhängigen Langzeit-Effekte von UFP auf Gesundheits-Endpunkte zeigen, sollte dies auf keinen Fall als ein Beweis für das Fehlen eines solchen Effekts missverstanden werden. Gegenwärtige Methoden zur Erfassung von UFP-Langzeitbelastungen sind nicht gut geeignet, um die räumliche Varianz von UFP zu erfassen. Daher sind dringend weitere Studien nötig, welche innovative Methoden zur Erfassung individueller UFP-Expositionen anwenden und evaluieren. Bedeutende Anwendungsfelder für neu zu entwickelnde Methoden zur Erfassung der Langzeitexposition gegenüber UFP sind verkehrsnahe Expositionen. Dabei sollten Erhebungen der Langzeitexposition auch das neu auftretende Problem in Bezug auf Expositionen gegenüber UFP in der Umgebung von Flughäfen angehen, wie vor Kurzem beschrieben (N. Hudda et al., 2016).

Unabhängigkeit von Effekten

Die Evidenz zu unabhängigen Effekten insgesamt weiterhin unzureichend. Wir haben festgestellt, dass insbesondere neuere Studien verstärkt Mehrschadstoffmodelle durchgeführt haben, was eine positive Entwicklung darstellt (e.g., Aguilera et al., 2016; Croft et al., 2017; Lanzinger et al., 2016a; Samoli et al., 2016; Stafoggia et al., 2017). Die verschiedenen Studien nutzen jedoch verschiedene Adjustierungen und es gibt noch keine Standardstrategie zur Adjustierung für weitere Luftschadstoffe. Derzeit scheint NO_2 im Allgemeinen einen größeren Effekt auf den UFP-Punktschätzer zu haben als andere Luftschadstoffe (e.g., Lanzinger et al., 2016a&b; Su et al., 2015; Samoli, Andersen et al., 2016, Zhang et al., 2013). Gründe hierfür sind die Überlappung der Quellen sowie die höhere Übereinstimmung der räumlichen und zeitlichen Varianz von UFP und NO_2, was zu instabilden Modellen und verzerrten Effektschätzern in Mehrschadstoff-Modellen führen kann.

Übertragbarkeit der Ergebnisse auf die Situation in Deutschland

Die Übertragbarkeit der oben beschriebenen Ergebnisse auf die Situation in Deutschland wird nach folgenden Kriterien bewertet: Lokalität der identifizierten Studien, Expositionslevel gegenüber UFP und anderen luftgetragenen Schadstoffen, der Basisprävalenz der untersuchten Endpunkte sowie die Auswahl der Studienpopulation.

Lokalität und Exposition

Die große Mehrheit der identifizierten Studien wurden in Nordamerika (n=37, 43.5%) oder Westeuropa (n=27, 31.8%) sowie in mehreren Weltregionen (n=5, 6%) durchgeführt (Tabelle 2). Wenn wir die Studienorte der Studien mit mehreren Studienzentren berücksichtigt, beobachten wir, dass die Mehrzahl der Studienstandorte in West- und Südeuropa zu verorten sind (n=44 von 101 Studienstandorten, 43.6%)(Tabelle 3). Die Konzentration der UFP variieren beträchtlich in Raum und Zeit, so dass direkte Vergleiche der Messungen zwischen einzelnen Studienstandorten großer Variabilität in Bezug auf Stunde, Tag und Jahreszeit der Messungen sowie der exakten Platzierung der Messstandorte (Verkehr, urbaner Hintergrund, regionaler Hintergrund) (Birmili et al., 2016; UFIPOLNET, 2008) unterliegen.

Im deutschen Messnetz für Ultrafeinstaub (German Ultrafine Aerosol Network; GUAN), wurden Langzeit-Messungen ultrafeiner und feiner Partikel an 17 Standorten innerhalb Deutschlands,

einschließlich alpiner Standorte (Zugspitze), ländlicher Standorte, Standorte urbanen Hintergrunds sowie straßennahen Messstellen durchgeführt (Birmili et al. 2016). Zu beachten ist, dass Partikel mit einer Größe von 20 bis 800 nm gemessen wurden und somit nicht die nucleation-mode Fraktionen, jedoch die accumulation mode Partikel, umfassten. Vorläufige Ergebnisse der GUAN-Messungen ergaben, dass stündliche mediane Partikelanzahlkonzentrationen zwischen 900/ ml (Zugspitze) und 9.000/ml an der straßennahen Messtation in Leipzig rangieren. Stündliche durchschnittliche Konzentrationen sind etwas höher mit 1.120/ml an der Zugspitze und 10.500/ml in Leipzig. Das 95. Perzentil der Verteilung stündlicher Werte erreicht 22.400/ml in Leipzig-Mitte. Alle drei straßennahen Messstationen hatten Maximalwerte oberhalb 19.900/ml, während die Werte der urbanen Hintergrund-Standorte zwischen 10.000 und 20.000/ml rangierten. GUAN demonstiert ebenfalls die substantielle Variation der Partikelgrößenverteilung im Laufe einer Woche an sechs hauptsächlich urbanen Standorten.

Die in Westeuropa durchgeführten eingeschlossenen Studien messen typischerweise ähnliche oder höhere durchschnittliche Gesamtpartikelanzahlen. Mit der verfügbaren Information sind direkte Vergleiche nicht möglich, da die Messinstrumente sich unterscheiden und verschiedene untere Messgrenzen haben. 16 von 27 Studien aus Westeuropa gaben als untere Messgrenze ihrer Messgeräte 10 nm oder geringer an. Einige Geräte gehen bis zu 3 nm als unterste Grenze. Da die Mehrzahl der Partikel eine Größe von 20 nm unterschreitet (nucleation-mode) (HEI perspectives, 2013), können geringe Unterschiede des unteren Grenzwertes zu beträchtlichen Unterschieden der durchschnittlichen Exposition führen. Zusätzlich variiert der obere Grenzwert beträchtlich, wobei nur wenige Studien UFP in engerem Sinne (<100 nm) untersuchen, sondern eher die Gesamtpartikelanzahl als Surrogat für UFP-Exposition nutzen. Dies stellt jedoch ein geringeres Problem dar, da die Gesamtpartikelanzahl von den Größenfraktionen unter 100 nm dominiert wird (HEI perspectives, 2013).

Im Rahmen von GUAN konnte die große Variabilität der Expositionen innerhalb Deutschlands dokumentiert werden. Ein direkter Vergleich absoluter Werte mit denen anderer Studien ist jedoch wegen der unterschiedlichen eingesetzten Messinstrumente schwierig. Die fünf Studien aus Deutschland basieren auf zentralen oder personenbezogenen Messungen (n=4) mit unteren Messgrenzen zwischen 3 und 10 nm. Diese Studien ergaben mittlere Expositionen zwischen 10.000/ml und 20.000/ml, was mit anderen Studien in diesem Review vergleichbar ist. Im Vergleich hierzu berichten die 13 Studien, welche in der westlichenPazifik-Region oder Süd-Ost-Asien in den Metropol-Regionen von China, Südkorea oder Taiwan durchgeführt wurden, ähnliche oder minimal höhere Werte der gemessenen Partikelanzahlkonzentrationen. Die einzige Modell-basierte deutsche Studie wandte das EURAD-Chemietransportmodell an und erhielt wesentlich höhere mittlere Expositionen. Dies ist auf den Modellierungsprozess zurückzuführen, welcher den vollständigen nucleation-mode und damit auch kurzlebige Partikel kleiner 3 nm umfasst. Aus den Messwerten des GUAN Netzwerkes und den in Deutschland durchgeführten Gesundheitsstudien zu UFP schließen wir, dass das Expositionsniveau der in dieser Übersichtsarbeit betrachteten Studien zwar sehr variabel in Raum und Zeit ist, jedoch generell vergleichbar für die Situation in Deutschland ist.

Erwartete Entwicklung der UFP-Belastung in Deutschland

Die Entwicklung der UFP-Belastung der Bevölkerung in den nächsten Jahren hängt von mehreren Faktoren ab: (1) Der Bildung und Emission dieser Partikel, (2) der räumlichen Verteilung der Bevölkerung und (3) der Konzentration von feinen Partikel in der Umgebungsluft.

Gemäß eines pan-europäischen Inventars anthropogener Partikelanzahlen ist Straßenverkehr die bedeutendste Ursache von Emissionen in städtischen Gebieten und entlang stark befahrener Straßen (Health Effects Insitute, 2013).Verkehrsbezogene Emittenten von primären UFP sind Direkteinspritzer in Fahrzeugen, deren Anzahl in der letzten Dekade angestiegen ist und wahrscheinlich weiter

ansteigen wird (Köllner, 2016). Andererseits wurden Diesel-Motoren, welche ebenfalls Partikel der ultrafeinen Größenbereiche ausstoßen, mit Partikelfiltern ausgestattet. Dadurch wurde der Ausstoß feiner Partikel beträchtlich reduziert (gemäß EURO5a auf weniger als 5 mg/km). Die EURO5b-Norm setzte erstmalig ein Limit für UFP, und zwar auf 6×10^{11} (European Union, 2007). Insgesamt ist aufgrund des wachsenden Verkehrs und der ansteigenden Anzahl von Stadtbewohnern (Vallance & Perkins, 2010) in Zufkunft mit einer zunehmenden Exposition von relevanten Bevölkerungsanteilen gegenüber verkehrsbezogenen UFP zu rechen.

Eine weitere Quelle hauptsächlich ultrafeiner Partikel ist der Luftverkehr. Mehrere Expositionsstudien haben erhöhte UFP-Expositionen in Windrichtung von Flughäfen weltweit berichtet world (Neelakshi Hudda et al., 2014; Keuken et al., 2015; Masiol et al., 2017; Shirmohammadi et al., 2017; Stafoggia et al., 2016). Die zunehmenden Kurzzeit-Belastungen sind zeitlich korreliert mit Flugzeug-Bewegungen und erreichen Konzentrationen von bis zu 50.000 Partikeln/ml (Keuken et al.. 2015) sieben km in Windrichtung des Amsterdamer Flughafens und bis zu 75.000 Partikeln/ml (Hudda et al.. 2014) acht km in Windrichtung des Flughafens in Los Angeles. Dieselben Studien zeigen, dass Langzeit-Belastungen sieben km in Windrichtung mit mehr als 200,000 betroffenen Einwohnern in der Nähe des Flughafens Schipohl/ Amsterdamum bis zu dreifach erhöht sind (Keuken et al., 2015) und bis um das vier- bis fünffache erhöht acht bis zehn km in Windrichtung in Los Angeles (Hudda et al., 2014). Ähnliche Expositionsstudien laufen in Deutschland und werden erste Informationen zur Belastung der Anwohner deutscher Flughäfen erbringen. Angesichts des wachsenden Luftverkehrs werden Expositionen aufgrund von Flugzeugemissionen wahrscheinlich in Zukunft eine zunehmende Rolle spielen.

Des Weiteren beeinflusst die Konzentrationen feiner Partikel in der Umgebungsluft die UFP Konzentrationen insofern, als dass UFP mit größeren Partikeln zusammentreffen und dabei koagulieren. Eine höhere Konzentration feiner Partikel in der Umgebung wird daher die Entfernung von UFP aus der Umgebungsluft unterstützen. Bei einer Reduktion feiner Partikel werden UFP wahrscheinlich länger in der Luft zirkulieren als in einer Umgebung mit höherer Feinstaubkonzentration.

Exposition gegenüber weiteren Luftschadstoffen

Die Höhe weiterer Luftschadstoffe ist von Bedeutung, da die meisten dieser Schadstoffe eigene Effekte auf den untersuchten Endpunkt haben. 78 der 85 identifizierten Studien (92%) erfassten die Höhe von mindestens einem weiteren Luftschadstoff, wenn auch nur 34 der Studien in ihren Analysen für mindestens einen Luftschadstoff adjustiert haben (siehe Kapitel 4.3). Die Erfassung von und die Adjustierung für weitere Luftschadstoffe in den Studien ist daher nicht auf vergleichbare Art und Weise durchgeführt worden.

Die Analyse der Mehrschadstoffmodelle zeigte, dass $PM_{2.5}$ and NO_2 den größten Einfluss auf die UFP-Schätzer zu haben scheinen. Oft - jedoch nicht immer - führt die Adjustierung für NO_2 zu einer Schwächung der Assoziation zwischen UFP und dem Gesundheitsendpunkt (Leitte et al. 2012; Meng et al. 2012; Stafoggia et a.. 2017; Su et al. 2015; Iskandar et al. 2012; Lanzinger et al. 2016; Rosenthal et al. 2013; Gong et al. 2014; Janssen et al. 2015; Steenhof et al. 2013). Die Adjustierung für PM_{10} and $PM_{2.5}$ schwächt die UFP-Assoziation ebenfalls in mehreren Studien, in den meisten Studien jedoch in geringerem Maße als die Adjustierung für NO_2.

Die Höhe der weiteren Luftschadstoffe, dabei insbesondere $PM_{2.5}$ and NO_2, kann innerhalb Europas mit dem Bericht der europäischen Umweltagentur “Air quality in Europe — 2017 report” (European Environmental Agency, 2017) verglichen werden. Gemäß dieses Berichts rangiert Deutschland unter den 28 Mitgliedsstaaten mit der höchsten durchschnittlichen Belastung an NO_2 (European Environmental Agency, 2017; Fig 6.1).

Ähnlich wie für UFP, können die jährlichen Durchschnittswerte der ausgewählten Messstationen keinen umfassenden Überblick über die Belastungen der Studienpopulationen der eingeschlossenen Studien widergeben, da NO_2-Konzentrationen hoher Variabilität in Raum und Zeit unterliegen. Von den 34 Studien, welche für weitere Luftschadstoffe adjustierten, wurden 15 in Westeuropa durchgeführt. Von diesen wurden drei in Augsburg/ Deutschland durchgeführt. Die übrigen Studien wurden hauptsächlich in größeren Städten in der Schweiz, den Niederlanden, Schweden und Finnland durchgeführt, welche vergleichbare Verkehrsexpositionen haben.

Daraus schließen wir, dass die Ergebnisse bezüglich einer teilweisen Überlappung der Effekte zwischen UFP und NO_2, die wir in den westeuropäischen Studien dieser Übersichtsarbeit beobachten (Iskandar et al. 2012; Janssen et al. 2015; Rosenthal et al. 2013; Stafoggia et al. 2017; Steenhof et al. 2013), ebenfalls für Deutschland zutreffen.

Prävalenz der Erkrankungen

Die Mehrzahl der in diesem Review identifizierten Studien sind in West-/Südeuropa und Nordamerika zu verorten. Die ursachenspezifischen altersadjustierten Sterberaten für alle nichtübertragbaren Erkrankungen und für respiratorische Erkrankungen im Jahr 2015 ähneln sich innerhalb der WHO-Region Amerika (inklusive Südamerika, was nicht in diesem Review eingeschlossen ist) und der WHO-Region Europa (World Health Organization, 2016b). Auf der anderen Seite unterscheiden sich die jährlichen ursachenspezifischen altersadjustierten Sterberaten für kardiovaskuläre Erkrankungen, mit einer erheblich geringeren altersspezifischen Sterberate für untere Altersklassen in Amerika (211/10,000) verglichen mit der europäischen Region (344/10.000). Der Unterschied in dieser Statistik ist primär auf die Kombination beider amerikanischer Kontinente zurückzuführen. Verglichen mit anderen europäischen Ländern und den USA, die in diesem Review eingeschlossen wurden, hat Deutschland eine vergleichbare Verteilung der Ursachen für vorzeitige Todesfälle wie die Niederlande bei ischämischen Herzerkrankungen, Lungenkrebs, Alzheimer-Erkrankung, zerebrovaskulären Erkrankungen und COPD als den fünf Hauptursachen. Dieses Ranking ähnelt der UK, Dälnemark, Schweden, Spanien und den USA stark. Darüber hinaus erforscht die Mehrheit der Studien kurzzeitige subklinische Endpunkte (Tabelle 8) und innerhalb dieser Kategorie kardiovaskuläre, respiratorische und Biomarker-bezogene Endpunkte (Tabelle 9). Die Erfassung der Endpunkte in diesen Studien betrifft nicht die länderspezifischen ICD-Kodierungsrichtlinien.

Wegen der Ähnlichkeit der Verteilung von Krankheiten in Deutschland und den Ländern, in denen Studien zu Mortalitäts- und Morbiditätseffekten von UFP durchgeführt wurden, kann eine Übertragbarkeit der Ergebnisse auf Deutschland angenommen werde3n. Dies gilt auch für Studien mit subklinischen Endpunkten, solange keine Unterschiede in den physiologischen Markern zwischen der Studienpopulation und der deutschen Bevölkerungen zum Zeitpunkt der Ersterhebung bestehen.

Studienpopulation

Die meisten Studien dieses Reviews basieren auf selektiven Studienpopulationen (n=62, 72.9%), und nur zehn (11.8%) bzw. dreizehn (15.3%) Studien wurden als repräsentativ oder zumindest teilweise repräsentativ für die Allgemeinbevölkerung (Tabelle 14) erachtet. Bei den Studien, welche als komplett repräsentativ für die Zielbevölkerung erachtet wurden, handelt es sich um Zeitreihenstudien, welche auf der Allgemeinbevölkerung der jeweiligen Studienregion basieren. Eine dieser Zeitreihenstudien (Diaz-Robles et al. 2014) zielte auf ausgewählte Altersgruppen innerhalb der Allgemeinbevölkerung ab. Von den übrigen Studien wählten 13 (15%) eine zufällige Stichprobe der Bevölkerung. Von den zehn Studien, die Langzeit-Effekte eruieren, basiert die Mehrzahl der Analysen auf mehreren Hunderten oder Tausenden von ausschließlich in Westeuropa oder Nordamerika lokalisierten Teilnehmern. Von diesen zielen sechs Studien auf Erwachsenen-Populationen eines oder beiderlei Geschlechts ab (Ostro et al. 2015), und weitere vier Studien wählten Kinder als

Zielpopulation (Laurent et al. 2014, 2016a and 2016b; Sunyer et al. 2015). Unter den Kurzzeit-Studien sind die Studienpopulationen zumeist hochgradig selektierte kleine Gruppen von entweder gesunden (jüngeren) Erwachsenen oder Teilnehmer mit respiratorischen oder kardiovaskulären Erkrankungen wie Asthma, COPD, Erkrankungen der Koronararterien etc.

Schlussfolgerungen - Übertragbarkeit

Basierend auf den oben beschriebenen Belastungshöhen, Exposition gegenüber weiteren Schadstoffen, Basisprävalenz der Erkrankungen sowie Repräsentativität der eingeschlossenen Studienpopulationen folgern wir, dass die Gesamtergebnisse dieses Reviews mit angemessenem Vorbehalt auf die Situation in Deutschland übertragen werden können.

Wichtige Einschränkungen sind (1) der Mangel an Studien mit Adjustierung für weitere Luftschadstoffe, was insbesondere angesichts der hohen NO2-Belastungen in Deutschland relevant ist und (2) die Wahl hochradig selektierter Gruppen in den Kurzzeit-Studien, da diese oft keine spezifischen vulnerablen Bevölkerungsgruppen wie Personen mit unzureichend therapierten Erkrankungen, Neugeborenen und Kindern berücksichtigen.

Gesamtfazit

Die Erforschung von UFP-Gesundheitseffekten in epidemiologischen Studien nimmt schnell zu. In den letzten sieben Jahren wurden erhebliche Fortschritte gemacht, welche zwei der dringendsten offenen Forschungsfragen betreffen: Es wurden mehrere Studien zu Langzeit-Gesundheitseffekten von UFP publiziert. Zweitens wurden insbesondere in den neueren Studien Bemühungen unternommen, um für weitere Luftschadstoffe zu adjustieren und unabhängige Effekte der UFP zu identifizieren.

Trotz der offensichtlichen Weiterentwicklungen in den oben genannten Bereichen hat sich das Gesamtfazit für den erforschten Zeitraum nicht erheblich verändert.

Zunächst bleibt die Evidenz zu Gesundheitseffekten für die meisten der untersuchten Endpunkte uneindeutig oder unzureichend. Von den Studien zu Mortalität und Krankenhausaufnahmen/Ambulanzkontakten ergaben die relativ wenigen Studien mit Adjustierung für weitere Luftschadstoffe gemischte Ergebnisse und damit derzeit uneindeutige Evidenz. Was die Anzahl der Studien betrifft, ist die größte Evidenz für Studien verfügbar, die subklinische Endpunkte erforschen. Innerhalb dieser Studiengruppe zeigen Studien mit kardiovaskulären Endpunkten sowie Endpunkten zu pulmonalen und systemischen Entzündungsprozessen die konsistentesten Muster mit Assoziationen, die im Allgemeinen auf adverse Gesundheitseffekte hinweisen. Nichtsdestotrotz bleibt die Evidenz für die Unabhängigkeit der Effekte für diese Endpunkte ebenfalls limitiert, da nur wenige Studien für weitere Luftschadstoffe adjustiert haben und dies häufig zu einer Reduktion der Effekte führt.

Zweitens bleibt die Expositionserfassung der Bevölkerung aufgrund der spezifischen Eigenschaften der UFP schwierig. Die Studien, welche die Belastung mittels zentralen Messstationen erfassen, verpassen wahrscheinlich einen großen Teil der UFP-Variabilität, da räumliche Varianz nicht berücksichtigt wird. Studien, die klassische räumliche Modellierungsmodelle anwenden, benötigen die Integration von Techniken, die räumliche und zeitliche Variabilität genauer erfassen. Null-Effekte oder die Abnahme von UFP-Effekten nach Adjustierung für weitere Luftschadstoffe können zumindest teilweise mit Expositions-Missklassifizierung und Messfehlern erklärt werden. Die Erfassung der Exposition sollte den Messtechniken, Größenfraktionen und Lokalisierung der Messstationen besondere Aufmerksamkeit widmen. Auch die Berichterstattung sollte standardisierter sein, um Studienergebnisse besser vergleichen zu können.

Drittens kann die Unabhängigkeit von UFP-Effekten derzeit aufgrund geringer Anzahlen an Studien mit Adjustierung und der oben erwähnten Einschränkungen bezüglich der Expositionserfassung für

UFP nicht bewertet warden. Eine positive Weiterentwicklung ist der Zuwachs an Studien, die diesem Thema Beachtung schenken und zeitgleiche Expositionen mit anderen Luftschadstoffen zu berücksichtigen suchen.

