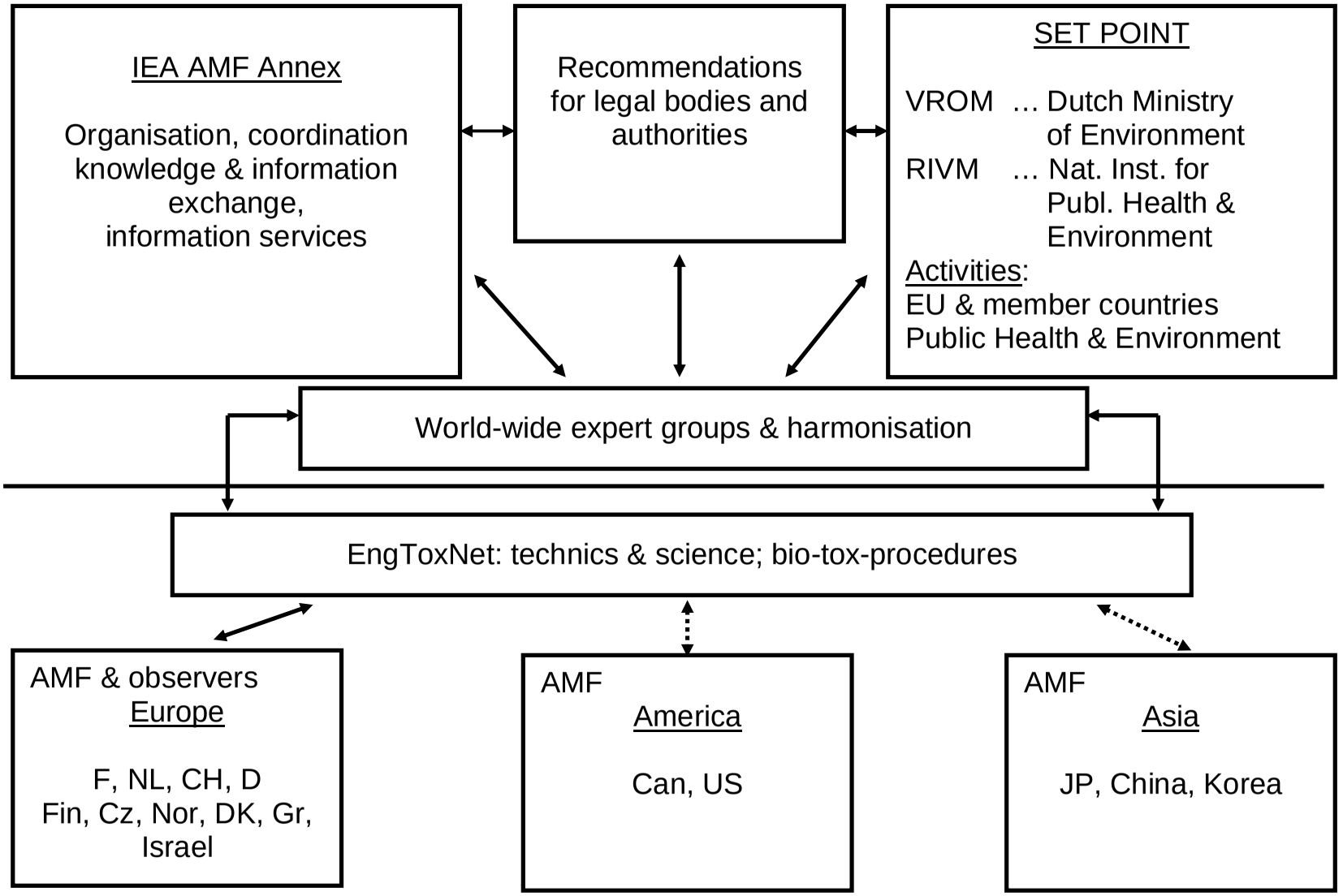
Efforts of Coordination and Information of the Worldwide Research on Toxicity of Exhaust Gases from Engines with Unified Methodology of Aerosol Exposure



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Toxicological tests – endpoints (examples)

LDH: estimate of membrane integrity which indicates cell viability (toxic conditions → leaky membrane → cytosolic proteins (as LDH) can leave the cell); more LDH – more potential of destroying cells.

WST-1: chemical which is used to measure proliferative ability of cells (do they grow as fast as, expected?) and cell viability. WST-1 is cleaved by mitochondrial activity in viable (healthy) cells and the product (formazan) can be detected colorimetrically. Mitochondrial activity is indicative for the metabolic functioning of a cell; more of the product formazan – cells healthier.