Viertens besteht weiterhin ein dringender Bedarf an Langzeitstudien zu Gesundheitseffekten von UFP. Die Durchführung von qualitativ hochwertigen Langzeitstudien wird eine Weiterentwicklung von Modellierungstechniken erfordern, welche sowohl räumliche als auch zeitliche Varianz berücksichtigen. Des Weiteren sollten spezifische Situationen mit Spitzenbelastungen identifiziert und detaillierter beschrieben werden um die Erfassung von Langzeit-bezogenen Gesundheitseffekten zu ermöglichen. Während straßennahe Expositionen bereits als wesentliche Faktoren erkannt wurden, fehlen insbesondere Studien zu Flughafen-bezogenen Belastungen, welche kürzlich in Verbindung mit erheblichen Konzentrationsanstiegen im Vergleich zur Hintergrundbelastung gebracht wurden.

Zusätzlich zu diesen allgemeinen Schlussfolgerungen forgern wir, dass die Gesamtergebnisse dieser Übersichtsarbeit mit angemessenem Vorbehalt auf die Situation in Deutschland übertragen werden kann. Wichtige Einschränkungen sind (1) der Mangel an Studien mit Adjustierung für weitere Luftschadstoffe, die angesichts hoher Belastung von NO2 in Deutschland insbesondere von Bedeutung ist, sowie (2) der Nutzung hochgradig selektierter Bevölkerungsgruppen in den Kurzzeitstudien.

1 Background

1.1 Scientific Background

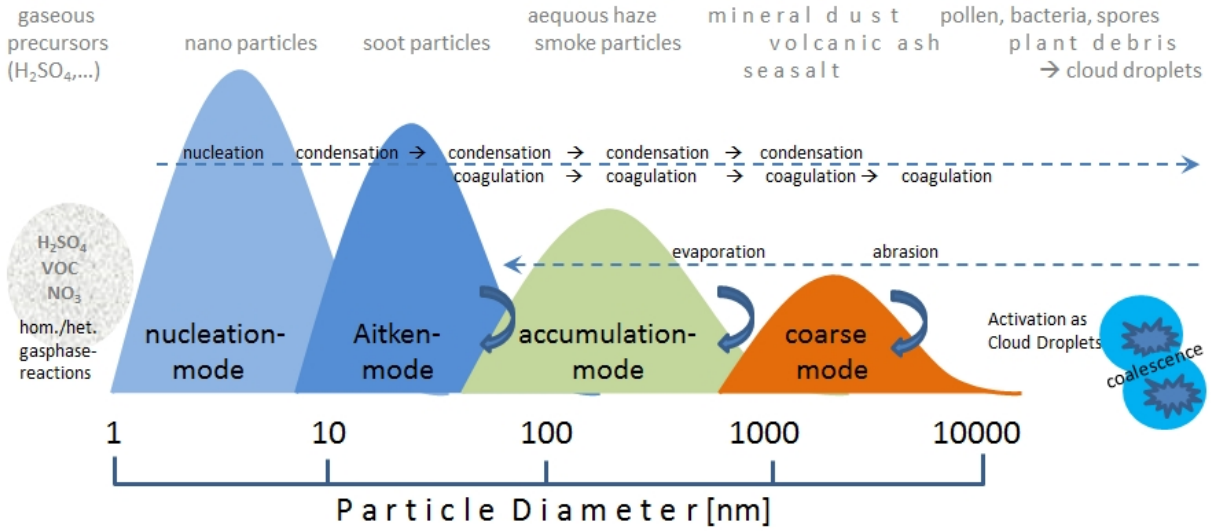
Environmental risks are important determinants of health and healthy ageing. Even if environmental risks are minor risks in relation to individual risk factors, their ubiquitous exposures may lead to a high attributable burden of disease at the population level (Cohen et al., 2017; Forouzanfar et al., 2016; World Health Organization, 2016a).

The pollution of ambient air, amongst others by particulate matter (PM, determined by different size fractions), increases all-cause mortality and has negative health impacts particularly on respiratory functions (e.g., asthma, COPD, lung cancer), the cardiovascular system (e.g. myocardial infarction, stroke, elevated blood pressure) as well as on metabolic changes and early childhood development (Thurston et al., 2017). It was estimated that ambient air pollution (AAP) accounts for more than four million premature deaths annually (Cohen et al., 2017) and thus is the most important environmental risk factors for mortality by chronic diseases worldwide (Forouzanfar et al., 2016). At the EU-level, AAP is estimated to account for about 400,000 premature deaths annually and to reduce life expectancy by nearly one year (European Environmental Agency, 2017).

AAP is a complex mixture of particulate and gaseous components. These include airborne particulate matter (PM), which can be divided by size and includes soot, and gaseous pollutants such as ozone (O_3), nitrogen oxides (NO_2, NO), sulphur dioxide (SO_2), volatile organic compounds (VOCs), and carbon monoxide (CO). Pollutants can be emitted from their source as primary pollutants, such as diesel soot and NO_2 from diesel powered combustion engines (referred to as primary pollutants), or they can be formed in the atmosphere from precursor substances (referred to as secondary pollutants). Ambient particulate matter in a specific city comes from a variety of sources, and can therefore differ widely in composition and extent. A detailed characterization of AAP is therefore useful when examining the consequences and potential abatement strategies for exposure reduction. By convention, airborne particles are often classified into three major groups by their size, irrespective of their sources or chemical composition, and measured as mass concentration. Particulate matter 10 (PM_{10}) is the mass of all particles with an aerodynamic diameter of <10 µm, $PM_{2.5}$ includes particles with an aerodynamic diameter of <2.5 µm. The mass of particles between 10 and 2.5 µm ($PM_{10-2.5}$) is usually called coarse PM. Ultrafine particles[8] are defined as 100 nm or less and measured mostly as particle number concentration, since they contribute only little to particle mass (Health Effects Insitute, 2013). Specifically in atmospheric modeling, further size fractions are defined according to the mode of generation (Figure 1). These modes include the nucleation mode (smallest particles with a diameter of up to approximately 20 nm) of particles that are formed from precursor substances, the so-called Aitken mode of particles formed by condensation (size range between approximately 10 and 80 nm), the accumulation mode particles which are formed by condensation and coagulation (size range approximately 50-1,000 nm) and the coarse mode, also formed by condensation and coagulation with a size range approximately 500-10,000 nm) (Baldauf et al., 2016). The coarse mode from atmospheric modeling is therefore not the same as the so-called PM_{coarse} (PM_{10}-$PM_{2.5}$),, which is derived from mass measurements and contains mostly larger particles from mechanistic processes (earth crustal material, break and tire wear).

[8] Nanoparticles which are often used in the context of ultrafine particles, represent industrially produced particles (N. Li et al., 2016)

Figure 1: Size-fractions of airborne particles (Source: Deutscher Wetterdienst, 2018)



In order to protect the human population against adverse health effects, mass concentrations of PM_{10} and $PM_{2.5}$ (particles with a diameter of less than 10 and 2.5 µm/m^3, respectively) are measured and evaluated in relation to air quality guidelines in many regions of the world. Up to now, routine measurements and regulations of the concentration of ultrafine particles (UFPs; particles with a diameter of less than 100 nm, see figure 1) are lacking.

UFPs vary with regard to their chemical composition and physical reactivity. They are emitted directly or are formed from precursors in atmospheric processes. In urban areas, UFPs mostly originate from combustion processes through motorized vehicles, particularly alongside roads (Health Effects Insitute, 2013; Kelly & Fussell, 2012).

Due to their small size, inhaled UFPs may enter into alveoli and are even capable to penetrate cell membranes. Consequently, UFPs may pass into the blood system, overcome the placental barrier, and finally diffuse into all organ systems including the brain and nervous system. Toxicological studies suggest that UFPs contribute to the development and progression of various diseases (Health Effects Insitute, 2013).

Epidemiological evidence for health effects of UFPs is scarce in comparison to that of larger particles. Nevertheless, an increasing number of epidemiological studies examining the exposure of the population and health effects of UFPs have been published in the last decade. Hypothesized health effects of UFP include cardiovascular and respiratory morbidity and mortality, the elicitation of local pulmonary and systemic inflammation and oxidative stress, and adverse actions on the brain and the metabolism. Two expert committees have reviewed and interpreted the epidemiological evidence base concerning UFPs (Health Effects Insitute, 2013; World Health Organization, 2013). The expert commissions of the Health Effects Institute (HEI) and of the World Health Organization (WHO) both concluded that scientific studies point towards adverse effects of UFPs on health. However, the evidence base on epidemiologic studies was not sufficient to recommend regulations on UFP concentrations.

Recently published epidemiologic studies now make it necessary to reevaluate the evidence base on the health effects of UFPs.

2 Hypotheses and Aims of the Study

The aims of this project were to systematically review the literature on the effects of UFPs on health, to evaluate the selected studies and to assess the transferability of the results to the situation in Germany. For this purpose, we focus on the following objectives:

(2) Conducting a systematic literature review
   a. Focus on health effects associated with ultrafine particles
   b. Emphasis on epidemiologic studies and quantitative effect measures (e.g., relative risks, dose-response relationships)
   c. Documentation of the literature search results and storage of all considered articles using a literature management database (EndNote).

(3) Evaluation of the identified literature
   a. Evaluation of individual study quality based on defined criteria
   b. Evaluation of the transferability of the identified findings to the present conditions in Germany

(4) Evaluation of the health relevance of ultrafine particles, specifically:
   a. Within the context of other air pollution exposures (e.g., PM_{10}, $PM_{2.5}$, ozone, nitrogen dioxide)
   b. With regard to the current German situation
   c. When considering the projected trajectory of ultrafine particle exposure in Germany

3 Methods

3.1 Selection Criteria for systematic review

We conducted a systematic literature review with a focus on epidemiologic studies that explore health effects of UFPs including quantitative effect measures *(work package 1 (a) search literature systematically in terms of health effects of UFP and (b) focusing on epidemiologic studies and quantitative effect measures (e.g., relative risk, dose-response-functions))*.

We included not only traditional/classic epidemiologic study designs such as cross-sectional studies, cohort studies and case-control studies, but also study designs often applied in environmental epidemiology such as time-series studies, panel studies, case-crossover studies, crossover studies and scripted exposure studies (novel study design in which participants are assigned to prespecified exposures, e.g. specific bike routes through a city). Furthermore, the studies had to comprise at least one of the following UFPs-measures: Particle numbers (PNC) for particles with a diameter of less than 100nm, $PM_{0.1}$, nucleation-mode, Aitken-mode particles as well as quasi-UFPs-measures: PNC for particles with a maximum diameter exceeding 100 nm, $PM_{0.25}$, surface area concentrations and accumulation mode particles. In terms of health outcomes, mortality, (international classification of diseases (ICD)-code determined) morbidity including symptoms, emergency/hospital admissions and subclinical outcomes were considered.

Toxicological studies were assessed only with regard to supporting evidence of the evaluation of UFP-related health relevance as stated in work package 3. Toxicological studies were not specifically considered in the search strategy.

Studies which investigate population related exposure to UFPs were assessed in order to evaluate the transferability of the reviewed results to the situation in Germany (work package 2b) and to evaluate the health related relevance of UFPs with regard to the situation in Germany (work package 3b) and in consideration of the potential trends of UFP exposure in Germany (work package 3c).

Studies focusing on occupational exposures to UFPs or to industrially engineered nanoparticles were excluded.

3.2 Databases

We systematically searched MEDLINE (Medical Literature Analysis and Retrieval System Online) and LUDOK for eligible studies investigating health effects of AAP related UFPs. The period included in the search was 1.1.2011 until 11.5.2017.

MEDLINE is a comprehensive database providing international literature in the fields of medicine, psychology and the Public Health system. Currently, MEDLINE contains more than 5,600 scientific journals. The search in MEDLINE was carried out through the search engine „PubMed“, published by the provider NLM (US National Library of Medicine).

In addition, we searched the LUDOK-database, which is provided by the Swiss Tropical and Public Health institute (Swiss TPH) on behalf of the Swiss Federal Office for the Environment. This database contains scientific literature on the effects of AAP on human health.

3.3 Search Strategies

3.3.1 HEI Search Strategy

In 2013, the Health-Effects Institute (HEI) published a comprehensive review on the health effects of ambient UFPs. The review was based on a literature search in the databases MEDLINE and Web of Science up to Mai 2011. In September 2011, the search was re-run and updated (Annex I, part 1).

The search of the HEI was performed by Dr. Stephanie Ebelt-Sarnat, Assistant Professor of Environmental Health at Rollins School of Public Health in Atlanta, Georgia. In the framework of our project, we replicated the search of the HEI. Divergences in the results were clarified in a phone call with Dr. Ebelt-Sarnat on 24.04.2017. The following issues were discussed:

- Applied keywords -> The HEI did not apply any truncations, only keywords documented in the search protocol (Annex I, part 1) were used.
- Eventually used field tags -> The HEI search did not include any special field-tags.
- As the HEI (1) imported the references from the Integrated Science Assessment – Particulate Matter (PM ISA) of the US EPA[9] in the Endnote database, followed by (2) references from the Web of Science and finally (3) imported the references from MEDLINE, we could not retrace in how far the references from the three different sources overlap.

The absolute number of retrieved references in the replicated search was about 2-3% higher, depending on the keyword. The search of the HEI could not be retraced thoroughly, as in the months following the HEI search, some references may not have been indexed in MEDLINE for the selected search period. When we replicated the search for the same time period, these references had been included. In order to tackle this problem in the current project, we set the starting point of our search on the 01.01.2011, i.e. about half a year earlier than the end point of the search period of the HEI.

[9] The National Center for Environmental Assessment of the United States Environmental Protection Agency (EPA) develops Integrated Science Assessments (ISAs) that summarize the science related to the health and ecological effects caused by these pollutants

3.3.2 LUDOK Search Strategy

The LUDOK database provides epidemiological and experimental original works studying the effects of „classical"/traditional ambient air particles on humans, as well as effects of further air pollutants that have an effect on the general population (i.e. excluding agents merely relevant in occupational settings). Additionally, meta-analyses and methodological work in this context is provided.

LUDOK performs a monthly search with a constant, very broad search strategy in PubMed. LUDOK uses the following keywords and field-tags: "Air Pollutants/adverse effects" [Mesh] OR "Air Pollution/adverse effects" [Mesh] OR "Air Pollutants" [Pharmacological Action] OR "Environmental Exposure/adverse effects" [Mesh] OR "air pollutants" OR "air pollution" OR "air pollutant".

Besides the regular search in PubMed, an intensive hand search was performed in over 20 relevant scientific and general journals as well as within the reference lists of publications (original works and reviews). Furthermore, LUDOK pursues notices from different sources as e.g., the Swiss TPH internal, the Bundesamt für Umwelt/ Schweiz (BAFU), the WHO and other research committees/teams.

A detailed description of the search strategy including a list of searched journals is provided in Annex 1, part 2.

3.3.3 Combined UKD Search Strategy

Our search strategy included a modified MEDLINE search of the HEI, a search in LUDOK and hand searches (Annex I, part 3).

MEDLINE Search

In the UKD search strategy, the keywords were extended in comparison to the HEI search keywords, following the very general search strategy of the LUDOK database (figure 2).

Figure 2: Search strategies of HEI, LUDOK and UKD (Source: own work, University hospital Düsseldorf)

	HEI	LUDOK	UKD
Database	**MEDLINE, Web of Science**	**MEDLINE (Embase was replaced by hand searches)**	**MEDLINE**
time period	until 09.05.2011	since 1929	01.01.2011 - 11.05.2017
Language	English	English, German, French, Italian	English, German
#1	"air pollution"	"air pollution" "air pollutant" "air pollutants" "Environmental Exposure/adverse effects" [Mesh] "Air Pollutants/adverse effects" [Mesh] "Air Pollutants" [Pharmacological Action] [Mesh] "Air Pollution/adverse effects" [Mesh]	"air pollution" "air pollutant" "Air Pollutants" "environmental exposure" "particulate matter" "Environmental Exposure/adverse effects" [Mesh] "Air Pollutants/adverse effects" [Mesh] "Air Pollution/adverse effects" [Mesh]
#2	"surface area" "number count" "number concentration" "particle count" "ultrafine"		"surface area" PNC "particle number" "ultrafine particle" "ultrafine particles" ultrafine "nano particle" "nano particles" nanoparticle nanoparticles PM0.1 PM0.25 "Accumulation mode" "Aitken mode" submicron*
#3	epidemiology health		epidemiology epidemiological epidemiologic health

The keyword „air pollution“, applied by the HEI, was expanded by the keywords „air pollutant“, „air pollutants“, „environmental exposure“, “particulate matter”, “air pollutants/adverse effects [Mesh], “environmental Exposure/adverse effects [Mesh], “air pollution/adverse effects” [Mesh] were complemented in our search.

In addition to the keywords for ultrafine particles used by the HEI search, the following keywords were added: “PNC”, “particle number”, „ultrafine particle“, nano particle“, „nanoparticle“, „PM0.1“, „PM0.25“, „accumulation mode“, “nucleation mode”, „Aitken mode“, submicron*. The keywords „surface area“ and “ultrafine”, which were applied by the HEI, were retained. The HEI keywords “particle count”, “number count” und “number concentration“ were already represented in our search by the keywords „PNC“ und „particle number“. A test search including the above named HEI-keywords did not result in further additional references.

The keywords related to health outcomes „health“ und „epidemiology“ applied by the HEI were extended by the adjectives „epidemiological“ and „epidemiologic“. In order to increase the specificity of our search (i.e. to reduce false-positive retrievals), we did not use the truncated keyword epidemiolog*). A truncation would have resulted in keywords that are not relevant or sensible for our project. Field-tags as [tw] were not used, as their usage did not influence the number of matches remarkably

Alternative search strategy in MEDLINE including health-specific outcomes

Based on the above described search strategy, an alternative search strategy including specific health outcomes has been applied. Instead of using the general keywords “health” and “epidemiology/ic/ical”, specific disease related keywords were used. A list with keywords provided by the UBA was extended (by keywords as allergi*, depression, dementia, vascular, asthma, COPD, inflammation, metabolic etc.) and reduced by keywords which did not yield further matches.

Further hand searches in reviews

Reviews of the last six years presented further sources of studies. The following reviews, which were known to the investigators, were searched a priori (Baldauf et al., 2016; Cassee, Heroux, Gerlofs-Nijland, & Kelly, 2013; Health Effects Insitute, 2013; Henschel & Chen, 2013; Rückerl, Schneider, Breitner, Cyrys, & Peters, 2011; Stone et al., 2016; World Health Organization, 2013):

- Rückerl R, Schneider A, Breitner S, Cyrys J, Peters A. 2011. Health effects of particulate air pollution: A review of epidemiological evidence. Inhal. Toxicol. 23:555–592; doi:10.3109/08958378.2011.593587.
- WHO, Regional Office for Europe. 2013. Review of evidence on health aspects of air pollution – REVIHAAP Project, Technical Report.
- Henschel S & Chen G, WHO, Regional Office for Europe. 2013. Health risks of air pollution in Europe – HRAPIE project. New emerging risks to health from air pollution – results from the survey of experts. World Health Organ. 65.
- HEI Review Panel. 2013. Understanding the health effects of ambient ultrafine particles. Heal. Eff. Inst. 122.
- Cassee FR, Héroux M-E, Gerlofs-Nijland ME, Kelly FJ. 2013. Particulate matter beyond mass: recent health evidence on the role of fractions, chemical constituents and sources of emission. Inhal. Toxicol. 25:802–812; doi:10.3109/08958378.2013.850127.
- Stone V, Miller MR, Clift MJD, Elder A, Mills NL, Møller P, et al. 2016. Nanomaterials vs Ambient Ultrafine Particles: an opportunity to exchange toxicology knowledge. Environ. Health Perspect.; doi:10.1289/EHP424.
- Li N, Georas S, Alexis N, Fritz P, Xia T, Williams MA, et al. 2016. A work group report on ultrafine particles (American Academy of Allergy, Asthma & Immunology): Why ambient

ultrafine and engineered nanoparticles should receive special attention for possible adverse health outcomes in human subjects. J. Allergy Clin. Immunol. 138:386–396; doi:10.1016/j.jaci.2016.02.023.

- Baldauf RW, Devlin RB, Gehr P, Giannelli R, Hassett-Sipple B, Jung H, et al. 2016. Ultrafine particle metrics and research considerations: Review of the 2015 UFP workshop. Int. J. Environ. Res. Public Health 13:1–21; doi:10.3390/ijerph13111054.

Besides these reviews, further reviews identified in our MEDLINE-search were screened for references.

Published abstracts from conference proceedings and search by authors

As being a young area of research, published abstract bands from the following relevant conferences and symposia were searched.

- UFP-Symposium 2016 of the TU Berlin und Umweltbundesamt on 22. and 23. September 2016 in Berlin. URL: http://www.tu-berlin.de/?167019
- ETH-conference „Combustion-generated nano-particles", 1997-2017. URL: http://www.nanoparticles.ch/conference_bibliography.html
- 6th International Symposium on Ultrafine Particles Air Quality and Climate Brussels, Belgium May 10 and 11, 2017. URL: http://ufp.efca.net/
- 20th Meeting of the Task Force on the Health Effects of Long-range Transboundary Air Pollution on 16–17 May in Bonn 2017. URL: http://www.euro.who.int/en/health-topics/environment-and-health/air-quality/news/news/2017/05/historic-20th-meeting-of-the-joint-task-force-on-the-health-aspects-of-air-pollution
- 3rd NANOAPP (Nanomaterials&Applications) 2017 (http://nanoapp.ios.si/) is a scientific meeting of acknowledged and renowned researchers, scientists and experts in the field of synthesis of various nanomaterials and their applications in Energy, Environment, Human Health, Sensors, Textiles, Medicine.
- 21st ETH-Conference on Combustion Generated Nanoparticles, June 19th to 22nd 2017, Zürich, Switzerland. URL: http://www.nanoparticles.ch/

3.4 Study selection by Inclusion and Exclusion Criteria

Ron Kappeler (RK) and Simone Ohlwein (SO) screened title, abstracts and – if needed – full texts of the studies with regard to the inclusion and exclusion criteria (see below). 10 % of the studies were screened by both reviewers. In case of uncertainties concerning the selection of a study the case was discussed by the whole team. If necessary, inclusion and exclusion criteria were clarified and extended. The process of the study selection is illustrated in a Flowchart (Annex I, part 4) and documented in a chart adapted to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) method (Figure 4).

Inclusion criteria

- Epidemiologic studies with an adequate study design, i.e.: cohort, case-control, cross-sectional, case-crossover, panel-studies, scripted exposures, time-series studies.
- Quantifiable measures of association containing at least one UFP measure/metric: Number (PNC) or size-fractioned PNC for particles < 100 nm, $PM_{0.1}$, nucleation-mode particles (NucMP) and Aitken-mode particles (AitMP) or containing at least one quasi-UFP effect measures: PNC < 3000, $PM_{0.25}$, $PM_{0.1}$, surface-area concentration or accumulation mode particles (AccMP).
- Quantifiable measures of association including at least one measure: Odds ratio, relative risk, hazard ratio, β-estimates of percent change or exposure-response functions.

- Health outcomes including mortality or ICD-coded diseases, symptoms, emergency/hospital admissions/visits, preclinical outcomes.
- Languages: English, German.
- Year: Studies published from 2011 onward until 11.5.2017 which were not included in the HEI review; studies published after the deadline are listed in the appendix (annex I, part 5)

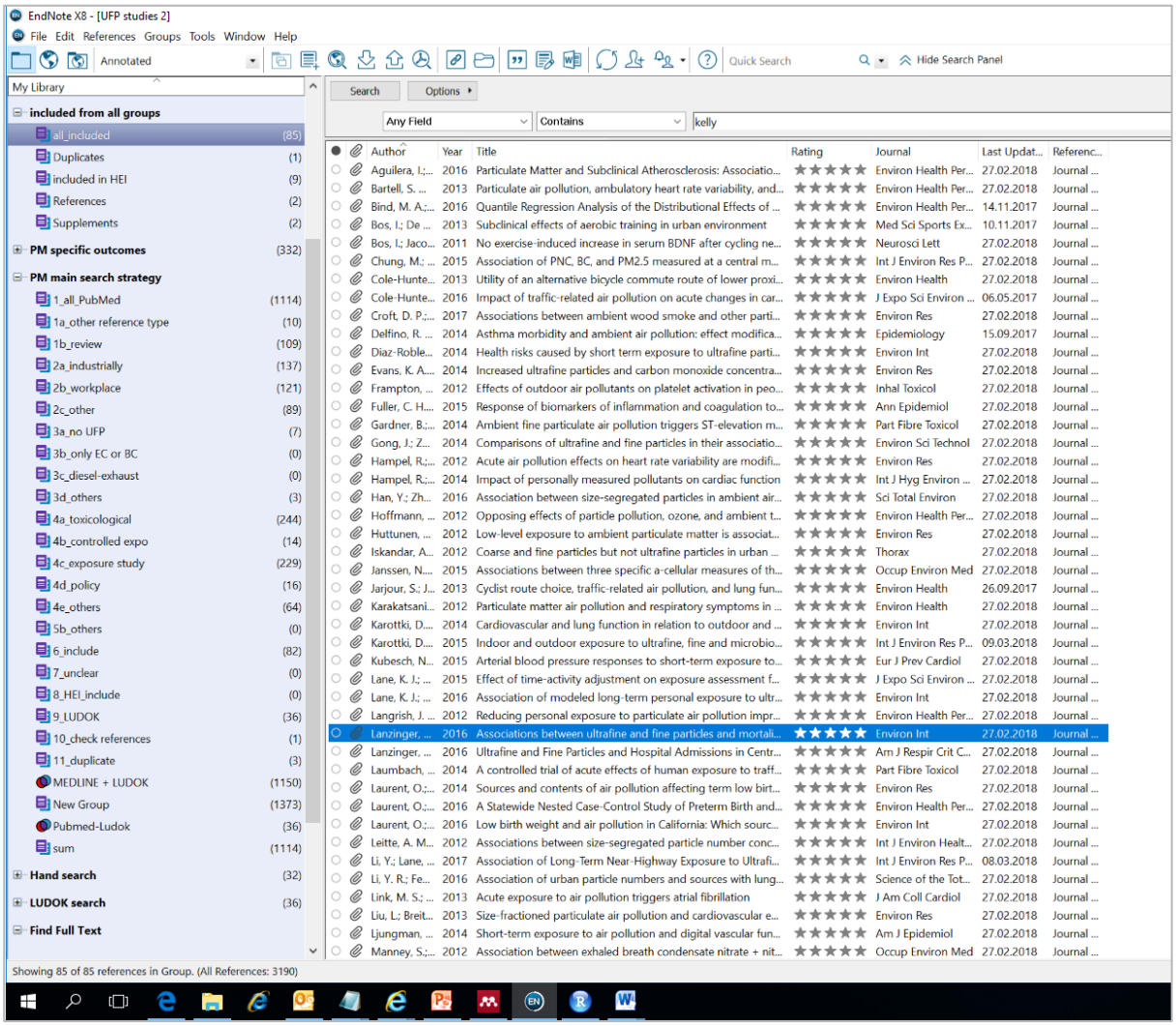
Exclusion criteria

- Toxicological studies, controlled exposure studies, animal experiments, in-vitro studies,
- Exposure to industrially engineered nanoparticles,
- Exposure to nanoparticles/ UFPs in occupational settings,
- Exposure to source-related indoor nanoparticles/ UFPs,
- Exposure to diesel particles, BC or EC only,
- Distance measures in substitution of exposure measurements
- Health outcomes of unclear health relevance, e.g. epigenetics, metabolomics, methylation.

3.4.1 Organization of the References

All references were organized within a library of the reference management program "Endnote". Access was provided for all project team members. Group sets have been created for the four different sources "MEDLINE main search strategy", "MEDLINE outcome specific search strategy", "Hand Search" (including search by author) and "LUDOK". Within each group set, separate groups have been created to document the assignment of the references analogous to the exclusion criteria (Figure 3). Furthermore, duplicates were documented within separate groups. To prevent mismatches, duplicates were not discarded automatically.

Figure 3: Example: Organization of the References in Endnote in separate groups (Source: own work, University hospital, Düsseldorf)



3.5 Data extraction to evaluate the studies quantitatively and qualitatively (WP II)

The identified articles were evaluated concerning their quality of report, significance and contents as well as their transferability to the German context. The established quality criteria (Annex I, part 6) are adapted from the Quality Assessment Tools of the National Heart, Lung and Blood Institute of the National Institute of Health (2014). When developing the different criteria, special attention was paid to exposure assessment. The available standardized instruments do not include this crucial element of studies in environmental epidemiology to the extent necessary and in the required depth. Therefore, new criteria had to be developed.

In particular, criteria to evaluate the applied measurement devices, the representativeness of the measurement sites for the exposure of the target population, the validity of used exposure models and for the assessment/modeling of several air pollutants.

Assignment of study designs

We assigned repeated measure analyses embedded within a cohort study as short-term cohort study. Scripted exposure studies are a relatively novel study design in which participants are assigned to prespecified exposures, e.g. specific bike routes through a city. Scripted exposure studies also contain so-called crossover studies, in which participants are exposed to different prespecified exposure scenarios.

There were single studies that measured outcomes in a weekly timeframe (Bind et al., 2016; Bos et al., 2013). By practical reasons, we decided to assign medium-term studies either to short- or long-term studies, depending whether their exposure assessment primarily relied on temporal variability (short-term studies) or whether it was based on spatial variability (long-term studies). This was done because these two design aspects determine the choice of the model in a major way.

4 Results

4.1 Literature search

Literature Research

The application of the main search strategy in MEDLINE yielded 1,114 references, the application of the alternative outcome-specific MEDLINE search strategy yielded 992 references, of which 332 were not included in the main search strategy (Figure 4). Together, the MEDLINE search yielded 1,446 references. The yield of the two MEDLINE searches was different regarding the focus of the resulting references: While the main search yielded many exposure studies, the alternative search strategy with specific health outcomes yielded many toxicological and animal experimental studies.

The search in the LUDOK database yielded 106 references, of which 30 were additional to the MEDLINE search. Another 8 additional references were identified through hand search in other sources such as the 142 identified reviews, original article reference lists, conference proceedings, and author search, yielding an overall total of 1,484 unique references that were examined for in- and exclusion criteria.

Exclusion of articles

Out of the 1,484 identified unique references, 1,399 were excluded by title and abstract and - if necessary by full-text - according to our predefined exclusion criteria, leaving an overall number of 85 orginal references for our further evaluation (Figure 4).

Exclusions were mainly due to type of study (review, toxicological, exposure, policy, other publication type). In detail, 744 studies were excluded due to study type. Of those, 142 references were excluded because they were reviews. 14 studies did not include appropriate UFP measures and 11 studies were already included in the HEI review. Further 475 studies were excluded due to source of investigated particles (industrial, occupational, indoor).

Yield of articles by search strategy

The final number of 85 original references included in this systematic review was achieved from the following sources: Of the 1,114 unique references identified by the main MEDLINE search strategy, 70 references were included in the analysis. Of the 332 unique references identified by the alternative outcome-specific MEDLINE search strategy, 3 additional references were identified for the review.

Of the 106 LUDOK references, 76 references were duplicates of already identified references through the combined MEDLINE search. 30 unique references that had not been identified through the MEDLINE search were further investigated: Of those, 14 references were assigned to the group of

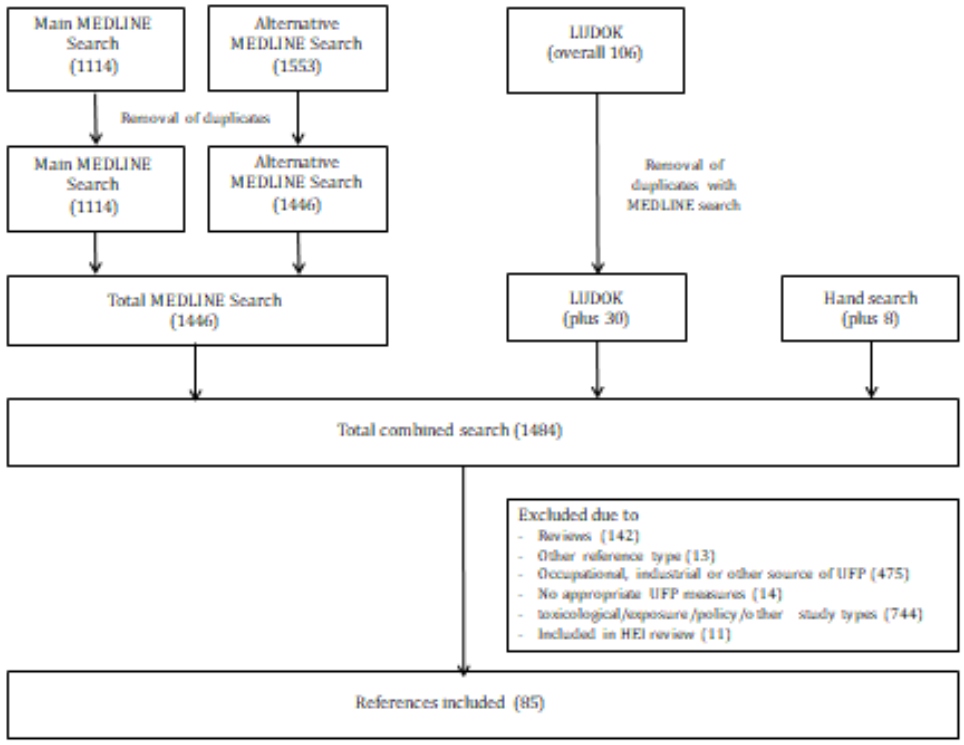
reviews and 8 references were excluded due to the other predefined exclusion criteria. Finally, altogether 8 relevant studies were identified additionally by the LUDOK database.

Of the 8 studies identified through hand search, 4 studies were added to the final analysis database.

Repeated search

In a repeated search on 23.02.2018, limited to articles published or accepted after the closing date of the full search, we identified another 13 articles, which are listed in the appendix (Annex I, part 5). These articles are not included in the detailed analysis of this report, but are added for the benefit of future evaluations.

Figure 4: Study selection process adapted to the PRISMA method (Source: own work, University hospital, Düsseldorf)



Evidence base from previous reviews

Our literature research and knowledge draws upon some relevant reviews published recently. The HEI provides the most thorough and complete information on the relationships between UFPs and various health effects. This report reviewed 79 primary research articles that examined the effects of UFPs and quasi-UFPs (>1,000nm) on health published after the U.S. EPA's 2009 Integrated Science Assessment for Particulate Matter until 2011. HEI found a growing number of studies assessing the health effects of UFPs as their main focus or as one of several pollutants of interest. They found 25 epidemiological studies assessing the short-term health effects of ambient UFPs characterized by particle number, which were not included in the 2009 PM ISA. However, HEI stated that "the evidence to date continues to lack consistency and coherence (…) whether ambient UFP's affect human health differently or independently from the effects of other particle or gaseous co-pollutants" (Health Effects Institute, 2013, p.63). The body of research, which has provided a suggestive but not definitive answer on the adverse health effects of UFPs on respiratory and cardiovascular outcomes was facing three issues according to HEI:

1) Inconsistency of Outcomes

Some studies on respiratory and cardiovascular outcomes report associations with UFP exposure (e.g. (Song et al., 2011) while others do not (e.g. De Hartog et al. 2010). Various factors such as different study designs, populations examined and UFP metric utilized or differences in pollutant composition might contribute to those inconsistencies.

2) Exposure assessment

There is a lack of larger epidemiologic studies of air pollution health effects because UFP monitoring data are scarce and when they are routinely assessed they are measured in different ways. Studies depending on only one monitor might miss the high spatial variability of UFP concentrations (Fanning et al., 2009; Terzano, Di Stefano, Conti, Graziani, & Petroianni, 2010; U.S. Environmental Protection Agency, 2009). Exposure misclassification may be at least partly responsible for null findings for health effects of UFPs.

3) Independence of UFP Effects

If UFP data is available, the high covariation with other combustion-related pollutants makes it difficult to disentangle the independent effects of UFPs from other pollutants. Therefore, studies adjusting their models for expected co-pollutant effects are conducted rarely. Even in studies where other metrics or gases were measured, co-pollutant exposures were not addressed in the analysis.

On top of those issues, HEI couldn't find any studies on long-term exposure effects of UFPs. Therefore the evidence base in 2013 on epidemiologic studies was not sufficient to recommend regulations on UFP exposure concentrations.

In February 2015 the United States Environmental Protection Agency invited experts from around the world to discuss and present evidence of health effects associated with UFP exposure, which has been summarized in 2016 (Baldauf et al., 2016). According to that workshop, short-term epidemiological studies provided evidence that exposure to traffic pollution (rich in UFPs) was associated with adverse cardiovascular outcomes, however, the effects still couldn't be reliably disentangled from other PM fractions or other gaseous pollutants. The scarce UFP monitoring networks still had not allowed for a comprehensive examination of long-term UFP exposures and adverse health outcomes in more locations. Similar to HEI's conclusion, epidemiological studies did not provide enough evidence that UFPs are more potent than other PM size fractions. Nevertheless, toxicological concerns about health effects of UFPs suggested that particle size may need to be considered in assessing potential adverse effects of exposures to PM (Baldauf et al., 2016).

Chen et al. (2016) thoroughly reviewed articles on composition of UFPs, their sources, typical characters, oxidative effects and potential exposure routes with a main focus on toxicology. Furthermore they also considered evidences emerging from nanotoxicology, as this research field contributes to the understanding of toxicity mechanisms of airborne UFPs in air pollution. They concluded that UFPs play a major role in adverse impacts on human health, but further investigations are required and efforts have to be made to raise awareness of the critical hazardous potential of UFPs among the public and authorities.

An American working group (Li et al. 2016) reevaluated the conclusions made by the HEI report by assessing experimental, epidemiological and clinical trial studies published in 2014 and 2015. The authors mentioned a critical knowledge gap in clearly identifying the impact of exposure to the nano-scale pollutants on human health. However, due to new evidence, especially from experimental and toxicological studies, they questioned the validity of HEI's conclusion that there is no evidence that the adverse health effects of UFP were dramatically different from those of $PM_{2.5}$. E.g., toxicological studies suggest that UFPs promote allergic lung inflammation and are capable of inhibiting the immune

response to infectious pathways. Nevertheless, the authors concluded that the issues of epidemiological studies assessing health effects of UFPs reported by the HEI Panel still remained.

Heinzerling et al. (2016), examining respiratory health effects of UFPs in children, identified 12 relevant articles from which four were not included in the HEI-report. In single pollutant models, exposure to UFPs were associated with incident wheezing, current asthma, lung function and emergency department visits due to exacerbation of asthma. Despite the recommendations from the HEI report, there were no long-term studies conducted since the publication of the report and only one study that reported a significant association between asthma emergency department visits and UFPs, also adjusted for co-pollutants (Halonen et al., 2008). In this study, the association was no longer significant after adjusting for NO_2 exposure. Even though the evidence between UFPs and children's respiratory health is accumulating, the authors concluded for the same reasons stated by the HEI Panel that the evidence remains inconclusive.

In addition, Clark et al. published in 2016 a study focusing on biological mechanisms of cardiovascular effects beyond the alveolar barrier within the body or in vitro tissues exposed to UFPs and quasi-UFPs of up to 500 nm size. They concluded that there is some (e.g. altered autonomic modulation with increases of heart rate in animal models) up to strong evidence (e.g. vasoconstriction induced by endothelium-dependent and independent pathways mediated through UFPs) for various cardiovascular outcomes (heart rate, vasoactivity, atherosclerotic advancement, oxidative stress, coagulability, inflammatory changes). The authors state that oxidative stress is important in mediating downstream cardiovascular outcomes such as vasoactivity, heart rate etc., and therefore this might be a good target to mitigate outcomes associated with UFP exposure.

Table 1: Previously conducted reviews including search period (ordered chronologically)

Reference	Title	Comments
HEI Perspectives 3, (2013)	Understanding the Health Effects of Ambient Ultrafine Particles	79 primary research articles, published after PM ISA until December 2011
Baldauf, R. et al. (2016)	Ultrafine Particle Metrics and Research Considerations: Review of the 2015 UFP Workshop	Summary of a workshop from February 2015
Chen, R. et al. (2016)	Beyond $PM_{2.5}$: The Role of Ultrafine Particles on Adverse Health Effects of Air Pollution	Thorough review of toxicity mechanisms of airborne UFPs
Li, N. et al. (2016)	A Work Group Report on Ultrafine Particles: Why Ambient Ultrafine and Engineered Nanoparticles Should Receive Special Attention for Possible Adverse Health Outcomes in Human Subjects	34 (mostly toxicological) studies that are not included in HEI
Heinzerling. et al. (2016)	Respiratory Health Effects of Ultrafine Particles in Children: A Literature Review	4 out of 12 epidemiological studies are published after HEI (until February 2015)
Clark et al. (2016)	The Biological Effects upon the Cardiovascular System consequent to Exposure to particulates of less than 500 nm in size	Focusing on biological mechanisms of cardiovascular effects beyond the alveolar barrier

Reference	Title	Comments
		(studies until January 2013)

4.2 Study characteristics

Location

Overall, 85 studies published between 29.06.2011 and 26.04.2017 were identified. Most of these studies were conducted in North America (n=37) or Western Europe (n=27) (see Tables 2 and 3). Further 12 studies were performed in the Western-Pacific region. Only very few studies were conducted in Middle/ South America (n=1), Eastern Europe (n=2) and South-East-Asia (n=1). Three out of five multi-center studies included studies conducted in several Western Europe countries (Karakatsani et al., 2012; Manney et al., 2012; Samoli, Andersen, et al., 2016), two multi-center studies included study sites located both in Western and Eastern Europe countries (Lanzinger et al., 2016a, 2016b).

Table 2: World regions of studies

World region	Number of studies	%
Africa	0	0.0%
North America	37	43.5%
Middle/ South America	1	1.2%
Western Europe	27	31.8%
Eastern Europe	2	2.4%
South-East-Asia	1	1.2%
Western-Pacific	12	14.1%
Multiple study regions	5	5.9%
Total	**85**	**100.0%**

Table 3: World regions of studies, with multi-center studies assigned to multiple study locations

World region	Number of studies	%
Africa	0	0.0%
North America	37	36.6%
Middle/ South America	1	1.0%
Western Europe	44	43.6%
Eastern Europe	6	5.9%
South-East-Asia	1	1.0%
Western-Pacific	12	11.9%
Total	**101**	**100.0%**

Time frame and study design

The majority of the studies were related to the investigation of short-term effects (n=75) measuring outcomes during hours to weeks after exposure. Ten studies investigated long-term associations

using exposure estimates averaged over a period of months to years. Among the included long-term studies, most studies used exposure time windows of one year. The study with the largest exposure window covered seven years (Ostro et al., 2015). Short-term studies are dominated by panel studies - 31 as repeated measures and one in a cross-sectional design, scripted exposure studies (n=16), and time-series studies (n=11). Further studies investigating short-term associations were case-crossover (n=8), cohort (n=4) and cross-sectional studies (n=4). The studies with a long-term study design consisted of cohort studies (n=4), cross-sectional studies (n=4), one case-cohort and case-control study, respectively (Table 4).

Table 4: Study design by long-term/ short-term studies

Design	Number of studies	%
Long-term	all=10	
Case-cohort study	1	1.2%
Case-control study	1	1.2%
Cohort study	4	4.7%
Cross-sectional study	4	4.7%
Short-Term	all=75	
Cohort study	4	4.7%
Cross-Sectional study	4	4.7%
Panel (cross-sectional)	1	1.2%
Panel (repeated measure)	31	36.5%
Case-crossover	8	9.4%
Scripted exposure	16	18.8%
Time-series	11	12.9%
Total	**85**	**100.0%**

% numbers are related to the sum of the long-/medium and short-term studies, respectively

Exposure assessment

Overall, most studies used measurement-based exposure assessments (87.1%) (Table 5). Model-based exposures were used in 10.6% of the studies. In long-term studies, mostly model-based exposure were used (9 out of 10), whereas the majority of short-term studies used measurement-based exposures (71 out of 75). This pattern is attributable to the fact, that model-based exposures are necessary to capture the spatial variation in exposure, which is the required exposure contrast for the assessment of long-term effects in cohorts studies (Aguilera et al., 2016; Ostro et al., 2015; Viehmann et al., 2015), cross-sectional studies and case-control/ case-cohort studies and less used in typically short-term study designs as time-series or scripted exposure studies. Among the identified studies, only two short-term studies applied model-based exposures, one cross-sectional study (Fuller et al., 2015) and one time-series study (Delfino et al., 2014).