ATP: is a key indicator for intact metabolism (the cells 'energy storage molecule'). The ability for ATP production is strongly affected by toxic conditions; more ATP – intact metabolism, cells OK.

MTT: works in a similar way as WST-1 (also product formazan).

Hoechst: is a dye (and a method) which can get into cells and is actively exported from cells. If the cell is not well, export will not work properly and the amount of the dye in a cell therefore indicates its viability; more Hoechst in the cell – worse condition.

PI exclusion: PI (propidium iodide) is only taken up by severely damaged cells. In principle a similar approach as LDH, but the other way round. Indicates membrane integrity; more PI in the cell is a sign of damage.

Glutathion, GSH: antioxidant molecule produced by the cell, which is sacrificed to oxidative molecules instead of e.g. DNA or important proteins and is used to protect proteins by binding to oxidation susceptible sites. Depletion of reduced GSH indicates high loads of oxidizing chemical species (e.g. ROS ... reactive oxygen species) and gives an estimate of the cell's antioxidant capacity.

NADPH: in principle the same as GSH, but NADPH is a reducing molecule which is used in metabolism (in part: reduce oxidized molecules that could not be protected by GSH); more NADPH means less oxidative stress.

TNF-a, IL-xy etc: cytokines, signal molecules (proteins), used for communication of cells with each other. Measurements of these proteins show the induction of inflammatory responses. ELISA is a method for quantification of such molecules, the amount indicates the strength of responses, (quantifies the crosstalk between cells, the signal exchange in relation to inflammation).

Flow cytometry (sophisticated analysis of shape and surface of the cells):
sorts and counts cells according to their state. E.g. cells in which an inflammatory response has been activated by cytokines have certain patterns/markers molecules on their surfaces, by which they can be sorted, (quantification of the outcome of the signal exchange measured by ELISA).

RT-PCR: reverse transcriptase polymerase chain reaction (analysis of intermediate molecules, which are produced by genes as reaction to the toxic influences):
measures the activity of genes, to what extent they are used by a cell. The information about gene function (e.g. used against oxidative stress) and information about gene activity indicates cellular responses to certain stimuli. Can be used for any response to any stimulus.

Comet assay (by a special method by moving the cells through a carrier substance):
measures the integrity of DNA. The extent of DNA strand breaks, which derive from oxidizing agents, radiation, errors during the process of replication (due to inhibitory chemicals, severe metabolic distortions and many more) can be estimated.

TUNEL: measures how many DNA breaks occurred by labeling the resulting free ends by means of an optical method.

H2AX: is a histone, a protein around which DNA is wrapped in the nucleus, and is involved in the repair of double strand DNA breaks (DSBs). If DSBs are present, H2AX becomes phosphorylated - ‘activated’ – which can be detected and used as an estimate of the occurrence of DSBs.

HMOX1 (heme-oxygenase 1)
is a gene that encodes for a protein involved in the regulation of the cellular redox-balance. It acts via the production of biliverdin and bilirubin, both of which have potent antioxidant properties. The production of the protein, that is the transcription of the gene HMOX1 is (among other stimuli) induced by oxidative stress, i.e. by a decrease in the concentration of cellular reducing equivalents such as for example glutathione (GSH).

SOD1 (superoxide dismutase 1)
is a gene encoding for a protein which converts superoxide radicals to oxygen and hydrogen peroxide and thereby acts against oxidative stress. The production of the protein is rapidly induced by oxidative stress i.e. by a decrease in the concentration of cellular reducing equivalents.

CASP7 (caspase7)
is a gene, that encodes for protein which is involved in apoptosis, a process during which a cell eliminates itself in a highly controlled manner without influencing the surrounding tissue (programmed cell death). In particular, caspases7 functions in apoptosis that is activated from inside the cell, i.e. by the cell itself, for instance as a consequence of severe damages in the genetic information (intrinsic stimulation of apoptosis).

FAS (tumor necrosis factor receptor superfamily member 6)
is a gene that encodes for a protein which is involved in apoptosis, a process during which a cell eliminates itself in a highly controlled manner without influencing the surrounding tissue (programmed cell death). In particular, FAS is involved in the induction of apoptotis that is stimulated by other cells (extrinsic stimualtion of apoptosis).