Table 5: Exposure assessment technique of medium-/long-term and short-term-studies

Exposure	Number of studies	%

Long-term	**10**	
Model based	9	10.6%
Measurements only	1	1.2%
Short-Term	**75**	
Model-based	2	2.4%
Measurements only	73	85.9%
Total	**85**	**100.0%**

The majority of the studies applied central-site measurements (n=45), followed by mobile measurement techniques (n=17) and combination of different modeling/ measurements (n=10), e.g central-site measurements in combination with spatio-temporal land-use regression models, residential measurements or microscale personal exposure models (Table 6).

Table 6: Type of expose models/ measurements used in the studies

Exposure model/measurement	Number of studies	%
Chemical-transport model	3	3.5%
Land-use regression model	1	1.2%
Dispersion model	1	1.2%
Measurement: Central site	45	52.,9%
Measurement: Residential	2	2.4%
Measurement: Mobile	17	20.0%
Microscale personal exposure model	2	2.4%
Other	4	4.7%
Combination of different types	10	11.8%
Total	**85**	**100.0%**

In most studies, UFPs were assessed as particle number concentrations (PNCs) per volume (Table 7). In about one third of the studies, PNCs sized up to 100 nm were used (29 out of 92[10]). In 63 studies, quasi-UFPs sized PNC up to 3,000 nm were used. In relation to different size modes, only one study used nucleation mode particles (n=1), representing particles with a diameter of less than 10 nm and Aitken-mode particles (n=1), representing particles with a diameter of 10-100 nm. In 14 studies, accumulation mode particles (AccMPs) were used, representing particles with a diameter of 100-1,000 nm[11] (see figure 1, p.13). Particles measured as mass per m^3 were used in 13 studies: In 6 studies, submicron $PM_{0.1}$ particles were assessed, in 7 studies, quasi-UFP $PM_{0.25}$ or $PM_{0.1}$ particles were assessed. Lung-deposited surface area (LDSA) was only used in two studies, of which one was long-term and one short-term.

[10] As many studies used various size-fractioned PNCs, the number of analyses using PNCs with a size up to 100 nm (n=29) and/or up to 3,000 nm (n=66) exceed the number of 75 included studies that assessed PNCs.

[11] In literature, different cutpoints are used to divide particles in the different modi.

Table 7: Particle metrics used in the studies

	Number of studies	%
PNC < 100 nm	29	23.6%
PNC < 3,000 nm	63	51.2%
NucMP	1	0.8%
AitMP	1	0.8%
AccMP	14	11.4%
$PM_{0.1}$	6	4.9%
$PM_{0.25}$	3	2.4%
PM_1	4	3.3%
LDSA	2	1.6%
Total	**123**[12]	**100.0%**

Type of outcome

We analyzed the number of studies according to the main type of outcome (mortality, hospital admissions/emergency, subclinical outcome measure, table 8) and by dividing outcomes according to major organ systems (Table 9). Eight studies assessing mortality (7 short-term, 1 long-term) analyzed the effects of UFPs on total, cardiovascular or respiratory mortality. Eleven (7 short-term, 4 long-term) studies analyzed the effects on cardiovascular, respiratory, or other morbidity outcomes. Eleven studies (all short-term) investigated UFP effects on cardiovascular or respiratory disease-related emergency calls/ hospital admissions. The vast majority of studies (n=60, 55 short-term, 5 long-term) used various subclinical measures as health outcomes, e.g. systemic inflammation. Three studies investigated several different types of main outcome types.

Table 8: Health outcome types of long-term and short-term-studies

	Number of studies	%
Long-term	All=10	
Mortality	1	1.1%
Morbidity	4	4.4%
Emergency/hospital call/admission	0	0.0%
Subclinical	5	5.6%
Short-term	All=80	
Mortality	7	7.8%
Morbidity	7	7.8
Emergency/hospital call/admission	11	12.2%
Subclinical	55	61.1%
Total	90	100.0%

[12] As many studies used various size-fractioned PNCs, the number of analyses using PNCs with a size up to 100 nm (n=29) and/or up to 3,000 nm (n=66) exceed the number of 75 included studies that assessed PNCs.

* Three studies analyze several types of outcome measures and were assigned to two different outcome types. Thus, the studies do not sum up to 85 studies.

Most studies measured cardiovascular organ system-related outcomes (4 long-term, 47 short-term), followed by inflammatory (3 long-term, 26 short-term) and respiratory/atopy (1 long-term, 24 short-term) health outcomes (Table 9). Few studies investigated total mortality (1 long-term, 4 short-term), oxidative stress (0 long-term,4 short-term) and other outcomes (e.g., preterm birth, term low birthweight, perceived stress, 3 long-term, 2 short-term).

Table 9: Health outcomes according to organ systems of long-term and short-term-studies

	Number of studies	%
Long-term	all=13	
Total mortality	1	0.8%
Cardiovascular*	4	3.3%
Respiratory*	1	0.8%
Inflammation	3	2.4%
Oxidative stress	0	0.0%
Neurocognitive	1	0.8%
Other	3	2.4%
Short-term	all=110	
Total mortality	4	3.3%
Cardiovascular*	47	38.2%
Respiratory + Atopy*	24	19.5%
Inflammation	26	21.1%
Oxidative stress	4	3.3%
Neurocognitive	3	2.4%
Other	2	1.6%
Total	**123**	**100.0%**

* includes mortality

Outcome type by particle metric

Tables 10 to 13 give an overview of the main exposure metrics for ultrafine and quasi-ultrafine particles used for the investigation of different outcomes. Table 10 shows the overall number of studies, while table 11 and 12 differentiate the studies by short- and long-term studies. Table 13 summarizes the studies according to the use of primarily ultrafine and / or quasi-ultrafine particle size.

Most studies (n=66) use total particle number concentrations as a surrogate for ultrafine particle exposure and therefore do not investigate ultrafine particles in the stricter sense (Table 10). A smaller group of studies (n=29) uses particle number concentration measures for “true” ultrafine particle exposure available. Among the other UFP metrics, only accumulation mode particle concentration is frequently assessed, while the other metrics are rarely used. Specifically lung deposited surface area has not been established as a routine metric in epidemiological studies yet.

Across outcome types, the majority of studies investigates short-term subclinical outcomes with total particle number measurements. Only in the relatively small group of mortality studies, about half of

the studies use true ultrafine particle exposure metrics (i.e. particles smaller than 100nm). Only few long-term studies are available yet, applying particle number either for total particles or for submicron particles or the mass-based measure $PM_{0.1}$.

Table 10: Number of studies (long and short term) by outcome and exposure assessment.

All studies	PNC<100 nm	PNC<3000 nm	NucM	AitM	AccM	$PM_{0.1}$	$PM_{0.25}$	PM_1	LDSA
Mortality	4	6	0	0	0	1	0	0	0
Morbidity	3	6	0	0	1	3	0	0	0
Emergency	5	10	0	0	3	2	0	1	0
Subclinical	17	44	1	1	10	0	3	3	2
Total	29	66	1	1	14	6	3	4	2

* PNC: particle number concentrations, nm: nanometer, NucM: nucleation-mode particles, AitM: Aitken-mode particles, AccM: Accumulation-mode particles, LDSA: long-deposited surface area, UFP: particles with a diameter sized less than 100 nm.

Table 11: Number of short-term studies by outcome and exposure assessment

Short-term	PNC<100 nm	PNC<3000 nm	NucM	AitM	AccM	$PM_{0.1}$	$PM_{0.25}$	PM_1	LDSA
Mortality	4	6	0	0	0	0	0	0	0
Morbidity	1	5	0	0	1	0	0	0	0
Emergency	5	10	0	0	3	2	0	0	0
Subclinical	16	40	1	1	10	0	3	3	1
Total	26	61	1	1	4	2	3	3	0

Explanations see Table 10

Table 12: Number of long-term studies by outcome and exposure assessment

Long-term	PNC<100 nm	PNC<3000 nm	NucM	AitM	AccM	$PM_{0.1}$	$PM_{0.25}$	PM_1	LDSA
Mortality	0	0	0	0	0	1	0	0	0
Morbidity	2	1	0	0	0	3	0	0	0
Emergency	0	0	0	0	0	0	0	0	0
Subclinical	1	4	0	0	0	0	0	0	1
Total	3	5	0	0	0	4	0	0	1

Explanations see Table 10

In sum, 33 studies used UFP exposure measures and 69 studies used quasi-UFP exposure measures (Table 13). In 19 of the above named studies, both UFP and quasi-UFP measures were used. Among long-term studies, the number of studies measuring UFP and quasi-UFP was equal with five studies each, whereas short-term study authors mostly applied quasi-UFP measures.

Concerning the different outcome types, subclinical outcomes were more frequently related to quasi-UFP measures than to UFP measures, both in long-term and short-term study designs. For mortality

and morbidity, the ratio of quasi-UFP versus UFP was nearly balanced, for emergency department/hospital admissions, quasi-UFPs were more frequent (10 versus 6).

Table 13: Number of studies by outcome and UFP versus quasi-UFP measurement

Studies	short-term			long-term			total		
Metric	**UFP**	**quasi-UFP**	**UFP + quasi-UFP**	**UFP**	**quasi-UFP**	**UFP + quasi-UFP**	**UFP**	**quasi-UFP**	**UFP + quasi-UFP**
Mortality	1	3	3	1	0	0	2	3	3
Morbidity	0	4	1	3	1	0	3	5	1
Emergency	1	5	5	0	0	0	1	5	5
Subclinical	7	36	10	1	4	0	8	40	10
Total	**9**	**45**	**19**	**5**	**5**	**0**	**14**	**50**	**19**

UFPs consist of the particle metrics PNC <100 nm, NucMP, AitMP and $PM_{0.1}$, quasi-UFP: particles with a diameter sized < than 3,000 nm, without a cutpoint at 100 nm, even though particles might me dominated by particles <100 nm. Here, quasi-UFPs consist of PNC <3,000 nm, AccMP $PM_{0.25}$ and $PM_{0.1}$.

The average particle numbers across the studies range from 1,646/ml PNC (no size range reported) in the warm season modelled by a dispersion model and assigned to study participants in California (Delfino et al., 2014) and 2,905/ml PNC (10-100 nm) assessed by a central monitor in Rochester (Croft et al., 2017) up to mean averages of 164,464/ml total PNC (10-1,000 nm) assessed by mobile measurement at a highly trafficked site in Barcelona (Kubesch et al., 2015). The highest central site-measured UFP-concentrations measured in Europe were assessed in Rome with 34,046/ml total PNC and and outside Europe in Beijing with 43,900/ml PNC (11.1-101 nm) (Song et al., 2013).

Quality indicators – general aspects and study population

Most studies clearly stated the research question (n=82). In most publications (n=82), the study authors specified the included participants clearly. In more than half of the studies (n=49), convenience samples were used. Six studies recruited participants from random samples of the population. Further seven studies used a combination of random and convenience samples. In 10 studies, other sample types were used, e.g. subsets of cohorts with specified health measurements or cohorts of specified groups.

In 10 studies, which were all time-series studies, the sample was completely representative for the general population as the data assessment of the health endpoint referred to the whole population in the respective study area. In thirteen studies, the study populations were representative samples of population groups, e.g., children or adults above a certain age. In most of the included studies (n=62, 72.9%), the study population was a selected group, not representative for the general population. A sample size justification was rarely provided (n=3). Most of the study participants (exposed and unexposed or cases and controls) were recruited from the same populations and the same time period (n=71 and n=82).

Table 14: Quality criteria of the UFP/quasi-UFP Studies concerning selection bias

Quality aspects – study population	n	%
Was the research question or objective in this paper clearly stated?		
Yes	82	96.5%
Not specified, reference given	2	2.4%
Not specified, no reference given	1	1.2%
	85	100.0%
Was the study population clearly specified and defined?		
Yes	82	96.5%
Not specified/ reference given	2	2.4%
Not specified/ no reference given	1	1.2%
	85	100.0%
Sample Type		
Random	6	7.1%
Convenience	49	57.6%
Random + Conv.	7	8.2%
Other	10	11.8%
NA	13	15.3%
	85	100.0%
Representativeness		
Completely	10	11.8%
Somewhat	13	15.3%
Selected group	62	72.9%
	85	100.0%
Was a sample size justification, power description provided?		
Yes	3	3.5%
Not reported/ reference given	1	1.2%
Not reported/ no reference given	77	90.6%
Not applicable	4	4.7%
	85	100.0%
Were all the subjects selected or recruited from the same or similar populations?		
Yes	71	83.5%
No	12	14.1%
Not reported/ reference given	0	0.0%

Quality aspects – study population	n	%
Not reported/ no reference given	2	2.4%
	85	100.0%
Were all the subjects selected or recruited from the same time period?		
Yes	82	96.5%
No	0	0.0%
Not reported/ reference given	3	3.5%
Not reported/ no reference given	0	0.0%
	85	100.0%

Quality indicators – Exposure assessment

Second, quality aspects concerning exposure assessment were investigated (Table 15). The majority of the studies (n=66, 77.6%) reported the size-ranges of the measured UFPs. Almost all studies (n=79, 92.9%) reported the technical device used to measure the particles. Less than half (n=34) of the studies assessing other air pollutants (n=78) adjusted for co-pollutants within multi-pollutant-models. Studies without adjustment for co-pollutant were considered as "high risk of bias". 66 studies adjusted for meteorology, from which the majority (n=64) were short-term studies.

Table 15: Quality of the UFP/quasi-UFP Studies concerning exposure assessment

Quality aspects - exposure	n	%
Reporting of UFP size ranges		
Reported	66	77.6%
NR/ reference given	6	7.1%
NR/ no reference given	13	15.3%
	85	100.0%
Reporting of technical device		
Reported	79	92.9%
NR/ reference given	4	4.7%
NR/ no reference given	2	2.4%
	85	100.0%
QA/QC for UFP measures described		
Yes	33	38.8%
Partly	1	1.2%
No	51	60.0%
Assessment of other air pollutants		
Yes	78	91.8%
No	7	8.2%
	85	100.0%
Adjustment for co-pollutants		
Yes	34	40.0%

Quality aspects - exposure	n	%
No	48	56.5%
unclear	3	3.5%
	85	100.0%
Adjustment for meteorology		
Yes	66	77.6%
No	19	22.4%
	85	100.0%

Quality indicators – Outcome assessment

Third, quality aspects concerning the outcome assessment were explored (Table 16). In all but one study (n=84) assigned exposure values were measured or modeled for time periods prior or parallel to the assessment of the outcome or for the time period of follow-up. In five of the included long-term studies, this was achieved by the use of chemical transport modeling, which allows the estimation of daily air pollutant concentrations for specific time periods. Furthermore, all but one study (n=84) defined and described the outcome measures clearly. In 68 of the studies, a blinding of the outcome assessors could be presumed. In 15 studies, no blinding was ensured. 13 of these studies were scripted exposure studies and we assumed that no blinding was conducted in these studies, unless specifically mentioned in the reported methods.

Table 16: Quality of the UFP/quasi-UFP Studies concerning outcome assessment

Quality aspects	n	%
Exposure measured prior or parallel to outcome assessment		
Yes	84	98.8%
No	1	1.2%
	85	100.0%
Outcome measures clearly defined and implemented		
Yes	84	98.8%
No/ reference given	1	1.2%
	85	100.0%
Outcome assessors blinded to exposure status resp. case-control status of participants		
Yes	68	80.0%
Partly	2	2.4%
No	15	17.6%
	85	100.0%

4.3 Health effects

4.3.1 Short-term effects

4.3.1.1 Mortality

Seven short-term time-series-studies (Lanzinger et al., 2016a; Leitte et al., 2012; Meng et al., 2013; Samoli, Atkinson, et al., 2016; Stafoggia et al., 2017; Su et al., 2015; Wolf et al., 2015) applying central-site measurements investigated effects of PNCs (four studies measured UFPs, two quasi-UFPs) on various mortality outcomes (total, cardiovascular, respiratory) (Table A1a). Two of the seven studies were conducted in a multi-center approach covering several countries (Lanzinger et al., 2016a; Stafoggia et al., 2017). Six studies adjusted for co-pollutants or constituents (Samoli, Atkinson, et al., 2016) for at least parts of the examined particle-outcome relationships (Table A3a).

All-cause mortality

For all-cause mortality, the evidence base is currently inconsistent: **Stafoggia** et al. (2017) investigated effects of PNC size ranges on non-accidental mortality in 8 western European cities from 1999 to 2013. The pooled percent changes were strongest for an increase of PNC of 10,000 particles at 7 days before death, with effect estimates of 0.37% (confidence intervals (CI) -0.03%; 0.78%) increase in total non-accidental mortality. These effects were mostly influenced by the Rome estimate. The city-specific non-significant effect estimates varied from about -0.8% (Athens) to 1.9% (Augsburg) (I^2 index for heterogeneity was below 50%). In Shenyang in China, **Meng** et al. (2013) observed consistent and weak significant positive associations between particles of six size fractions between 250 and 500 nm and all-natural-cause mortality with a 2-day moving average interqurartile range (IQR) incremental change (e.g., percent change per 2,600 PNC250–280/ml: 2.41% (1.23%, 3.58%)).

These associations were only present in the larger size fractions up to 10 µm in the warm season and absent in the cold season. Within the framework of the UFIREG[13]-project, **Lanzinger** et al. (2016a) did not find associations between UFPs/quasi-UFPs averaged over lag 0-2 and natural mortality (percent changes of relative risks (RRs) per 2,750 PNCs: 0.1% (−2.0%; 2.4%)). Exposures averaged over lag 2-5 (delayed) and averaged over lag 0-5 (cumulated) yielded similar non-significant results. Finally, **Samoli** et al. (2016) investigated single-site measured, 1-day lagged PNC > 6nm related mortality among approximately 9 million Londoners and found estimates close to zero.

Two of the above named studies adjusted for co-pullutants (Meng et al., 2013; Stafoggia et al., 2017): When **Stafoggia** et al. (2017) adjusted their models for NO_2, $PM_{2.5}$ and $PM_{2.5-10}$ for 5-, 6- and 7-lagged exposures, estimates decreased considerably or even turned into a negative direction (e.g., for NO_2 and lag 7: -0.25% (-0.72%; 0.22%)), whereas estimates decreased to a minor extent upon adjustment of PM_{10}, CO or O_3 (e.g., for PM_{10} and lag 7: 0.28% (-0.13%; 0.68%)). The effect estimates for quasi-UFPs in the study by **Meng** et al. (2013) decreased only moderately and remained statistically significant upon adjustment for SO_2, NO_2, $PM_{2.5}$ and PM_{10}, respectively. The lowest two-pollutant estimate was 1.66% (0.14%, 3.17%) for the model including NO_2. Upon adjustment for $PM_{2.5-10}$ effect estimates became stronger.

Respiratory mortality

The up-to-date body of evidence for respiratory mortality is similarly inconsistent. Strong associations were observed only in two studies conducted in China (Leitte et al., 2012; Meng et al., 2013). **Leitte** et al. (2012) explored associations between various size-fractions of PNC and respiratory mortality in

[13] UFIREG: Ultrafine Particles - an evidence based contribution to the development of regional and European environmental and health policy

about 8,000,000 residents in Beijing, China, from 2004 to 2005. They found slightly negative (1-day lag) to non-significant positive (same day, lag of 2 days, average of 4 days and average of 5 days) changes per IQR of 13,000 particles/ml in the UFP range (PNC3-100nm). Associations with PNC sized 3-1,000 nm ranged from close to zero to significantly positive (percent increase per IQR of 14,000 particles/ml: 9.3 (1.3–17.9)) for a 2-day lag. **Meng** et al. (2013) observed associations slightly above the null effect between particles of eight size fractions between 250 and 1,000 nm and respiratory mortality. These associations increased substantially in the warm season, but did not reach statistical significance. **Lanzinger** et al. (2016a) found positive but non-significant associations of UFPs and quasi-UFPs with respiratory mortality for the 2-day lag, lag 2-5 and lag 0-5. **Stafoggia** et al. (2017) observed inconsistent estimates, ranging from significantly negative (lag 3) to positive associations (e.g., lag 6, lag 10). The study by **Samoli** et al. (Samoli, Atkinson, et al., 2016) indicated slightly inverse percent changes of 2-day lagged quasi-UFPs related respiratory mortality.

Of the above stated studies, two adjusted for co-pollutants (Lanzinger et al., 2016a; Leitte et al., 2012) and one for constituents and total PNC within the source-related estimates (Samoli, Atkinson, et al., 2016). Upon adjustment for SO_2, NO_2 or PM_{10}, the significant associations in the study by **Leitte** et al. (2012) remained positive but lost significance for PNC sized 300-1,000 nm averaged over 4 and 5 days. The decline in effect estimates was strongest after adjustment for NO_2. The association with PNC total as an average of two days didn't change upon adjustment for any of the co-pollutants and remained significantly positive. The effect estimates for 6-day averaged PNC < 100nm in **Lanzinger** et al. (2016a) decreased only slightly after adjusting for $PM_{2.5}$ and increased and became significant upon adjustment for NO_2. In the two-pollutant models by **Samoli, Atkinson** et al. (2016) co-source adjustment was conducted, i.e. estimates for single sources were calculated and these estimates were adjusted for all other sources. Furthermore, co-pollutant models were adjusted for PNC total minus the investigated source. The inverse effect estimate for respiratory mortality was generally robust to co-source adjustment. Mutual adjustment for all sources generally exerted a greater influence on the estimates compared with estimates from two-source models.

Cardiovascular mortality

The six studies investigating short-term effects of UFPs on cardiovascular (CV) mortality indicate inconsistent evidence: **Meng** et al. (2013) observed significant positive associations with a 2-day average IQR incremental change in PNCs fractions between 250 and 650 nm in Shenyang, China. These associations were stronger in the warm season and lost significance in the cold season. **Su** et al. (2015) also found significant positive associations between UFP particles of differend size fractions and cardiovascular mortality with a 1-day lag and cumulated 5-day average in Beijing. For lag 0, associations were weakly positive. A German single-centre time series study analyzed measured PNC sized between 10 and 2,000 nanometers with fatal myocardial infarction (**Wolf** et al., 2015). The authors found slightly positive to quasi null associations with same day exposure, previous day exposure and with mean exposures of the 4 preceding days. **Lanzinger** et al. (2016a) reported slightly inverse effects of UFPs and quasi-UFPs on cardiovascular mortality for 2-day lag, lag 2-5 and lag 0-5. Likewise, Stafoggia et al. (2017) found non-significant inverse associations of UFPs for the lags 0 to 3, 8 and 9, zero effects at lags 4 and 10 and slightly positive effects at lags 5 and 6 with the strongest effect at lag 7 for CV mortality. Similar to their results for respiratory mortality, **Stafoggia** et al. (2017) observed inconsistent estimates, ranging from negative (lags 0-3, 8,9) to positive associations (lag 7). **Samoli**, **Atkinson** et al. (2016) observed inverse effect estimates for the association between 1-day lagged quasi-UFPs and cardiovascular mortality (RR: -1.86 (-4.50, 0.86) per 5,180/ml).

Of the above stated studies, two studies applied two-pollutant models (Lanzinger et al., 2016a; Su et al., 2015). In **Lanzinger** et al. (2016a), the null association of PNC < 100 nm averaged over day 2-5 with cardiovascular mortality remained close to zero after adjusting for $PM_{2.5}$ and turned to a significant inverse association upon adjustment for NO_2. **Su** et al. (2015) observed only slightly reduced (by 1-2%) and still significant effect estimates for 5-day averaged PNC < 100 nm upon

adjustment for PM_{10} and $PM_{2.5}$. However, upon adjustment for NO_2, effect estimates lost significance and decreased by about 5%.

Summary: Mortality

In comparison to the prior evidence, seven additional studies have been conducted with overall mixed results. For all-cause mortality, only two out of four studies found positive estimates in analyses not adjusted for co-pollutants. Of these, only one study showed positive associations for quasi-ultrafine particles after adjustment for other pollutants, while in the other study, elevated point estimates decreased towards the null upon adjustment.

The evidence of respiratory mortality is also scarce and inconsistent. Out of the 5 studies on respiratory mortality, four studies found positive, though mostly non-significant associations for UFPs or quasi-UFPs. Three studies adjusted for co-pollutants, with opposite effects after NO_2 adjustment, leading either to an enhancement or to an attenuation of effect estimates after adjustment for NO_2. The studies presented two-pollutant associations only for those models/ lags/ size fractions showing the strongest associations. Thus, the specific effect estimates are difficult to compare and consistency of the results can't be fully assessed.

Similar to the overall results for respiratory mortality, associations of UFP/quasi-UFP with CV mortality are inconsistent. The six single exposure studies observe positive (three studies) as well as inverse associations (three studies) with CV mortality. In the two multi-pollutant studies, adjustment for NO_2 led to a decrease in effect estimates, causing the loss of significance in one study and a decrease to a significantly inverse relationship in the other study. Adjustment for $PM_{2.5}$ only caused small or no changes in the UFP estimate.

Evidence from this as well as from prior reviews suggests that effects may be larger in the warm season; therefore possible effect modification by season is an important factor to consider in future short-term effect studies. Moreover, the observed effects at least partially overlap with other air pollutant effects, most clearly seen for NO_2. Due to differences in investigated size fractions, no conclusions can be made about the most important fractions.

Table 17: Summary table of conducted analyses in the seven mortality studies

Study	All-Cause	Single pollutant associations	Multipollutant associations	Respiratory	Single pollutant associations	Multipollutant associations	Cardiovascular	Single pollutant associations	Multipollutant associations
Lanzinger et al. 2016a	✓	0	0	✓	(+)	+	✓	(-)	-
Leitte et al. 2012				✓	UFP: (+), quasi-UFP: +	UFP: 0 quasi-UFP: (+)			
Meng et al. 2013, (only quasi-UFP)	✓	+	+	✓	(+)	nc	✓	+	nc
Samoli et al. 2016	✓	0	0	✓	-	-	✓	(-)	nc
Stafoggia et al.,2017	✓	(+)	(-)	✓	+	nc	✓	(-)/(+)*	nc
Su et al. 2015							✓	+	(+)
Wolf et al. 2015							✓	(+)	nc

0 indicates no association. (+) and (-) indicates primarily non-significant associations, + and - indicate significant associations. nc: not conducted

4.3.1.2 Morbidity

Associations between UFPs/quasi-UFPs and acute morbidity outcomes was assessed by one panel study (Karakatsani et al., 2012), two case-crossover studies (Cole-Hunter et al., 2013; Link et al., 2013), a scripted exposure study (Langrish et al., 2012), a time-series study (Wolf et al., 2015) and two cohort studies (A. J. Mehta et al., 2015; Y. Wang et al., 2014). Exposure measurement was conducted by a central-site monitor, except for the scripted exposure and one case-crossover study (Cole-Hunter et al., 2013, Langrish et al., 2012) which determined personal pollution using monitoring equipment within a backpack. One study used a multi-center approach covering several states within the EU (Karakatsani et al., 2012) (Table A1b). None of the studies additionally adjusted for co-pollutants.

Respiratory morbidity

One panel study within the framework of the "Relationship between Ultrafine and fine Particulate matter in Indoor and Outdoor air and respiratory Health" (RUPIOH)-project explored total PNC related effects on respiratory symptoms (**Karakatsani** et al., 2012). Respiratory health of 136 participants aged 35 and older and with either chronic obstructive pulmonary disease (COPD) or asthma from Amsterdam, Athens, Birmingham and Helsinki was monitored for six months by daily symptoms diary. Daily diary records contained breathing problems after wake up, shortness of breath, wheeze, cough, phlegm as well as limitations of vigorous activities, moderate activities and walking due to breathing problems. Karakatsani et al. (2012) did not find significant associations between PNC and daily respiratory symptoms over lag 0-2 (e.g. lag1 OR for cough per 10,000 particles/cm^3: 1.009 (0.944; 1.079)) and a 6-day moving average (OR 0.894 (0.714; 1.119). They found significant inverse effect estimates for shortness of breath (lag1: OR 0.91 (0.844; 0.982) and limitations in walking (ma0-6: OR 0.804 (0.658; 0.981)).

In contrast, **Cole-Hunter** et al. (2013) found significantly increased respiratory symptoms in healthy adults commuting in an urban environment of high air pollution. 35 participants completed two return trips, one each in a highly polluted area near busy roads and one alternative route of lower proximity to motorized traffic. Participants reported significantly more nose and throat irritation between high and low pollutant trips (mean ± standard deviation: 1.82 ± 0.33 vs. 1.53 ±0.23, $p<0.01$ and 2.00 ± 0.4 vs. 1.56 ± 0.24, $p<0.01$, respectively). An evaluation of independent effects of UFP, however, is not possible.

Langrish et al. (2012) conducted a study with patients suffering from coronary heart disease measuring quasi-UFP PNC by mobile devices. In this scripted exposure study 98 patients walked on a predefined route in central Bejing, once while using a respiratory mask and once while not using the mask. At the beginning of the study day, 2 hours and 24 hours after the walk they were asked to report physical symptoms (headache, dizziness, nausea, tiredness, cough, breathlessness, irritation of the throat or nose, unpleasant smell and bad taste in the mouth). In the presence of the mask, there were significantly lower self-reported symptoms except for dizziness and breathlessness, but since the exposure with the mask was determined based on measurements of mask filter efficacy and the authors only conducted a group comparison (mask vs. no mask), they were not able to disentangle the effect of total PNC from other pollutants.

Cardiovascular morbidity

Utilizing a case-crossover design, **Link** et al. (2013) investigated the onset of atrial fibrillation in patients with dual chamber implantable cardioverter-defibrillators (ICDs) associated with total PNC 2 and 24 hours before the event. The authors observed increased odds for atrial fibrillation with 12% (-19; 56) for a moving average of 24h per 8,400 particles/cm^3 and an even higher odds with 24% increase (-4; 61) for a moving average of two hours per 10,900 particles/cm^3.

Within the Cooperative Health Research in the Region Augsburg (KORA)-framework **Wolf** et al. (2015) investigated in a registry-based time-series study the effect of PNC (10-2,000 nm) on nonfatal myocardial events in the general population. A total of 8,298 coronary events were recorded and thereof 3,303 were recurrent events. An non-significant increased risk of 2% (-1.5; 5.8) per IQR change of 6,800 particles/cm^3 of the four preceding days in nonfatal myocardial events and a significantly increased risk of 6% (0.6; 11.7) in recurrent myocardial events were found. However, while most effect estimates for different lags and outcomes were above the null, only one estimate for recurrent events was significantly elevated.

Mental health

Between 2005 and 2008, adults 65 years of age and older without cognitive impairment were recruited for the MOBILIZE Cohort in Boston and followed until 2010. During two in-home interviews the presence of depressive symptoms using the 20-item CESD-R Scale was assessed by trained staff (**Wang** et al., 2014). There was no association between the presence of depressive symptoms and the proximity to major roadways as an indicator for long-term traffic exposure. They also found no evidence suggestive of a positive association between depressive symptoms and mean PNC exposure in the preceding two weeks. **Mehta** et al. investigated the association between AP and non-specific stress in a cohort of 987 elderly men in the Veterans Administration Normative Aging Study. Stress was quantified with a 14-item Perceived Stress Scale (PSS), which scores stress experienced in the previous week from 0-56. PNC (7-3,000 nm) at moving averages of 1, 2 and 4 weeks were associated with increased stress. An interquartile range increase of 15,997 particles/ml in one week average PNC was significantly associated with a 3.2 point (2.1; 4.3) increase in perceived stress score.

Summary short-term studies on morbidity outcomes

Of the few studies investigating short-term effects of UFPs/quasi-UFPs on morbidity outcomes, only two studies observed significantly elevated estimates with a marker of perceived stress and with recurrent coronary events. Since none of the above mentioned studies adjusted for co-pollutants or were by design able to disentangle the independent effects of different constituents of the air pollution mixture, we cannot conclude an independent effect of UFPs on morbidity outcomes. The evidence base for CV morbidity outcomes is scarce with only two studies available on different outcomes. This evidence suggests that participants with preexisting cardiovascular disease might be more susceptible to adverse associations with elevated UFP/quasi-UFP concentrations.

However, while both studies show generally positive associations, no inference on the independence on the reported UFPs effect can be made. The evidence for associations with short-term changes in mental health symptoms is insufficient.

4.3.1.3 Emergency department/ hospital call/visit/admission

The use of emergency health care services was investigated by five case-crossover studies (Evans, Halterman, Hopke, Fagnano, & Rich, 2014; Gardner et al., 2014; Iskandar et al., 2012; Rosenthal et al., 2013; Wichmann et al., 2013) and six time-series studies (Delfino et al., 2014; Diaz-Robles et al., 2014; Lanzinger et al., 2016b; Liu et al., 2013; Samoli, Andersen, et al., 2016; Samoli, Atkinson, et al., 2016) (Table A1c). Six studies adjusted for co-pollutants (Evans et al., 2014; Iskandar et al., 2012; Lanzinger et al., 2016b; Rosenthal et al., 2013; Samoli, Andersen, et al., 2016; Samoli, Atkinson, et al., 2016) (Table A3c).

Respiratory disease

Three studies (Delfino et al., 2014; Evans et al., 2014; Iskandar et al., 2012) investigated UFP-related effects on asthma symptoms or hospital/emergency department visits in children. **Evans** et al. (2014) found adverse associations between central-site measured PNC<100nm and the occurrence in

pediatric asthma visits in 74 asthmatic children aged 3-10 years (OR up to 1.27 (0.90; 1.79), for an average of 4 days per 2,088/ml PNC in Rochester. Other lags and accumulation mode particle associations were null or inverse. A register-based study from Copenhagen (**Iskandar** et al., 2012) explored the effects of central-site measured PNC sized 10-700 nm on hospital admissions for asthma in 8,226 children aged 0-18 years. In this case-cross-over study, the authors observed non-significant associations for an average of 5 days, being strongest for 0-1-year-old infants (OR: 1.08 (0.97; 1.22) per 3,812.86/ml PNC). In a case-crossover analysis of hospital admissions in children with asthma, **Delfino** et al. (2014) investigated, whether high exposure to traffic-related air pollution, measured among others as UFP, modified the air pollution-outcome association. They found that generally, associations were stronger in high traffic exposure situations, especially in the cold season. Direct associations with UFPs/quasi-UFPs were not reported.

One of the four respiratory illness related time-series studies investigated associations between $PM_{0.1}$ and outpatient visits for respiratory illness in the general population of Temuco, Chile (Diaz-Robles et al., 2014). **Diaz-Robles** et al. (2014) reported a significant associations primarily for longer lags of 3,4 and 5 days (e.g., RR for lag 4: 1.07 (1.04; 1.10) per 4.73 $\mu g/m^3$ $PM_{0.1}$). The study by **Samoli,** Atkinson et al. (2016) (short description provided in 4.3.1.1) indicated positive associations in 0-14-year old children (percent increase per 5,180/ml 1.86 (-0.28; 4.05) for a two-day lag, but not in the 15+ age groups (15-64-year olds: -1.14 (-2.66; 0.41) in a study in the UK. Moreover, two multi-center time-series studies investigated this issue. A large study by **Samoli**, Andersen et al. (2016) analyzed central site-measured PNCs of various size fractions in relation to hospital admissions in approximately 9 million persons from five Western European cities during 10 years. Inconsistent and non-significant pooled effect estimates were observed across different lags ranging from a percentage change of -0.44 (-1.73; 0.87) per 10,000 particles/ml for lag 7 up to 0.43 (-0.58; 1.45) for lag 5. One aspect of this study was the lack of a harmonized approach to UFP measurements, relying on site-specific measurements that had already been in place, at least partly explaining the heterogeneity of the results. **Lanzinger** et al. (2016b) investigated respiratory UFP effects in 2,582,000 habitants of five Western and Eastern European cities in the UFIREG study. The authors found consistent pooled non-significant increased relative risks up to 3.4% (-3.2; 7.3) in the 6-day average submicron PNC per increment of 2,750 particles/ml. One strength of this multi-center study was the attention given to a harmonized exposure assessment in all study centers.

Four studies investigating UFP-related emergency department visits/hospital admissions for respiratory disease adjusted UFP-associations for co-pollutants and one study adjusted for co-sources (Samoli, Atkinson, et al., 2016) (Table A3c). Two studies found no major effect of adjustment for co-pollutants on the original estimates (Samoli, Andersen et al., 2016; Evans et al. 2014): While single day lags changed slightly in most of the two-pollutant models, the general direction of the inconsistent associations remained inverse in **Samoli, Andersen** et al. (2016). When adjusting for NO_2, the mostly inverse effect estimates remained stable or became closer to the null for the 0 to 2-day lags. However, for 3 to 7-day lags, most effect estimates decreased and turned into a negative direction, specifically when adjusting for NO_2. For the 5-day lagged exposure, the previously positive point estimate turned into a significantly inverse direction upon adjustment for NO_2. Adjustment for $PM_{2.5}$ and PM_{10} led mostly to decreased effect estimates, but most point estimates remained positive. **Evans** et al. (2014) conducted two-pollutant models using the pollutants shown to be associated with asthma exacerbation. In the two-pollutant models including carbon monoxide and O_3, the authors state that the effect estimates in these models did not differ substantially from those in the single-pollutant models without presenting any data. However, no NO_2 or $PM_{2.5}$ adjustments were performed, and the original, un-adjusted estimates were already inconsistent and not significantly different from the null.

The other two studies found decreases to the null after adjustment for co-pollutants: In the study by **Lanzinger** et al. (2016b), adjusting UFPs for $PM_{2.5}$ and NO_2 in the models averaged for 5 days, led to weakened effect estimates which turned negative in Ljubljana and Prague, while they stayed slightly

above the null in Augsburg and Dresden. The pooled estimate, however, also reversed into an inverse relationship. In general, the two-pollutant models with NO_2 showed stronger decreases in effect estimates than with $PM_{2.5}$. **Iskandar** et al. (2012) conducted two-pollutant models for 4-day averaged UFP and asthma-related hospital admissions. After adjustment for PM_{10}, $PM_{2.5}$, NO_2 and NO_x, the positive associations disappeared.

Cardiovascular disease

Three case-crossover studies (Gardner et al., 2014, Rosenthal et al., 2013, Wichmann et al., 2013) and three time series studies (Lanzinger et al., 2016b; Liu et al., 2013; Samoli, Atkinson, et al., 2016) explored the effects of central-site measured PNCs of various size fractions on the use of health services due to acute cardiovascular conditions. None of the case-crossover studies adjusted for co-pollutants, while all of the time-series studies did.

In their case-crossover study, **Gardner** et al. (2014) used medical records on cardiac catheterizations from a hospital in New York and analyzed UFP-related effects on 677 myocardial infarctions, classified in ST-elevation[14] myocardial infarction (STEMI) and non-ST-elevation myocardial infarction (NSTEMI). The authors observed non-significant positive effect estimates for STEMI (OR for an average of 24h per 3,284 PNC/ml: 1.06 (0.89; 1.26). Accumulation mode particles were associated with slightly higher point estimates (OR for an average of 24h per 755 PNC/ml: 1.12 (0.92; 1.38). The study did not indicate any association for NSTEMI with PNCs or accumulation mode particles. Two studies investigated effects of PNCs and accumulation mode particles on out-of-hospital cardiac arrests. **Rosenthal** et al. (2013) studied effects of submicron PNCs and accumulation mode particles on 2,134 cases of out-of-hospital cardiac arrests due to all cardiac causes, MI and other cardiac causes in Helsinki/ Finland. Effect estimates across differently lagged and cumulated submicron PNCs for all cardiac arrests were close to zero and mostly positive but never significantly elevated for UFP and for accumulation mode particles (e.g., 2h-lagged OR: 1.04 (0.98; 1.10) per 1,007 accumulation mode particles/ml). For myocardial infarction, ORs were frequently positive in relation to PNCs, being significantly elevated for the average exposure of the previous 24 hours (OR of PNCs < 100 nm: 1.27 (1.05; 1.54) per 10,624 particles/ml and for accumulation mode particles: 1.19 (1.04-1.35) per IQR). Another Scandinavian study (**Wichmann** et al., 2013), assessing out-of-hospital cardiac arrests in relation to different particle metrics sized 10-700nm (PNC, PAC, PVC) in 4,657 patients from Copenhagen, found non-significant effect estimates close to zero.

Three studies used a time-series design to examine the effects of PNC of different size fractions on cardiovscular hospital admissions (Lanzinger et al., 2016b; Samoli, Atkinson, et al., 2016) and emergency room visits (Liu et al., 2013). In the multi-site study by **Lanzinger** et al. (2016b), the relative risk for cardiovascular hospital admissions decreased slightly for an average of 2-day (percent change: -0.6 (-2.4; 1.1) and 6-day (-0.1 (-2.6; 2.4)) exposure to UFP PNC. A delayed exposure to UFP (mean average: 2-5 days) led to minimally increased RRs. Associations with PNC sized 20 to 800 nm were slightly elevated for delayed (ma 2-5 days) and cumulated (ma 0-5 days) lags. **Liu** et al. (2013) explored different size fractions of PNC in relation to total cardiovascular emergency room visits. The authors found delayed (ma 0-10 days) associations between number concentration of ultrafine particles and cardiovascular emergency room visits, mainly from lag 4 to lag 10, mostly contributed by 10–30 nm and 30–50 nm particles. Increase in 2-day average number concentration of 30–50 nm particles led to a 2.4% (1.5–6.5%) increase in cardiovascular emergency room visits per IQR of 2,269 particles/ml. A study investigating quasi-UFP-related effects on cardiovascular hospital admissions (**Samoli**, Atkinson, et al., 2016) reported non-significantly increased effect estimates for 15 to 64-year

[14] ST segment on an electrocardiogram normally represents an electrically neutral area of the complex between ventricular depolarization (QRS complex) and repolarization (T wave

old London residents (percent changes: 0.81 (-0.78; 2.42) and close to zero effect estimates for residents equal/older than 65 years for 1-day lagged exposures.

Three studies investigated independent effects of UFPs with adjustment for co-pollutants (Lanzinger et al., 2016b; Rosenthal et al., 2013; Samoli, Atkinson, et al., 2016). **Lanzinger** et al. (2016) explored $PM_{2.5}$ and NO_2-adjusted effect estimates for delayed (mean average: 2-5 days) UFP exposure. The close to zero single-pollutant effect estimates decreased to the null upon adjustment for $PM_{2.5}$ or NO_2. **Rosenthal** et al. (2013) conducted multi-pollutant models for UFPs and accumulation mode particles, adjusting for $PM_{2.5}$, in relation to out-of-hospital cardiac arrest due to myocardial infarction and due to other cardiac causes. For accumulation mode particles associated with myocardial infarction, most effect estimates declined upon adjustment for $PM_{2.5}$, strongest for the average of 24 previous hours and the average of the lags 0 to 3. For UFPs, associated with myocardial infarction, the results were inconsistently slightly decreasing or remaining similar for short-term lags of 0, 1, 2 and 3 hours and the average of the 7 previous hours and slightly increasing for 1- to 3-day lagged exposures and the average of the previous three days. Furthermore, Rosenthal et al. (2013) conducted two-pollution models for PNC and accumulation mode particles adjusting for O_3 in relation to other cardiac causes. For accumulation mode particles, most effect estimates declined. For UFPs, the results were again inconsistent across the different lags and averaged time periods. No adjustments for NO_2 were conducted. In the study by **Samoli**, **Atkinson** et al. (2016), effect estimates for immission factors derived from particle size and number distribution decreased for background and nucleation sources and increased for traffic and secondary sources after adjustment for the other factors.

Summary of short-term associations with emergency department visits/hospital admissions

The evidence base for UFP-related effects on utilization of the healthcare system due to respiratory symptoms is scarce (Tables A1c, A3c). Possible associations seem to be most probable for children as a susceptible subgroup. While single-pollutant associations were observed in few studies, multi-pollutant models of the studies could not verify independent associations of UFPs/quasi-UFPs with respiratory hospital admissions/emergency department visits. Specifically adjustment for NO_2 led to a decrease in estimates, which mostly reached the null in co-pollutant models.

Most studies investigating cardiovascular disease-related use of the healthcare system indicate weak associations being stronger for shorter time lags of up to 24 hours. These associations decreased upon adjustment for co-pollutants with no clear evidence for independent associations of UFPs/quasi-UFPs with cardiovascular emergency department visits/hospital admission. .