DNA (deoxyribonucleic acid)
: is the molecular material on which cells store their genetic information. DNA is basically made up of four molecules, the nucleotides (abbreviated A, G, C and T), that are linked together in higly specific orders, thereby providing the DNA- or the genetic sequence. This sequence serves as blueprint for the production of proteins, which are the molecular machines that provide all biochemical and regulatory functions in a cell.

mRNA (messenger ribonucleic acid)
: is a molecule that serves as an intermediate in the process of protein production from genetic sequences. Proteins consist of small molecules (amino acids) that are linked together in highly protein-pecific sequences. The blueprint of these sequences is stored in the cellular genome inform of DNA (deoxyribonucleic acid), which basically consists of a long sequence four different molecules (nucleotides). Basically, for each protein an organism is able to produce, there is a gene present in the genome that provides the needed sequence. For the synthesis of a protein, the cell needs to copy the genetic sequence and convert it to the according protein sequence. These processes are called gene transcription and protein translation. mRNA is the product of gene transcription and the substrate for protein translation, i.e. an intermediate information-storing molecule.

Abbreviations:

LDH:	Lactate dehydrogenase
WST-1:	Water soluble Tetrazolium salt 1
ATP:	Adenosin triphosphat
MTT:	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
PI:	Propidium iodid
CCK-8:	Cell counting kit-8
GSH:	reduced glutathion, antioxidant molecule
ROS:	reactive oxygen species
NADPH:	Nicotinamid adenin dinucleotid phosphat
TNF-α:	Tumor necrosis factor-alpha
IL:	Interleukin
ELISA:	Enzyme linked immunosorbent assay
RT-PCR:	reverse transcriptase polymerase chain reaction
TUNEL:	Terminal dUTP nick end labeling (dUTP = deoxyuridine triphosphate)
EMSA:	Electrophoretic mobility shift assay
H2AX:	Histon 2A family, member X
HMOX1	heme-oxygenase 1
SOD1	superoxide dismutase 1
CASP7	caspase7
FAS	tumor necrosis factor receptor superfamily member 6
DNA	deoxyribonucleic acid
mRNA	messenger ribonucleic acid

Introduction in test methodologies and some biological processes

Gene expression and proteins

1) A certain signal acts on the promoter region of a target gene.
2) The signal activates the promoter, protein complexes are recruited which transcribe the gene (transcription = production of RNA from a DNA template). Depending on the gene and the signal, a certain lag time between the stimulus and the activation of the gene can be observed.
3) The mRNA is processed and transported out of the nucleus.
4) The mature mRNA is translated to a polypeptide by the action of ribosomes (translation = production of polypeptides from an mRNA template). Depending on the protein and the state of the cell, translation may not occur immediately.