Table 18: Summary table of conducted analyses in the 7 studies on emergency department visits/hospital admissions

Study	Respiratory	Single pollutant associations	Multipollutant associations	Cardiovascular	Single pollutant associations	Multipollutant associations
Evans et al., 2014	✓	(+)	(+) (no NO_2 adjustment)			
Gardner et al., 2015				✓	(+)/0	nc
Iskandar et al., 2012	✓	(+)	0			
Rosenthal et al., 2013				✓	(+)/+	0
Wichmann et al., 2013				✓	(+)/0	nc
Delfino et al., 2014	✓	nr	nr			

Study	Respiratory	Single pollutant associations	Multipollutant associations	Cardiovascular	Single pollutant associations	Multipollutant associations
Diaz-Robles et al., 2014	✓	+				
Lanzinger et al., 2016	✓	(+)	0	✓	(+)/0	0
Samoli UK, 2016	✓	(+)/(-)	(+)	✓	(+)	(-)/(+)
Samoli EU, 2016	✓	(+)/(-)	(-)/-			
Liu et al., 2013				✓	+/(+)	nc

0 indicates no association. (+) and (-) indicates primarily non-significant associations, + and - indicate significant associations. nc: not conducted, nr: not reported

4.3.1.4 Subclinical outcomes

Respiratory markers

Lung function related indices were investigated in one cross-sectional study in Denmark (Karottki et al., 2014), one case-crossover study in Australia (Cole-Hunter et al., 2013), four panel-studies, conducted in South Korea (Song et al., 2013), Taiwan (Y. R. Li et al., 2016), Denmark (Karottki et al., 2015) and Atlanta/ USA (Sarnat et al., 2014) and five scripted exposure studies, conducted in Atlanta/ USA (Mirabelli et al., 2015), California/ USA (Jarjour et al., 2013; Park, Gilbreath, & Barakatt, 2017) and The Netherlands (Janssen et al., 2015; Strak et al., 2012) (Table A1d). Three of these studies applied two-pollutant-models (Table A3d).

In a cross-sectional approach, **Karottki** et al. (2014) found non-significant, slightly elevated effect estimates for the ratio of forced expiratory volume in 1 second (FEV_1) to forced vital capacity (FVC) in relation to central-site measured PNC sized 10 to 280 nm. Using a case-crossover design, **Cole-Hunter et** al. (2013) found non-significant lower peak-flow rates for high versus low inbound traffic exposure in healthy cycling adults.

Four panel studies investigated UFP effects on peak flow rates and spirometry indices in different groups. The study by **Song** et al. (2013) observed lower peak flow rates for central-site measured PNC sized 11 nm to 110 nm for 1-day lagged exposures in children with atopic diseases. For PNC sized 111 to 930 nm. Song et al. (2013) found consistently decreased peak flow rates for 1-day lagged exposures as well as exposures averaged over two to three days among children with atopic disease. Peak flow rates increased in response to 1-day lagged exposures of PNC sized 111 to 930 nm and decreased in response to cumulated exposures in healthy children. Associations were similar for particles sized 11 to 110 nm In contrast, **Li** et al. (2016) observed an increase in lung function indices FVC, FEV_1, and different FEF-values related to increases in UFP and accumulation mode particles of the previous day in children with asthma or allergic rhinitis. When separating different contributors, only secondary aerosol contributors yielded decreased lung function indices (in contrast do diesel vehicle emissions and aged vehicle emissions). **Karottki** et al. (2015) found decreases in the lung function parameter FEV_1:FVC (percent changes: -4.0 (-8.1; 0.5) per 3,000 particles/ml) in a panel of 48 adults for a lag period of 48 hours. **Sarnat** et al. (2014) observed slightly elevated FEV_1 levels relative to baseline levels among asthmatic participants at the 1 h and 2 h post-commute time points.

Five scripted exposure studies investigated effects of PNC with varying size fractions on lung function indices both in vulnerable as well as healthy participants. **Mirabelli** et al. (2015) observed slightly reduced FEV_1 % predicted values per IQR of mobile measured total PNC in young asthmatic adults. This adverse association could not be observed in young non-asthmatic participants. In a study comparing health effects of high versus low traffic routes in healthy regular cyclists, the authors did

not observe significant changes in spirometry indices (**Jarjour** et al., 2013). On the other hand, a study with healthy cyclists in California/USA (**Park** et al., 2017) found significant adverse associations between mobile measured PNC > 10 nm and the spirometry indices FVC, FEV_1 and PEF in 32 healthy cyclists immediately after exposure. However, for FEV_1: FVC, the authors did not find any associations. Similarly to Park et al. (2017), a study within the framework of the RAPTES-study in the Netherlands (**Janssen** et al., 2015) found significant adverse associations between exposure to mobile measured total PNC and percentage change in FEV_1 2 hours after exposure in outdoor sites (-1.5 per 23,000 particles/ml) in healthy students. Another study of the RAPTES project (**Strak** et al., 2012) indicated significant associations between PNC and FVC immediately after exposure (-1.19 per 23,000 particles/ml).

Three studies adjusted for co-pollutants: Two scripted exposure studies (Janssen et al., 2015; Strak et al., 2012) found similar effects upon adjustment for $PM_{2.5}$ and PM_{10} but decreased estimates upon adjustment for NO_2. **Li** et al. (2016) observed a similar protective effect of 1-day lagged UFP exposures on FVC, FEV_1, and different FEF-values upon adjustment for O_3. However, models with accumulation mode particles adjusted for O_3 yielded non-significant inverse associations.

Summary of subclinical respiratory endpoints

Most of the above reviewed studies have only limited sample sizes (15-84 participants). Moreover, study samples were frequently selective, either representing healthy young adults or persons suffering from atopy and/or asthma. The investigated lags and averaging periods differ across studies, but generally, most associations were found in a time range of 0-48 hours after increased exposure. Finally, results of the studies are mostly inconsistent in relation to the specific respiratory endpoints. With regard to peak-flow endpoints, measurement error could be an issue in this self-monitored endpoint, especially in the study by Cole-Hunter et al. (2013) which could not be blinded. Due to the lack of adjustment for co-pollutants, little can be concluded regarding the independence of effects. The scarce evidence on studies with co-pollutant adjustment suggests an at least partial overlap of UFP, respectively PNC effects with NO_2-effects.

Blood pressure indices

Blood pressure indices have been assessed in a panel analysis within a cohort study located in Massachusetts/USA (Bind et al., 2016), seven panel studies located in Massachusetts/USA (Chung et al., 2015; Hoffmann et al., 2012), Beijing (Gong et al., 2014; J. Zhang et al., 2013) (Gong et al., 2014; J. Zhang et al., 2013), New York/USA (Rich et al., 2012; M. Wang et al., 2016), Belgium (Pieters et al., 2015), and four scripted exposure studies, conducted in Spain (Kubesch et al., 2015), New Jersey/USA (Laumbach et al., 2014), Canada (Weichenthal, Hatzopoulou, & Goldberg, 2014) and China (Langrish et al., 2012) (Table A1d). Four of the studies investigated effects in two-pollutant models (Pieters et al., 2015; Rich et al., 2012; Weichenthal et al., 2014; J. Zhang et al., 2013) (Table A3d).

The cohort study by **Bind** et al. (2016) investigated effects of central-site measured PNC sized 7-3,000 nm on various cardiovascular endpoints in a sample of 1,112 veterans. Bind et al. observed increased levels of diastolic blood pressure (DBP) and systolic blood pressure (SBP) in response to extended concentrations of PNC.

While associations for DBP was consistently positive, SBP was positive in the lower and medium quantiles of the outcome's distribution (e.g., 10th percentile estimate = 4.9 mmHg (1.4; 8.6)).

Chung et al. (2015) investigated PNC related effects on systolic, diastolic and pulse pressure (PP) in 220 participants residing near a highway. The authors observed significantly increased DBP of 2.4 mmHg per 10,000 particles/ml in response to central-site measured PNC during the averaged 24h prior to measurement. The association with SBP was non-significantly positive, with pulse pressure slightly inverse. **Gong** et al. (2014) investigated associations of single-site measured PNC and AccMPs with blood pressure in a panel of 125 healthy young Chinese adults. The authors observed inconsistent

associations for SBP, DBP and HR in response to PNC sized 13 to 108 nm across 0 to 6-day lagged exposures in young adults. One significant positive association was observed for a 4-day lagged exposure to PNC associated with SBP. A panel-study with 125 young adults indicated associations between quasi-UFPs and SBP for 3- and 4-day lagged exposures, being significant at 4-day lagged exposure. Effect estimates for 0- to 3-day and 5- to 6-day lagged exposures were close to zero (J. Zhang et al., 2013). **Hoffmann** et al. (2012) found non-significantly increased SBP per IQR of central-site measured PNC total averaged over the 1–5 days before examination in a panel of diabetic persons. Effect estimates for DBP were close to zero and only slightly elevated for central mean pressure. **Rich** et al. (2012) studied effects of PNC sized 10 to 100 nm and AccMPs on different blood pressure indices in a panel of 76 individuals with previous myocardial infarction or unstable angina. The authors reported increases in SBP at almost all lags ranging from 1 to 5 days, being significant for a lag of 24-47h (ß-estimates: 0.89 mmHg (0.06; 1.72) per 2,680 particles UFP/ml and 0.94mmHg (0.02; 1.87) per 897 accumulation mode particles/ml). In the same panel, **Wang** et al. (2016) explored effects of SBP in relation to AccMPs lagged for 5 hours up to 4 days. The authors found consistent increases in SBP, being most pronounced for 0-23h lagged exposures (ß-estimates: 1.48 (0.09; 2.86)). In relation to UFP PNC exposure, SBP decreased for 5h and 4-day lagged exposures and increased for 0-23h up to 72-95h, being strongest for 0-23h (1.38 (0.07; 2.68)) and 24-47h lags (1.60 (0.32; 2.89)). A study exploring UFP effects on SBP in schoolchildren observed most pronounced effects of increased BP estimated for the PNC size fraction 20-30 nm (**Pieters** et al., 2015). Whereas increases in PNC total were linked to elevated SBP measurements, PNC sized < 100 nm did not have an effect. DBP was not associated with PNC.

Four scripted exposure studies measured indices of autonomic function applying mobile measured PNC. **Kubesch** et al. (2015) found statistically significant increases in SBP and DBP in healthy adults 2 hours post exposure of PNC sized 10-1,000 nm. **Laumbach** et al. (2014) did not find any associations at any time point after a ride of healthy adults in a passenger vehicle. In a study with 53 middle-aged female cyclists, **Weichenthal** et al. (2014) observed borderline significant increases in DBP in relation to PNC sized 10 to 100 nm and slightly positive associations with DBP three hours post exposure. **Langrish** et al. (2012, study described in 4.3.1.2) found statistically significant differences in mean arterial blood pressure, but not in DBP, SBP in participants wearing a respiratory mask in comparison to participants not wearing a mask.

Only four studies examining associations between UFP and blood pressure indices applied two-pollutant models (Pieters et al., 2015; Rich et al., 2012; Weichenthal et al., 2014; J. Zhang et al., 2013). **Pieters** et al. (2015) found nearly unchanged adverse effects of PNC on SBP after adjusting for PM_{10}. **Zhang** et al. (2013) observed only slightly decreased SBP values upon adjustment for CO, O_3 and SO_2. The decrease was slightly more pronounced with NO_2 used as co-pollutant. **Weichenthal** found stronger effects for SBP, DBP upon adjustment for $PM_{2.5}$, NO_2 and O_3. **Rich** et al. adjusted single pollutant models of AccMP for $PM_{2.5}$. The authors observed reduced estimates for SBP upon adjustment for $PM_{2.5}$.

Summary of blood pressure indices

The majority of studies found adverse associations between blood pressure indices and exposure to UFP/quasi-UFP, indicating increases in BP. These results differed across different endpoints (SBP, DBP, PP), different size fractions and lag periods. Apart from one study with more than 1,000 participants, the studies consisted of smaller study populations. In addition, all study samples represented selected group, impeding a transfer to the general population. Apart from these limitations, the evidence from two-pollutant studies is too scarce to draw conclusions on independent UFP effects on blood pressure indices.

HRV indices

HRV indices have been assessed in a panel analysis within a cohort study located in Massachusetts/USA (Bind et al., 2016), 11 panel studies located in Spain (Cole-Hunter et al., 2016), Germany (Hampel et al., 2012; Peters et al., 2015), California/USA (Bartell, Longhurst, Tjoa, Sioutas, & Delfino, 2013), New York/USA (Rich et al., 2012; M. Wang et al., 2016), Georgia/ USA (Sarnat et al., 2014), China (Gong et al., 2014; Sun et al., 2015; J. Zhang et al., 2013) and Taiwan (Wu et al., 2012). Furthermore, four scripted exposure studies were conducted in New Jersey/USA (Laumbach et al., 2014), Canada (Shutt et al., 2017; Weichenthal et al., 2014) and China (Langrish et al., 2012). Finally, one ultra-short-term panel study (Hampel et al., 2014) was reviewed (Table A1d). Five studies examining HRV indices in response to UFPs applied two-pollutant models (Table A3d).

The evidence on HRV is mixed with studies showing adverse associations for at least single indices (Bind et al., 2016; Cole-Hunter et al., 2016; Gong et al., 2014; Hampel et al., 2012; Hampel et al., 2014; Shutt et al., 2017) and those with null or even protective associations (Bartell et al., 2012; Laumbach et al., 2014; Wu et al., 2012; Weichenthal et al., 2014; Zhang et al., 2013). Several studies on HRV were conducted in susceptible groups (i.e. diabetics or patients with coronary artery disease), showing mostly small, and in part significant adverse associations with various markers of HRV (Hampel et al., 2012; Peters et al., 2015; Sun et al., 2015; Rich et al.,2012; Wang et al., 2016; Sarnat et al., 2014; Langrish et al., 2012). Most associations of UFPs or quasi-UFPs with indicators of HRV were within a very short to short time-frame (5 minutes to 95 hours), with two studies showing changes within minutes of increased exposure (Peters et al., 2015; Hampel et al., 2014). One study investigated HRV indices in a medium-term time-frame (Bind et al., 2016).

Five studies examining associations between UFP and HRV indices applied two-pollutant models (Peters et al., 2015, Rich et al.,2012, Sun et al., 2015, Weichenthal et al., 2014, Zhang et al., 2013). **Peters** et al. (2015) observed unchanged effects for the standard deviation of all NN beat interval (SDNN) and root mean square of the sucessive differences in ms (RMSSD) upon adjustment for ambient $PM_{2.5}$. **Rich** et al. adjusted single pollutant models of AccMP for $PM_{2.5}$. The authors observed increased effect estimates for Tpeak– Tend (Tp/Te) and similar effect estimates for heart rate turbulence upon adjustment for $PM_{2.5}$. **Sun** et al. (2015) found similar UFP-related effect estimates for SDNN upon adjustment for NO_2 and O_3. In the study by **Weichenthal** et al., (2014) associations with SDNN decreased by more than half in size in two-pollutant models upon adjustment for $PM_{2.5}$, NO_2 and O_3. **Zhang** et al. (2013) adjusted his models for NO_2 and O_3. Whereas effect estimates for HR remained similar, effect estimates for high-frequency power turned negative upon adjustment for NO_2 and decreased upon adjustment for O_3.

Summary of HRV studies

A relatively large body of evidence is available for HRV indices, mostly observing effects on at least on one HRV outcome. This evidence base adds to prior evidence from the HEI report (2013), which already included six studies with mixed results. Upon adjustment for co-pollutant, associations changed in both directions. Across studies, different time-windows and different co-pollutants were examined, so that no clear pattern can be observed.

Indices of arrythmia

Bartell (2013) assessed PNC (5-3,000 nm) related to arrhythmia and HRV (Table A1d). The authors observed non-significant associations, with a slight decrease in daily ventricular tachycardia (VT) counts and mostly positive effect estimates and inconsistent effect estimates for hourly VT absence/ presence across different lags and for daytime versus nighttime. One, three and five-day lagged PNC was inversely associated with daily ventricular tachycardia. Day- and nighttime measured hourly tachycardia for 1-hour to 5-day lagged PNC yielded non-significant inverse associations for 8- and 24h lagged PNC with hourly daytime ventricular tachycardia all hourly nighttime ventricular tachycardia with the exception of 8 hours. On the other hand, associations with $PM_{0.25}$ and daily VT counts yielded

consistent and stronger effect estimates across 0-, 1- and 2-day lagged exposures. No adjustment for co-pollutants was conducted.

Summary of studies on arrhythmias

Considering the limited number of studies with only one study, the evidence base is still insufficient.

Indices of vascular function

Vascular function has been assessed within one cohort study (Amar J. Mehta et al., 2014), two cross-sectional studies (Karottki et al., 2014; Ljungman et al., 2014) three panel studies (Karottki et al., 2015; Zanobetti et al., 2014; X. Zhang, Staimer, Tjoa, et al., 2016) and one scripted exposure study (Weichenthal et al., 2014) (Table A1d). Two of these studies further adjusted for co-pollutants (Zhang, Staimer, Tjoa, et al., 2016; Weichenthal et al., 2014) (Table A3d).

Seven studies considered effects of UFP in relation to vascular function indices. One cohort study (Amar J. **Mehta** et al., 2014) with 370 elderly US veterans investigated associations between central-site measured PNC sized 7-3,000 nm and augmentation pressure and augmentations index. The authors observed consistent increases per IQR for both endpoints, being significant for mean average lag periods of 1, 3 and 14 days. Two cross-sectional studies conducted in Denmark (Karottki et al., 2014) and in Massachussets (Ljungman et al., 2014) explored central-site modelled PNC in relation to vascular endpoints. **Karottki** et al. (2014) observed a significant decrease in microvascular function in relation to 2-day lagged central-site measured PNC sized 10-280 nm in 49 adults. In a large sample of 2,072 adults being part of the Framingham Heart Study Offspring and Third Generation Cohorts, **Ljungman** et al. (2014) analyzed micro-vessel function measured by peripheral-arterial tonometry in relation to total PNC. The study showed significant increases in baseline pulse amplitude, but did not observe any consistent patterns for hyperemic response measured by PAT ratio across mean average exposures of 1 to 7 days.

Three panel studies investigated central-site measured UFP related effects on vascular function. In a panel of 48 adults in Denmark, **Karottki** et al. (2015) found significant decreases in MVF in relation to central-site measured PNC sized 20-280 nm. Incontrast, **Zanobetti** et al. (2014) did not observe associations between total PNC and brachial artery diameter in a panel of 64 adults with type 2 diabetes mellitus (T2DM). **Zhang** et al. (2016b) found no associations between measured $\mathbf{PM_{0.18}}$ in relation to reactive hyperemia index (RHI), but for $PM_{2.5}$.

Finally, one scripted exposure study (**Weichenthal** et al., 2014) applying mobile measurements of PNC sized 10 to 100 nm in 53 healthy female cyclists in Canada found significant decreases in RHI in response to UFP exposure.

Only two of the studies investigating vascular effects applied two-pollutant models. In the study by **Zhang** et al. (2016b), previously zero effect estimates for RHI turned in a positive direction upon adjustment for O_3. In the study by **Weichenthal** et al. (2014), effect estimates for RHI remained significantly inverse upon adjustment for $PM_{2.5}$ and NO_2.

Summary of studies on vascular function

The majority of the studies examining associations between UFP/quasi-UFP and vascular function indicate a possible association. However, a lack of consistency regarding the study design, specifically the outcome parameters, as well as missing co-pollutant models do not allow overall conclusions.

Biomarkers - Pulmonary inflammation

Nine panel studies located in Beijing (Gong et al., 2014; J. Zhang et al., 2013) Shanghai (Han et al., 2016), The Netherlands (Manney et al., 2012), Belgium, (Pieters et al., 2015), Boston/ USA (Peng et al.,

2016) Atlanta/ USA (Sarnat et al., 2014), Atlanta/USA (X. Zhang, Staimer, Gillen, et al., 2016) and four scripted exposure studies located in New Jersey/ USA (Laumbach et al., 2014) , Georgia/USA (Mirabelli et al., 2015), Belgium (Bos et al., 2013) and The Netherlands (Strak et al., 2012) investigated UFP related effects on markers of pulmonary inflammation. Pulmonary indices were fractional exhaled nitric oxide (FeNO), nitric oxide in exhaled breath condensate pH (NO_x EBC), nitrite and/or nitrate, malondialdehyde (MDA) and IL-1ß in exhaled breath condensate (Table A1d). One of the studies adjusted for co-pollutants (Table A3d).

Nine studies investigated effects of UFPs and quasi-UFPs on fractional exhaled NO with time windows ranging from immediately after exposure up to 7 days after exposure. Five central-site measured panel studies (Gong et al., 2014; Han et al., 2016; Peng et al., 2016; X. Zhang, Staimer, Gillen, et al., 2016) indicate positive associations for shorter lag periods. A panel study using a microscale personal exposure model (Sarnat et al., 2014) also found significant associations. One panel study assessing $PM_{0.18}$[15], averaged across five days, observed significant positive effect estimates for exhaled NO in relation to $PM_{0.18}$ (X. Zhang, Staimer, Gillen, et al., 2016). Three scripted exposure studies using mobile measurements found significant increases immediately after exposure to total PNC (Bos et al., 2013; Mirabelli et al., 2015; Strak et al., 2013). However, all studies focusing on fractional exhaled NO used selected groups and PNC of different size fractions.

Five studies explored UFPs and quasi-UFPs in relation to MDA in exhaled breath condensate. The effects were less pronounced than for exhaled NO. Two panel studies (Gong et al., 2014; Sarnat et al., 2014) found inconsistent associations in response to PNC across lags, ranging from significant inverse to non-significant positive, or being non-significantly positive. A panel study assessing quasi-UFP $PM_{0.18}$ averaged across five days, found non-significant positive associations (X. Zhang, Staimer, Gillen, et al., 2016). A scripted exposure study by Mirabelli et al. (2015) observed slightly positive associations immediately after exposure.

Few studies assessed nitrite (Gong et al., 2014; Laumbach et al., 2014), or nitrite and nitrate (Laumbach et al., 2014; Manney et al., 2012) in exhaled breath condensate. In summary, effect estimates for EBC nitrite + nitrate were mostly positive (except for (Manney et al., 2012)) and were strongest for shorter lag-periods, i.e. immediately after exposure (Laumbach et al., 2014) or in 0 to 2-day lagged exposures (Gong et al., 2014). Most effect estimates were inverse or null for EBC NO_x in Manney et al. (2012), suggesting a decrease in pulmonary inflammatory markers.

One study (Pieters et al., 2015) observed significant increases in the pulmonary inflammation marker IL-1ß in exhaled breath condensate for concurrent exposure to UFP PNC, being strongest for the smallest fraction of 20-30 nm.

Two-pollutant models were conducted in the study by Strak et al. (2012). Significant effect estimates remained unchanged after adjustment for $PM_{2.5}$, and increased after adjustment for NO_2, immediately after exposure. In the study by Zhang et al. (2013) effect estimates remained significant and attenuated only slightly upon adjustment for NO_2, O_3 and SO_2.

Summary of studies on pulmonary inflammation

The studies which have been investigated UFP-effects on pulmonary inflammations suggest positive associations between UFP and adverse changes in the pulmonary inflammation marker, in particular immediately after exposure. Nevertheless, the evidence base for pulmonary inflammation in response to UFP is still limited as the studies used different subgroups, exposure metrics, outcome measures

[15] $PM_{0.18}$ is a standard UFP metric measured by MOUDI impactors. It describes particles with a cut-off diameter of 18 nm.

and time frames. The two studies that conducted two-pollutant models observed overall robust effect estimates.

Biomarkers - Systemic inflammation

One cohort study located in Massachusetts/ USA (Bind et al., 2016), two cross-sectional studies located in Massachusetts/ USA (Fuller et al., 2015) and Denmark (Karottki et al., 2014), eleven panel studies conducted in New York/ USA (Croft et al., 2017; Rich et al., 2012; M. Wang et al., 2016), Finland (Huttunen et al., 2012), Denmark (Karottki et al., 2015), Germany (Rückerl et al., 2014; Rückerl et al., 2016), Georgia/USA (Sarnat et al., 2014), California/ USA (Wittkopp et al., 2013), China (Gong et al., 2014; J. Zhang et al., 2013), one Australian case-crossover study (Cole-Hunter et al., 2013) plus four scripted exposure studies conducted in Belgium and the Netherlands (Bos et al., 2013; Steenhof et al., 2014; Steenhof et al., 2013; Strak et al., 2013) investigated UFP-related effects on systemic inflammation markers.

The above named studies used various markers of systemic inflammation C-reactive protein (CRP), interleukin-6 (IL-6) and myeloperoxidase are the most commonly used markers of systemic inflammation, while fibrinogen was the most investigated measure for coagulation. Systemic inflammation and coagulation are considered as mediators for cardiovascular diseases.

The most common investigated inflammatory marker among our identified studies was hs-CRP. The largest study sample in which hs-CRP measures were realized was a repeated measures study which was embedded in the Normative Ageing Cohort study including 1,112 veterans (**Bind** et al., 2016). The quantile regression yielded significantly elevated hs-CRP values for the 70. to 90th percentiles (referring to the distribution of CRP in the cohort) per IQR of central-site measured total PNC averaged over 28 days. The lower quantiles yielded zero to non-significant positive effect estimates. The cross-sectional studies found mostly non-significant positive associations between central-site measured quasi-UFP PNC and hs-CRP with exposure time windows of averaged 28 days (**Fuller** et al., 2015) and a 2-day lag (**Karottki** et al., 2014). Fuller et al. (2015) observed significantly decreased CRP estimates in relation to a second, near highway measurement site. However, this second measurement site had considerable missings in exposure data. Of the eleven mostly central-site measured PNC-related panel-studies, five studies (**Croft** et al., 2017; **Huttunen** et al., 2012; **Karottki** et al., 2015; **Rich** et al., 2012; **Sarnat** et al., 2014; **Rückerl** et al., 2014; **Wang** et al. (2016); Wittkopp et al. (2013)) did not observe strong associations. The effect estimates ranged mostly from slightly inverse to positive relations being significant for single lag periods. Rich et al. (2012), Wang et al. (2016) and Wittkopp et al. (2013) found the strongest associations for 1 and 2 day lag periods and Huttunen et al. (2012) for a lag period of 3 days. One scripted exposure study (**Strak** et al. (2013) found non-significant inverse relationships between total PNC and CRP 25 hours post exposure.

Of the studies investigating UFP-related effects on hs-CRP and described above, two adjusted their models for co-pollutants. In the study by **Rückerl** et al. (2014), associations lost significance after adjustment for $PM_{2.5}$. **Strak** et al. (2013) observed decreased effect estimates upon $PM_{2.5}$ which gained significance upon adjustment for NO_2. In contrary, adjustment for PM_{10} yielded less inverse associations.

The evidence base from the studies with regard to fibrinogen is inconsistent. Mostly positive associations were found in the studies by **Croft** et al. (2017) being strongest for shorter lag periods (0-11h, 0-47h), **Rich** et al. (2012) and **Wang** et al. (2016), most pronounced for lag hours 24-47. Inconsistent effect estimates were found in **Bind** et al. (2016), showing highest effect estimates for the 10., 20. and 90th percentile. **Gong** et al. (2014) and **Zhang** et al. (2013) found inconsistent effect estimates across 0 to 6-day lags, without a specific direction. In other studies, mostly inverse associations were observed (**Huttunen** et al. (2012) and **Strak** et al. (2013)). The inconsistent

evidence base may originate from different subgroups being selected and different exposure metrics and time periods being assessed.

Four studies investigating UFP-related effects on fibrinogen in two-pollutant models. In the study by **Strak** et al. (2013), the negative effect estimates for fibrinogen even decreased upon adjustment for $PM_{2.5}$ and PM_{10}. Upon adjustment for NO_2, effect estimates became significantly inverse. Similar to Strak et al. (2013), **Zhang** et al. (2013) found decreased effect estimates upon adjustment for NO_2, SO_2, O_3 and CO. **Croft** et al. (2017) observed significantly increased effect estimates upon adjustment for $PM_{2.5}$, Delta-C and BC. **Rich** et al. (2012) found unchanged significant positive effect estimates for accumulation mode particles with a lag period of 24-47 hours upon adjustment for $PM_{2.5}$. As expected, estimates became insignificant upon adjustment for UFP and accumulation mode particles (vice versa).

Some panel and scripted exposure studies (Gong et al., 2014; Huttunen et al., 2012; Karottki et al., 2014; Rich et al., 2012; Steenhof et al., 2013; J. Zhang et al., 2013) measured blood cell counts as markers for systemic inflammation. The short-term studies indicate inconsistent results for the most frequent used white blood cell counts. In a group of young university workers, **Gong** et al. (2014) and **Zhang** et al. (2013) found positive association for shorter lag periods of 0 and 1-days, being significantly elevated at lag 0. However, 2 to 6-day lagged exposures to UFP PNC yielded inverse associations. **Huttunen** et al. (2012) oberserved non-significant effect estimates being lowest for 0-day lagged and most pronounced for 2-day lagged exposure to quasi-UFP PNC in a panel of elderly IHD patients. The study by **Rich** et al. (2012) suggests slightly positive associations between UFP PNC and white blood cell counts and generally (except from 4-day lag) slightly inverse associations with accumulation mode particles for 0 to 4-day lag periods. **Steenhof** et al. (2014) found significantly positive associations between quasi-UFP PNC and white blood cell count 2 hours post exposure for all and outdoor sites and significantly negative associations 18h post exposure. The same pattern was observed for neutrophiles. However, monocyte, lympohocyte and eosinophile counts were consistently and significantly inverse associated. **Karottki** et al. (2014) found also (non-significant) associations for neutrophils and adverse associations for leukocyte, monocyte and lymphocyte counts. Eosinophiles were positively associated with 2-day lagged PNC sized 10-280 nm.

Associations with myeloperoxidase were explored in three studies (Croft et al., 2017; Huttunen et al., 2012; Rückerl et al., 2014). **Croft** et al. (2017) observed consistently inverse associations, being significant for a lag period of the previous 12 hours. **Huttunen** et al. (2012) found non-significant positive associations for 0 to 3-day lagged exposures. In the group of genetically susceptible persons, **Rückerl** et al. (2014) found significant positive associations for a 5-day averaged exposure to UFP PNC. However, effect estimates were non-significant inverse in the group of individuals suffering from T2DM or IGT.

Numerous studies investigated further inflammatory markers. **Bind** et al. (2016) found increasing effect estimates for Interleukin-6 along larger averaged lag periods, finally being significant at a mean average of 28 days. TNR-II was positively associated with PNC for lag periods from 7 to 28 days in comparison to lag days 0 to 3. Furthermore, the intercellular adhesion molecules-1 ICAM-1 was consistently and significantly positive associated with PNC whereas associations with the vascular cell adhesion molecule-1 VCAM-1 were less pronounced across the different quantiles. **Huttunen** et al. (2012) investigated the inflammation markers interleukin IL-8 and IL-12. He found strongest associations for 3-day lagged exposures compared to 0 to 2-day lagged exposures. 3-day lagged quasi-UFP PNC associations with IL-12 was significantly elevated. For urinary MDA and urinary 8-OHdG. **Wittkopp** et al. (2013) found non-significantly inverse associations for IL-6sR as well as positive associations for tumor necrosis factor TNFRII for 0 to 5 day-lagged exposures. In a scripted exposure approach, **Steenhof** et al. (2013) observed elevated levels of IL-6 in healthy participants 2h after

exposure in a real-world scenario. **Gong** et al. (2012) found elevated effect estimates for 2 to 5-day lagged (urinary MDA) and 3- to 6-day lagged 8-OHdG.

Summary of associations with systemic biomarkers

Overall, the majority of the studies investigating UFP effects on systemic inflammation markers indicate inconsistent associations. Effects of UFP on indices for hs-CRP, fibrinogen, blood cell counts, myeloperoxidase varied, which may originate from different compositions of participants, assessed PNC fractions and exposure assessment types. In most studies, effects seem to be most pronounced for shorter lag periods. Only few multi-pollutant models do not allow statements on independent effects of UFPs/ quasi-UFPs.

Neurocognitive indices

Two scripted exposure studies conducted in Brussels (Bos et al., 2013; Bos et al., 2011) explored associations between mobile measured quasi-UFP exposures and serum brain-derieved neurotropic factor (BDNF) in a short-term (Bos et al., 2011) and medium-term time-frame of 12 weeks (Bos et al., 2013). In the later study, Bos and collegues additionally assessed cognitive tests. Whereas the short exposure of 20 minutes cycling along a busy road did result in any association with BDNF, the 12-week aerobic training program resulted in higher BDNF-levels differed significantly in participants exercising in a rural area versus participants exercising in an urban area. Due to the lack of co-pollutant adjustment, it is not possible to disentangle UFP associations from other pollutant effects.

Table 19: Summary table of conducted analyses in the 55 studies on subclinical outcomes

Outcome	Number of studies	Number of studies with single-pollutant--associations in expected direction	Number of studies with multi-pollutant associations in expected direction	Comments (i.e. studies with significant results in the non-expected direction)
Respiratory indices	11	4/11	3/3	Li et al. (2016) found significantly positive associations between UFP and FEV_1 & FVC
Blood pressure	13	9/13	2/4[16]	Two of the nine studies with associ. showed inconsistent results across lags
HRV	16	12/16	3/5	In Zhang et al. (2013), effect estimates decreased upon adj. for NO_2 and increased upon adj. for O_3
Arrhythmia	1	1/1	nc	Strong associations with $PM_{0.25}$, nearly protective associations between PN and hourly nighttime measured tachycardia
Vascular function	7	4/7	1/2	
Pulmonary inflammation	12	12/12	2/2	Most studies investigated effects on FeNO

[16] One of the four studies did not show assoc. in single-pollutant models, either. A further study (Rich et al., 2012) did not show all results, therefore rated as non-associated here

Outcome	Number of studies	Number of studies with single-pollutant--associations in expected direction	Number of studies with multi-pollutant associations in expected direction	Comments (i.e. studies with significant results in the non-expected direction)
Systemic inflammation (incl. fibrinogen)	18	7/18[17]	2/5	Significant inverse associations between fibrinogen & PNC upon adjustment for NO_2 (Strak et al., 2013)
Neurocognitive outcomes	2	1	nc	-

HRV: Heart rate variability, Nc: not conducted.

4.3.2 Long-term effects

4.3.2.1 Mortality

One long-term study explored relations between all-cause, cardiovascular, ischemic heart disease and pulmonary mortality (**Ostro** et al., 2015) (Table A2a). The large cohort study included 101,884 female participants of the California Teachers Study and was realized from the beginning of 2001 to mid-2007. Ultrafine PNC sized 10 to 100 nm was applied using a chemical-transport model taking into account meteorological fields and emissions estimates for different sources to predict airborne particulate matter concentrations. Mortality outcomes were assessed via linkage to an administrative database. The authors adjusted their models extensively for individual covariates. Ostro et al. (2015) observed slightly increased hazard ratios for all-cause mortality (1.01 (95% CI 0.98; 1.05)), cardiovascular mortality (1.03 (95% CI 0.97; 1.08)), and no associations for pulmonary mortality (95% CI 1.01 (0.93; 1.10)). Among cardiovascular causes of death, hazard ratios for IHD mortality were significantly elevated (1.10 (95% CI 1.02; 1.18)). The study did not adjust for co-pollutants but for constituents of UFP, therefore prohibiting the evaluation of independent effects of UFPs. A further limitation of the study is the lack of representativeness of the participants. Moreover, the spatial resolution of the exposure model was relatively large with 4x4 km^2, which prevents the assessment of small scale differences in exposure to UFPs/quasi-UFPs, which are characterized by a high spatial variability. Underestimation of the true association is therefore possible.

4.3.2.2 Morbidity

Four studies investigated associations between morbidity and long-term UFP exposure based on chemical transport or land regression models (Laurent et al., 2014; Laurent et al., 2016a, 2016b; Li et al., 2017). All four studies were carried out in North America with two cross-sectional, one nested case control and one case-cohort design. Three of these studies investigated birth outcomes, one cardiovascular and cerebrovascular morbidity. None of them conducted co-pollutant models.

Cardiovascular/ cerebrovascular/ metabolic morbidity

Three communities near Boston, representative for highway or urban background air pollution, were selected for the CAFEH study. Data from a subset of 435 participants which attended a field clinic was cross-sectionally analyzed by **Li** et al. (2017). Mobile monitoring and spatial-temporal regression models were used to estimate PNC (>4 nm) at each residential address (resolution of 20m). Subsequently, time activity information from all participants was used to assign individualized time activity adjusted annual PNC exposure. Self-reported prevalences of stroke or ischemic heart disease,

[17] Most positive associations relate to fibrinogen

hypertension and diabetes were non-significantly associated with ORs of 1.35 (95% CI 0.83; 2.22), 0.71 (95% CI 0.46; 1.1) and OR 1.14 (95% CI 0.81; 1.62). Due to lack of adjustment for co-pollutants, the independence of UFP associations cannot be evaluated.

Birth outcomes

Two studies explored associations between low birth weight (LBW) in term born infants (>37 gestational weeks) and submicron particle mass $PM_{0.1}$. **Laurent** et al. (2014) evaluated cross-sectional data of 960,945 singleton live births in Los Angeles between 2001 and 2008. Odds of having a term LBW infant was significantly increased with OR 1.03(95% CI 1.02; 1.03) per IQR of 0.4271 µg primary $PM_{0.1}/m^3$. In addition, the model was able to deliver broad source categories for $PM_{0.1}$, namely gasoline, diesel, shipping, high sulfur combustion sources, commercial meet cooking, wood burning and other sources. The source most strongly associated with term LBW was gasoline, followed by wood burning, meat cooking, diesel and high sulfur sources. In a very similar study setting with the same exposure assessment, **Laurent** et al. (2016b) were able to partly confirm their results with a case-cohort approach with over 70,000 term born LBW infants in California between 2001 and 2008. Even though primary $PM_{0.1}$ mass was not associated with increased odds for term LBW (OR 0.996 (95% CI 0.98; 1.01) per IQR 1.359 $\mu g/m^3$), when broken down by sources, odds for term LBW were significantly higher for on-road gasoline (OR 1.05 (95% CI 1.02; 1.09) per IQR), commercial meat cooking (OR 1.03 (95% CI 1.01; 1.06) per IQR) and on-road diesel (OR 1.03 (95% CI 1.0; 1.06) per IQR). In this second study, PNC (<100 nm) was also modeled with the line-source roadway dispersion model (CALINE4), however there was no association with term LBW (OR 1.001 (0.989; 1.014) per IQR 6,444 particles/cm^3). Using a nested matched case-control approach within the same cohort, **Laurent** et al. (2016a) studied the association between preterm birth (n= 442,314) and PM applying the same exposure measurements. ORs for preterm birth in association with IQR increases in average exposure during pregnancy were significantly elevated for primary $PM_{0.1}$ and its components organic carbon (OC), elemental carbon (EC) and secondary organic aerosols (SOA): 1.02 (95% CI 1.02; 1.03) per 1.39 µg primary $PM_{0.1}/m^3$, 1.02 (95% CI 1.01; 1.03) per 0.99 µg OC in $PM_{0.1}/m^3$, 1.04 (95% CI 1.04; 1.05) per 0.13 µg EC in $PM_{0.1}/m^3$ and 1.13 (95% CI 1.12;1.143) per 0.061 µg SOA in $PM_{0.1}/m^3$. As for sources of primary $PM_{0.1}$, strongest and statistically significant associations per IQR increase in exposure were observed for on-road gasoline, followed by on-road diesel and commercial meat cooking. However, preterm birth was negative and significantly associated with submicron particle mass from wood burning. Furthermore, inverse effect estimates were observed for the association between preterm birth and PNC in all subjects with OR 0.99 (95% CI 0.99; 1.00) for a 6,480 particles/cm^3 increase in PNC. In a separate analysis, the authors explored the influence of geocoding accuracy with a subgroup of births, geocoded at the tax parcel level. In this subgroup, the effect estimate for an IQR increase in PNC was significantly elevated with OR 1.03 (95% CI 1.02; 1.04) for 6,770 particles/cm^3. Due to lack of adjustment for co-pollutants, the independence of UFP associations cannot be evaluated.

4.3.2.3 Emergency department visits

No studies found.

4.3.2.4 Subclinical outcomes

Three cohort studies located in Switzerland (Aguilera et al., 2016), Spain (Sunyer et al., 2015) and Germany (Viehmann et al., 2015) and two cross-sectional studies located in Boston/USA (Lane et al., 2015; Lane et al., 2016) investigated long-term subclinical health effects in response to quasi-UFP and UFP exposures (Table A2c). The study by Aguilera et al. (2016) additionally adjusted for co-pollutants (Table A4b).

Cardiovascular endpoints

Aguilera et al. (2016) explored associations between land-use regression modeled PNC 10-300 and LDSA and carotid intima-media thickness in 1,503 participants of the SAPALDIA cohort located in different areas in Switzerland. The cross-sectional analysis resulted in increased effect estimates for carotid intima-media thickness (2.06% (95% CI 0.03%; 4.10%) per increment of 10. to 90th percentile) and LDSA (2.32 (95% CI 0.23; 4.48)). Upon adjustment for lifestyle variables and T2DM prevalence, SBP, HDL cholesterol and different medications, effect estimates increased. Upon further adjustment for NO_2, the effect estimate in relation to PNC turned into a negative direction. However, the effect estimate for LDSA increased but lost its significance.

Inflammation markers

Three studies investigated long-term effects of quasi-UFP on inflammatory biomarkers within a time window of one year. **Viehmann** et al. (2015) analyzed repeated measures of hs-CRP, fibrinogen, white blood cell counts and platelets in relation to PNC sized 5 to 2,200 nm modeled by a chemical transport model with a spatial resolution of 1x1km^2. In their cohort with multiple measurements of 3,213 participants of the HNR cohort, Viehmann et al. (2015) observed consistently increased effect estimates for all endpoints being most pronounced for hs-CRP (3.8% (95% CI −0.6%; 8.4%) per IQR of 27,000 particles/ml) and fibrinogen (1.0% (95% CI 0.0%; 2.0%) per IQR of 27,000 particles/ml) and white blood cell counts (1.0 (95% CI −0.1; 2.1) per IQR of 27,000 particles/ml). Two cross-sectional studies by **Lane** (2015; 2016) investigated effects of a land-use regression model in combination with a time-activity pattern on hs-CRP, IL-6, TNFRII and fibrinogen within participants of the CAFEH cohort. In the first study with 140 participants, **Lane** et al. (2015) used personal exposure model including the residential annual average + work + other + highway + air-condition at home at specific temperature in relation to hs-CRP and fibrinogen. His models yielded positive associations for hs-CRP (ß-estimate: 1.26 (95% CI −0.02; 2.75)) and (IL-6 0.65 (95% CI −0.26; 1.55)) in the fully adjusted models (increments unclear). The subsequent study with a larger cohort of 408 participants, **Lane** et al. (2016) observed elevated effect estimates for the markers of systemic inflammation hs-CRP 14.0% (95% CI -4.6%; 36.2%), IL-6 (8.9% (95% CI -2.6%; 21.8%) and TNFRII (5.1% (95% CI -0.4; 10.9) per 10,000 particles/ml in the fully adjusted models. For the coagulation marker fibrinogen, the authors reported inverse changes (-1.9 (95% CI -5.5; 1.6)). Due to lack of adjustment for co-pollutants, the independence of UFP associations cannot be evaluated.

Neurocognitive health endpoints

A Spanish cohort study in the framework of the BREATHE[18] project investigated quasi-UFP effects on the cognitive function of 2,715 children attending schools in low and high polluted areas (**Sunyer** et al., 2015). PNC sized 10-700 nm was assessed at school during two measurement campaigns complemented by exposures at the home address estimated by a land-use regression model. At baseline and within one year, cognitive function in children of high polluted areas developed less in schools of high polluted areas versus low polluted areas. Quasi-UFP exposure at the courtyard was related to inverse associations in working memory, superior working memory and positive associations in inattentiveness at baseline and after 12 months being significant for superior working memory and inattentiveness (e.g., difference of 3.9 (95% CI 0.31; 7.6) per increase of 6,110 particles/ml). The models were adjusted for maternal education and socioeconomic status, but not for co-pollutants, which prevents the investigation of independent associations of UFPs/quasi-UFPs.

[18] Brain Development and Air Pollution Ultrafine Particles in School Children

Summary of long-term health effects

The above described study results of long-term studies on UFP health effects are summarized in table 20. A limited number of studies, varying outcome measures and exposure assessment methods as well the lack of or co-pollutant adjustments do not allow drawing final conclusions.