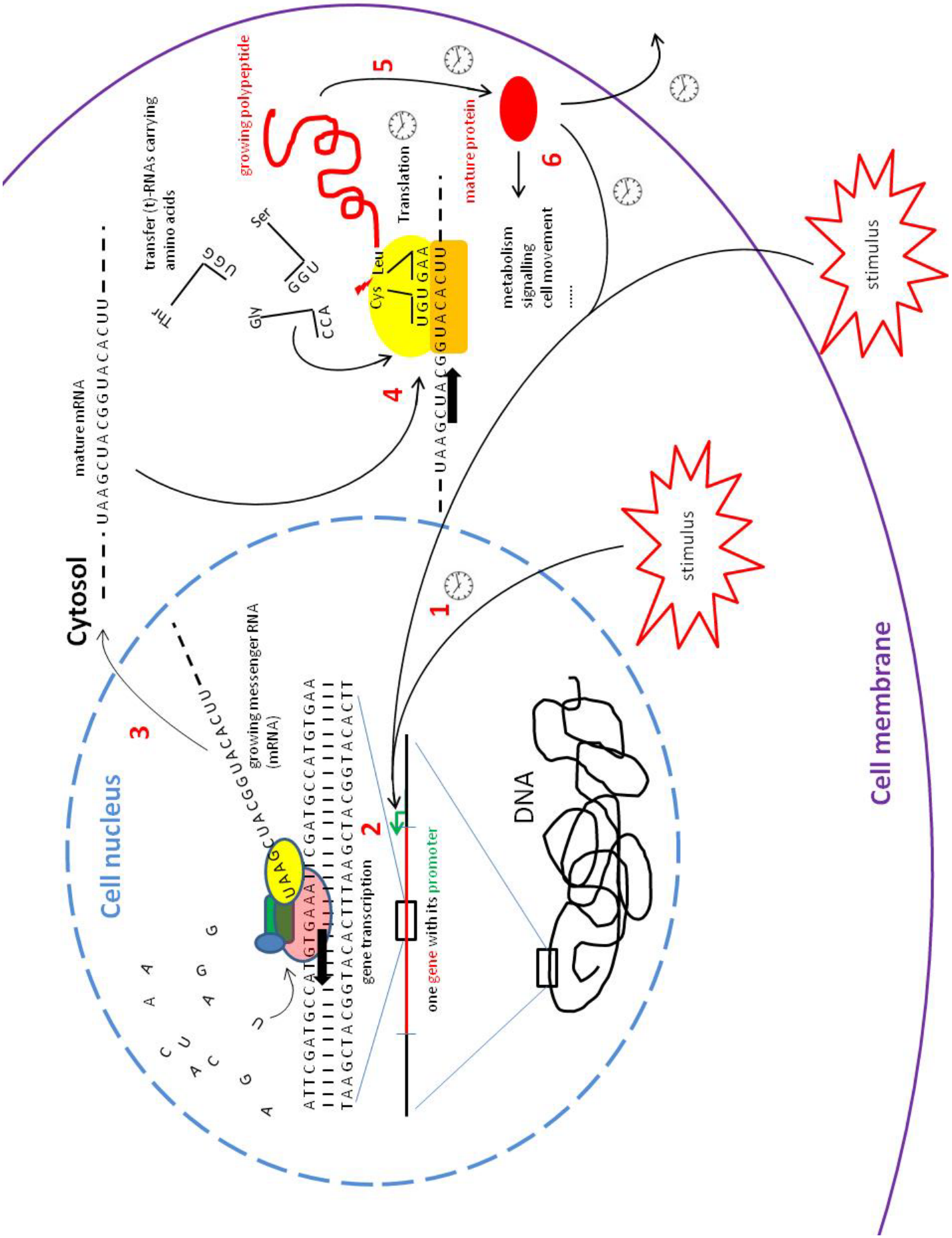
using real-time RT-PCR, we measure the amount of the mRNA of a specific gene relative to the amount of mRNA of a reference gene for which a change in expression has not to be expected upon the experimental treatment.

1) The polypeptide is processed and folded into the protein with the proper conformation. This also may take time.
2) The mature polypeptide exerts its biological action, which in many cases includes the regulation of its own production. Genetic responses are often delayed (depending on the function of the protein).

Proteins can be detected:

- By quantification of the biochemical action (enzymatic activity) of a given protein in a sample, by measuring the amount of the product of the chemical reaction the protein catalyzes. LDH is detected like this

    - By the use of specific antibodies which bind to the protein of interest. Chemical labels attached to the antibodies then allow quantification of how much of the protein is in a sample. This is basically how ELISA (enzyme linked immunosorbent assay) works, which we use for the quantification of TNF-a and IL-8


Cytosol
mature mRNA
U A A G C U A C G G U A C A C U U
transfer (t)-RNAs carrying amino acids
Thr
UGG
Ser
GGU
Gly
CCA
growing polypeptide
Cys
Leu
UGU GAA
Translation
mature protein
metabolism
signalling
cell movement
.....
stimulus
stimulus
Cell membrane
Cell nucleus
growing messenger RNA (mRNA)
gene transcription
one gene with its promoter
DNA
1
2
3
4
5
6

Cytokines (TNF-a and IL-8)

Cytokines are small soluble proteins which are released by cells in order to communicate with other cells. When cytokines bind to specific receptors (also proteins) on the cell surface, the receptor triggers a signal cascade inside the cell, finally leading to changes in the gene expression patterns and the behaviour (e.g. movement) of the cell.

Tumor necrosis factor (TNF)-a is a pro-inflammatory cytokine. It is released by cells (most importantly macrophages) upon encountering various kinds of injury and foreign material (antigens). Binding of TNF-a to TNF-receptors on a cell induces (among others) inflammatory reactions which include the production and the release of other cytokines such as IL-8.

IL-8 is produced and released in response to binding of TNF-a to the TNF-receptor. It is a chemotactic factor, meaning that it attracts other cells (immune cells) to the site of injury or infection.

We measure the amount of released cytokine as well as the gene expression level of the two cytokines TNF-a and IL-8. Because mRNA processing, RNA translation , protein processing and release requires time, the proteins can be detected only a certain time after gene expression has started. Furthermore, since proteins are quite stable, they can still be detected after gene expression has stopped.