Table 20: Summary table of conducted analyses in the 10 long-term studies

Outcome type/ study	Outcome	Single pollutant associations	Multipollutant associations
Mortality/ Ostro et al. 2015	- all-cause - cardiovascular/ IHD - pulmonary	0 (+)/0 0	nc nc nc
Morbidity / Li et al. 2017 Laurent et al. 2014/2016b Laurent 2016a	- cardiometabolic - low birth weight - preterm birth	(+) +/(+) -/+	nc nc nc
Subclinical/			
Aguilera et al. 2016	- carotid-intima-media thickness (PNC/LDSA)	+/+	-/(+)
Viehmann et al. 2015	- hs-CRP/ fibrinogen/ WBC	(+)/+/(+)	nc
Lane et al. 2015	- hs-CRP/ IL-6	(+)/(+)	nc
Lane et al. 2016	- hs-CRP/ IL-6/ TNRFIII/ fibrinogen	(+)/(+)/(+)/(-)	nc
Sunyer et al. 2016	- working memory, - superior working memory - inattentiveness	(+) + +	nc

IHD: Ischemic heart disease, 0 indicates no association. (+) and (-) indicates primarily non-significant associations, + and - indicate significant associations. Nc: not conducted.

4.4 Summary of health effects

An overview on all included short-term and long-term studies reflects the inconsistency of the results (Table 21). More than half (n=49) of the studies on short-term effects (n=79) reported at least one significant effect in the single pollutant model, especially those studying mortality or subclinical outcomes. For less than half of the single-pollutant associations (21 of 49), the general pattern of the association was consistent regardless of the significance level. The associations in multi-pollutant studies (n=32) remained consistent in about half of the studies (n=7).

Associations between health outcomes and long-term exposure with ultrafines were more consistent in the single pollutant models even though there were considerably fewer studies. Nevertheless, long-term studies adjusting for other pollutants are still lacking with only one study, which did not show effects in the multipollutant model.

Table 21: Summary table of all included studies in single- and multipollutant associations

Outcome	Single pollutant effect	Consistency of general pattern	Multipollutant effect	Consistency of general pattern
Short-term	49/79*	21/49	18/32	7/18
Mortality	5/7	2/5	4/6	1/4
Morbidity	3/7	0/3	-	-
Hospital admission	4/10	2/4	0/5	-

Subclinical	37/55	17/37	14/21	6/14
Long-term	8/10	1/1	0/1	-
Mortality	1/1	1/1	-	-
Morbidity	3/4	-	-	-
Hospital admission	-	-	-	-
Subclinical	4/5	-	0/1	-

5 Discussion

5.1 Literature search

We conducted a systematic comprehensive search of relevant epidemiological studies on ultrafine and quasi-ultrafine particles for the period from 01.01.2011 until 11.05.2017. The different strategies of our search consisted of a MEDLINE search, using two alternative strategies, a search in the specialized data base LUDOK, and a hand search in review articles and reference lists of identified publications. Overall, the additional yield of the alternative MEDLINE search strategy, and of the complementary search strategies (LUDOK and hand search), and of the repeated search was substantial, with altogether 15 additional references added to the final analysis data base and an additional 13 articles identified per MEDLINE and hand search in February 2018. This relatively high yield reflects the lag in indexing newly published studies in large literature data bases as well as the fast development of an emerging scientific field. More specialized data bases such as the dedicated LUDOK literature data base are therefore very useful for targeted and timely research.

5.2 Evaluation of health relevance of ultrafine particles

Our evaluation of the health relevance of ultrafine particles is based on the above described epidemiologic studies and how they add to the available the evidence since the comprehensive review conducted by the HEI, published in 2013. Overall, the epidemiological evidence is quickly increasing and it can be expected, that the next few years will bring a substantial increase in relevant studies. Currently, we are still in the beginnings of health-related research of UFPs, which is in part due to the still developing methods (see sections below on exposure assessment).

The HEI concluded in its review that "the current database of experimental and epidemiologic studies does not support strong and consistent conclusions about the independent effects of UFPs on human health" (Health Effects Institute, 2013). Major reasons for this lack of evidence, specifically for epidemiologic studies, lie in the difficulty of assessing population-based exposure to UFPs for short-term as well as for long-term studies. Due to the specific properties of UFPs with a high temporal and spatial variability, common exposure assessment strategies, which have been developed for the more homogeneously distributed larger particle fractions, will lead to larger exposure misclassification when applied to UFPs. Nevertheless, HEI does not conclude that independent effects of UFPs can be ruled out, but rather recommends the exploration of alternative exposure metrics, spatial modeling techniques, and statistical methods.

In this review, we use similar design- and outcome-specific categories as in the HEI review to be able to integrate our findings with the prior evidence. Since independence of effects is the key question regarding the health relevance of UFPs, we specifically focus on studies with co-pollutant adjustment.

Inconcistency of results by endpoint

Previous evaluations have concluded, that the combined results for respiratory as well as for cardiovascular endpoints are still inconsistent (Health Effects Institute, 2013). When considering the newly acquired evidence during the years from 2011 to 2017, this picture has not changed substantially. Even though there is a growing number of specifically designed studies to investigate health effects of UFP/quasi-UFP, we cannot identify a consistent pattern of health effects on either respiratory or cardiovascular disease across the different endpoints including mortality, morbidity, emergency department visits/hospital admissions or subclinical endpoints. For other outcomes such as mental disorders, neurocognitive function or birth outcomes, the evidence base is still too small to derive firm conclusions.

Even though results are not consistent across different outcomes types, the majority of the 11 studies investigating short-term effects on BP, the major risk factor for cardiovascular disease, indicate an association with increased blood pressure. Once again, evidence from the three co-pollutant-adjusted studies is mixed, which underscores the necessity of further studies with co-pollutant adjustments.

The lack of consistent findings can be explained by a number of factors. These include differences in exposure assessment (see below), endpoint assessment, study design and size, and different confounder control, specifically differences in the adjustment for co-pollutants (see below).

Long-term exposure and health effects

In contrast to the last prior comprehensive review by HEI (2013), ten studies have been published investigating long-term effects of UFPs on various health outcomes. While most of these studies found elevated point estimates for associations of UFPs with adverse health outcomes, only one study adjusted for co-pollutants, including NO_2. Adjustment with NO_2 led to a decrease in the effect estimate to an inverse association.

While the current evidence base does not support an independent effect of UFPs on health outcomes, this should by no means be mistaken for a proof of the absence of such an effect. As will be discussed below, current exposure assessment techniques are not well suited to describe and investigate long-term exposure to UFPs. More studies applying novel methods for individual-level exposure to UFPs are therefore urgently needed. Important applications are next to road traffic-related exposures also the emerging problem regarding exposure to UFPs in the vicinity of airports, which has only recently been described (N. Hudda et al., 2016).

Exposure assessment

Overall, the number of studies including the assessment of exposure to and the investigation of health effects of UFPs is rapidly increasing. One important factor contributing to this rapid increase is the development of new instrumentation, which enables a less expensive assessment of UFP/quasi-UFP for example with condensation particle counters. However, research is still at the beginning and new exposure assessment methods need to be defined and employed in epidemiological studies.

Challenges of exposure assessment for UFPs include the high spatial and temporal variability of UFP/quasi-UFP, which necessitate different exposure assessment designs than the “classical” air pollutants like $PM_{2.5}$ and PM_{10} with a much more homogeneous spatial distribution. This high spatial variability is of concern not only for long-term health effects studies, which are based on long-term spatial differences in exposure, but also for short-term studies with a central-site measurement. These studies assume that the temporal changes from day to day are evenly distributed across the sometimes very large study areas; an assumption that might not hold true for UFPs. Given the possibility of a larger exposure estimation error for UFPs compared to other pollutants, a systematic bias towards the null in single-pollutant studies and in multi-pollutant studies is probable (Dionisio et al., 2014).

In the future, the development of enhanced spatiotemporal models can contribute to a more precise exposure assessment across larger areas. Current models such as the German EURAD model need to be adapted to incorporate specific sources, validation measurements and increase the spatial resolution.

A further challenge of UFP/quasi-UFP exposure assessment is the non-standardized equipment and the non-standardized use of size fractions in the studies. The commonly used measurement devices have different lower cutpoints for the particle size. Since the majority of particles are located in the nucleation mode (< 20 nm) of the particle size distribution, even small differences in the lower cutpoint between 1 and 20 nm can lead to substantial differences in particle number concentration.

Futhermore, the reporting of the exposure assessment often does not include the exact size range of particles, which prevents direct comparisons of exposure between studies.

Independence of effects

Even though several studies across the investigated endpoints have observed positive associations of UFP/quasi-UFP with various health effects, the overall evidence for independent effects is still insufficient. We noticed, that specifically the newer studies conduct multi-pollutant models with a higher frequency than the older studies, which is a positive development (e.g., Aguilera et al., 2016; Croft et al., 2017; Lanzinger et al., 2016a; Samoli et al., 2016; Stafoggia et al., 2017). However, the type of adjustment still varies substantially between studies and there is no standard strategy for co-pollutant adjustment yet. At the moment, adjustment for NO_2 generally seems to exert a greater effect on the point estimate than other co-pollutants (e.g., Lanzinger et al., 2016a&b; Su et al., 2015; Samoli, Andersen et al., 2016, Zhang et al., 2013). One reason for this is the overlap in sources and spatial/temporal distribution of UFP/quasi-UFP and NO_2, which can lead to instability in the models and biased effect estimates in two-exposure models.

5.3 Transferability of results to the situation in Germany

The transferability of the above reported results to the situation in Germany will be judged according to the following criteria: Localizations of identified studies and level of exposure to ultrafine particles, level of exposure to airborne co-pollutants, baseline prevalence of investigated diseases and selection of study populations.

5.3.1 Exposure

The vast majority of the identified studies are located in North America (n=37, 43.5%) or Western Europe (n=27, 31.8%) and five studies (6%) located in more than one world region (Table 2). When examining the study sites of studies with multiple study centers, we can observe that the majority of study sites are located in Western and Southern Europe (n=44 of 101 study sites, 43.6%)(Table 3). The concentrations of ultrafine particles vary considerably in time and space and direct comparisons of single center measurements are subject to large variation depending on hour, day and season of measurement as well as exact placement of the measurement site (traffic, urban background, regional background site)(Birmili et al., 2016; UFIPOLNET, 2008). In the German Ultrafine Aerosol Network (GUAN), long-term measurements of ultrafine and fine particles have been conducted at 17 sites across Germany, including alpine sites (Zugspiptze), rural sites, urban background and roadside measurement sites (Birmili et al. 2016). Of note, the size of the measured particles ranges from 20 to 800 nm, thereby not encompassing the nucleation mode of particles and including the accumulation mode particles. Preliminary results of GUAN measurements indicate a range of hourly median concentrations of particle number (sized 20-800 nm) between 900/ml (Zugspitze) and 9,000/ml at the roadside in Leipzig. Hourly mean concentrations are higher with 1,120/ml at the Zugspitze and 10,500/ml in Leipzig. The 95 percentile of the distribution of hourly values reaches 22,400/ml in Leipzig-Mitte. All three roadside measurement sites had P95 values above 19,900/ml, while the urban background sites ranged between 10,000 and 20,000/ml. GUAN also demonstrates the substantial variation in particle size distribution during the course of a week at six mainly urban sites.

The identified studies conducted in Western Europe typically have similar or higher mean total particle counts. A direct comparison is not possible with the available information, since instruments for measurements differ and have different lower cutpoints. 16 out of the 27 studies in Western Europe report the lower cutpoint of their measurement device as 10 nm or lower. Some devices go down as far as 3 nm as their lower cutpoint. Since the majority of particles is sized below 20 nm (nucleation mode) (HEI perspectives, 2013), small differences in the lower cutpoint leads to

substantial differences in mean exposures. In addition, the upper cutpoint also varies considerably, with only few studies examining ultrafine particles in the more strict sense (<100 nm), but rather use the surrogate of total particle number concentration as the exposure of interest. This, however, presents a minor problem as total particle number is dominated by the size fraction below 100 nm (HEI perspectives, 2013).

For the benefit of this review, GUAN primarily demonstrates the large variability of exposures within Germany, but it is not well suited to compare absolute values with other studies, which used different measurement devices. The five studies from Germany included in the review are based on central-site or personal measurements (n=4) with lower cutpoints ranging between 3 and 10 nm. These studies yield mean exposures between 10.000/ml and 20.000/ml, which is comparable to other studies in this review. In comparison, the 13 studies located in the Western Pacific region or in South-East-Asia, in the metropolitan areas of China, South Korea or Taiwan, report measured mean particle number concentrations in similar or slightly higher ranges. The only German study based on modelled exposures applying the EURAD CTM yielded substantially higher mean exposures due to the modelling process, which included the complete nucleation mode and therefore also encompasses short-lived particles sized below 3 nm. We therefore conclude that the level of exposure in the identified studies, while very variable across time and space, is generally comparable to the German situation.

The development of population exposure to ultrafine and quasi-ultrafine particles in Germany in the coming years depends on several factors: (1) the formation and emission of these particles, (2) the spatial distribution of the population, and (3) the concentration of fine particles in ambient air.

According to a size-resolved pan-European anthropogenic particle number inventory, the most important sources of emissions are road traffic in urban areas and alongside highly trafficked roads (Health Effects Insitute, 2013) . Traffic-related emitters of primary UFP are direct injection engines in vehicles, which have increased in number during the last decade and will probably increase further (Köllner, 2016). On the other hand, vehicles with Diesel-powered engines, which also emit particles in the ultrafine and quasi-ultrafine size range, have been equipped with particle filters. This has reduced the emission of fine particles substantially (according to EURO5a less than 5 mg/km). For UFP, the EURO5b norm for the first time sets a limit at 6×10^{11} (European Union, 2007). Overall, with increasing traffic and a rising number of city dwellers expected in the future (Vallance & Perkins, 2010), exposure to road traffic-related UFPs is likely to increase in Germany in the next decade.

A further source of mostly ultrafine particles is aircraft traffic. Several exposure studies have documented increased UFP exposure downwind of airports around the world (Neelakshi Hudda et al., 2014; Keuken et al., 2015; Masiol et al., 2017; Shirmohammadi et al., 2017; Stafoggia et al., 2016). The increased short-term exposure is correlated with aircraft movements over time and reach concentrations up to 50,000 particles/ml (Keuken et al., 2015) 7 km downwind of the airport in Amsterdam and up to 75,000 particles/ml (Hudda et al., 2014) 8 km downwind in Los Angeles. The same studies show that long-term concentrations are elevated up to 3-fold 7 km downwind with more than 200,000 exposed inhabitants close to Schipohl airport, Amsterdam (Keuken et al., 2015) and up to 4-5-fold in Los Angeles, 8-10 km downwind (Hudda et al., 2014). Similar exposure studies are ongoiong in Germany and will yield first information about the exposure of residents close to German airports. Given the increase in air travel, the exposure due to airdraft emissions is likely to play an increasing role in the future.

Moreover, the concentration of fine particles in ambient air is a determinant of UFP in a way that UFP will collide and coagulate with larger particles. A high concentration of ambient fine particles will therefore support the clearance of UFP in ambient air. With the reduction of fine particles, UFP will likely stay longer airborne than in an environment with high PM concentrations.

5.3.2 Exposure to co-pollutants

The level of airborne co-pollutants are important, as most of these co-pollutants have own effects on the outcomes of interest. 78 of the 85 identified studies (92%) assessed the level of at least one other air pollutant; however, only 34 studies adjusted for at least one co-pollutants in their analysis (see section 4.3). Assessment of and adjustment for airborne co-pollutants is therefore not conducted in a comparable way across the identified studies.

Analysis of the multi-pollutant models revealed, that $PM_{2.5}$ and NO_2 are the co-pollutants which tend to influence the UFP/quasi-UFP estimate the most. Often, but not always, does the adjustment for NO_2 lead to an attenuation of the association of UFP/quasi-UFP with the health outcome (Leitte et al. 2012; Meng et al. 2012; Stafoggia et a.. 2017; Su et al. 2015; Iskandar et al. 2012; Lanzinger et al. 2016; Rosenthal et al. 2013; Gong et al. 2014; Janssen et al. 2015; Steenhof et al. 2013). Adjustment for PM_{10} and $PM_{2.5}$ also attenuates the UFP/quasi-UFP association in several studies, but in most cases less than the NO_2 adjustment.

The level of co-pollutants, and specifically $PM_{2.5}$ and NO_2, can be compared across Europe using the "Air quality in Europe — 2017 report" by the European Environmental Agency (European Environmental Agency, 2017). According to this report, Germany ranks top among the 28 member states regarding the annual mean of NO_2 at the included monitoring sites (European Environmental Agency, 2017; Fig 6.1). Similar to UFP/quasi-UFP, the annual mean at selected monitoring sites is not able to give a comprehensive overview of the exposures of the study populations in the included studies, as NO_2 concentrations are subject to a high variability across time and space. Of the 34 studies that adjusted for co-pollutants, 15 were conducted in Western Europe. Of those, three were conducted in Germany, Augsburg, and all other studies were conducted in mostly major cities in Switzerland, the Netherlands, Sweden, Denmark and Finland with comparable traffic exposures.

We therefore conclude that the findings of an at least partial overlap of effects between UFPs and NO_2, which we observe in the Western European studies included in this review (Iskandar et al. 2012; Janssen et al. 2015; Rosenthal et al. 2013; Stafoggia et al. 2017; Steenhof et al. 2013), hold true for Germany as well.

5.3.3 Disease prevalence

The majority of the studies identified in this review is located in Western/Southern Europe and North America. The cause-specific age-adjusted death rates for all non-communicable diseases and for respiratory diseases for 2015 are similar for the WHO Region of the Americas (including South America, which is not included in this review) and the WHO European Region (World Health Organization, 2016b). On the other hand, the annual cause-specific age-adjusted death rates for cardiovascular diseases differ, with a substantially lower age-specific death rate in the Americas (211/10,000) compared to the European Region (344/10,000). This difference is primarily due to the combination of both Americas in this statistic. Compared to other European countries and the USA included in this review, Germany has a similar distribution of causes of premature deaths as the Netherlands with ischemic heart disease, lung cancer, Alzheimer disease, cerebrovascular disease and COPD ranking 1 to 5 in both countries. This ranking is very similar in the UK, Denmark, Sweden, Spain and the USA.

Moreover, the majority of studies investigate short-term sublinical outcomes (Table 8) and of those, cardiovascular, respiratory and biomarker outcomes present the focus of the included studies (Table 9). The outcome assessment of these studies is not subject to country-specific ICD-coding conventions. Unless baseline differences in physiological markers exist between the populations included in this review and the German population, which we have evidence for, transferability on results for Germany can be inferred.

5.3.4 Study population

Most studies included in this review are based on selected study populations (n=62, 72.9%) and only 10 (11.8%), respectively 13 (15.3%) studies were deemed representative or at least somewhat representative of the general population (Table 14). The studies deemed to be completely representative of the target population are the time-series studies, which are based on general populations of the city of study. One of these time-series studies (Diaz-Robles et al. 2014) targeted selected age-groups within the general population. Of the other studies, 13 (15%) studies include at least one random sample of the source population. Almost all identified articles describe the study population well. The 10 studies investigating long-term effects are mostly analyses based on existing cohorts of several hundreds to thousands of participants, exclusively located in Western Europe or North America. Of these, six studies target the adult population of either sex or limited to one sex (Ostro et al. 2015), and four studies target children (Laurent et al. 2014, 2016a and 2016b; Sunyer et al. 2015). Among the short-term studies, the study populations are mostly highly selected small groups of either healthy (younger) adults or participants with a respiratory or cardiovascular disorder such as asthma, COPD, coronary artery disease, etc..

5.3.5 Transferability – conclusions

Based on the above descriptions of exposure level, co-pollutant exposure, baseline disease prevalence and included study populations we conclude that the overall results of this review can be transferred with the appropriate caution to the German situation.

Important limitations are (1) the paucity of studies with co-pollutant adjustment, which is specifically important because of the high NO_2 exposures in Germany, and (2) the use of highly selected groups in short-term studies, as these often do not include specifically vulnerable populations such as patients with badly controlled disease, newborns and children.

5.4 Overall conclusions

The investigation of health effects in epidemiological studies is a rapidly increasing field of research and substantial developments have been made during the last seven years, tackling two of the most urgent open questions of research: First, several studies on long-term health effects of UFPs have been conducted and published. Second, specifically the more recent studies have undertaken efforts to control for co-pollutants to identify the independent effect of UFPs.

Despite the obvious development in the field, the overall conclusions have not changed substantially over the time period investigated in this study.

First, the evidence on health effects remains inconclusive or insufficient for most of the studied outcomes. Specifically, while a number of studies have investigated mortality and emergency department/hospital admission outcomes, the relatively few studies with co-pollutant adjustment reveal mixed and, up to now, inconclusive evidence. In terms of number of studies, most evidence is available from studies investigating subclinical outcomes. Within this group of studies, cardiovascular outcomes and outcomes of pulmonary and systemic inflammation show the most consistent patterns with associations generally pointing into the direction of the adverse health outcome. Nevertheless, the evidence for independence of effects remains limited here as well, as only few studies have adjusted for co-pollutants.

Second, exposure assessment in the population remains difficult, due to the specific characteristics of UFPs. Studies using central-site exposure assessment probably miss a large part of the variability. Studies using classical spatial modeling techniques need to incorporate the very high spatial and temporal variability. Null findings or reductions in UFP/quasi-UFP effect estimates upon co-pollutant

adjustment can at least in part be explained by exposure misclassification and measurement error. Exposure assessment has to devote special attention to measurement techniques, size-fractions and localisations of monitor placement. Reporting needs to be standardized to make studies more easily comparable.

Third, the independence of UFPs cannot be evaluated at the moment, due to the low number of studies with adjustment and the above mentioned limitations to exposure assessment for UFPs. A positive development is the increase in studies paying attention to this issue.

Fourth, there is still an urgent need for long-term studies on health effects of UFPs. This will require the development of modeling techniques. Furthermore, specific high-exposure situations need to be identified and described in more detail to be able to assess long-term health effects. Specifically, while near road exposures have already been recognized as important factors, airport-related exposures, which have recently been shown to be substantially above background concentrations, have not been included in health effects studies yet.

In addition to these general conclusions, we conclude that the overall results of this review can be transferred with the appropriate caution to the German situation. Important limitations are (1) the paucity of studies with co-pollutant adjustment, which is specifically important because of the high NO_2 exposures in Germany, and (2) the use of highly selected groups in short-term studies.

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