HMOX1 and SOD1

HMOX1 and SOD1 both are proteins involved in the defence against oxidative stress. The production of both proteins is induced by a large array of stimuli, including radiation, heat, mechanical stress, heavy metals and of course reactive oxygen species. Also nitric oxides are known to be important players. Polyaromatic hydrocarbons have been shown to act antagonizing on HMOX1 and SOD1 production.

SOD1 converts the superoxidide anion O_2^- to O_2 and H_20.

The action of HMOX1 relies in the cleavage of the biomolecule porphyrin, the products of which act anti-oxidative, anti-inflammatory and anti-apoptotic. Importantly, the production of HMOX1 is induced by inflammatory cytokines and HMOX1 induces the production of anti-inflammatory cytokines and represses the production of pro-inflammatory cytokines.

Apoptosis

Apoptosis = programmed cell death, a highly (genetically) regulated energy dependent process in which a cell undergoes a series of changes including for example the breakdown of proteins and DNA and the disintegration of the cell into multiple membrane-enclosed fragments.

This is in sharp contrast to what happens during necrosis. During necrosis, a cell is a passive victim and follows an energy independent mode of death. The cell disintegrates in an uncontrolled way, the cell membrane eventually disrupts, leading to the release of various factors into the surrounding tissue. This cell debris usually affects other cells and causes inflammation.

The biological roles of apoptosis include renewal and shaping of tissues (e.g. the tissue between the fingers is eliminated during the embryonal development via apoptosis), elimination of self-intolerant immune cells, and elimination of damaged and infected cells.

FAS is a protein which is released by cells that get into contact with cell that should be eliminated. On the surface of such cells, the FAS receptor (also a protein) is present. Binding of FAS to the receptor triggers a chain of signals within the cell which finally result in apoptosis. This apoptotic pathway is referred to as the extrinsic one.
Severly damaged cells can induce their own apoptosis by signals originating from intracellular components that detect metabolic imbalances, DNA damages, and regulatory defects. This pathway is referred to as the intrinsic induction of apoptosis.

The intracellular apoptotic signal cascades of both pathways involve a large array of proteins which translate the apoptotic stimulus into the execution of apoptosis. Caspases are the most prominent group of these proteins and their production is transiently up-regulated during certain stages of apoptosis (which is also true for FAS and the FAS receptor).

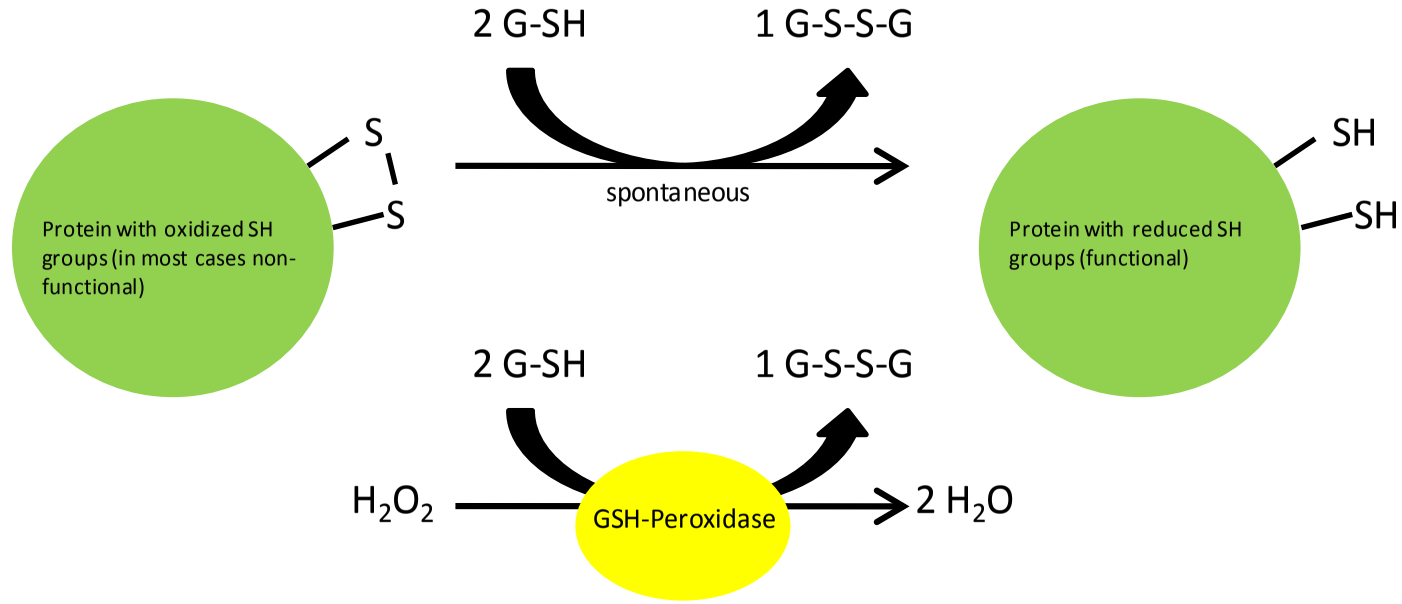
We measure apoptotic responses by real-time PCR and not on the protein level. Since the upregulation is only transient, it can happen that the peak of the expression of such pro-apoptotic genes is missed if samples are only taken at a certain time point.

Changes in the expression levels of CASPASE7 indicate an apoptotic (or anti-apoptotic) response independently on whether the intrinsic or the extrinsic pathway is active. When a change in FAS expression is detected as well, it must be assumed that the extrinsic pathway is active. No changes in FAS expression imply the activity of the intrinsic pathway.

GSH

Glutathion is a tripeptid, composed of the three amino acids glutamate, cystein and glycin.
The important feature of this molecule is the presence of a sulfhydryl (–SH) group in cystein
Its main functions are:

1) protection of SH groups in proteins from oxidation
2) detoxificatio of H_2O_2 (by the enzyme GSH-peroxidase)



The cellular pool of reduced GSH is continuously replenished by the action of the enzyme GSSG reductase. For this, NADPH (the cell's main reducing agent) is needed as an electron donor. Strongly oxidizing conditions may overburden the kinetics of GSH-peroxidase or may result in the depletion of the NADPH pool. Measurement of the concentration of reduced GSH gives a measure for how oxidative a cell experiences a certain condition.

LDH

Lactate dehydrogenase is a protein involved in the glucose metabolism and based on ist biochemical function has nothig to do with cytotoxicity. Under normal conditions, it is present in high amounts as a soluble protein in the cytosol.

Cytotoxic conditions affect the integrity of the cell membrane. This may happen directly (the cell membrane is damaged, for example by peroxidation of membrane lipids) and indirectly (the cellular membrane synthesis, maintenance and repair mechanisms are inhibitied).

LDH detection outside cell therefore gives a measure of the extent to which the membrane is damaged, which in turn is indicative for the the overall cytotoxicity of a certain treatment.

If a high LDH release is detected, it must be assumed that the cell is not able to show normal responses anymore. This is because damages in the cell membrane affect all regulatory mechanisms and the whole cellular homeostasis. Therfore, if high LDH release is observed, the cells should not be used for further endpoint measurements.

Toxicological properties of emission particles from heavy duty engines powered by conventional and bio-based diesel fuels and compressed natural gas

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Abstract

Background
One of the major areas for increasing the use of renewable energy is in traffic fuels e.g. bio-based fuels in diesel engines especially in commuter traffic. Exhaust emissions from fossil diesel fuelled engines are known to cause adverse effects on human health, but there is very limited information available on how the new renewable fuels may change the harmfulness of the emissions, especially particles (PM). We evaluated the PM emissions from a heavy-duty EURO IV diesel engine powered by three different fuels; the toxicological properties of the emitted PM were investigated. Conventional diesel fuel (EN590) and two biodiesels were used − rapeseed methyl ester (RME, EN14214) and hydrotreated vegetable oil (HVO) either as such or as 30% blends with EN590. EN590 and 100% HVO were also operated with or without an oxidative catalyst (DOC + POC). A bus powered by compressed natural gas (CNG) was included for comparison with the liquid fuels. However, the results from CNG powered bus cannot be directly compared to the other situations in this study.

Results

High volume PM samples were collected on PTFE filters from a constant volume dilution tunnel. The PM mass emission with HVO was smaller and with RME larger than that with EN590, but both biofuels produced lower PAH contents in emission PM. The DOC + POC catalyst greatly reduced the PM emission and PAH content in PM with both HVO and EN590. Dose-dependent TNFα and MIP-2 responses to all PM samples were mostly at the low or moderate level after 24-hour exposure in a mouse macrophage cell line RAW 264.7. Emission PM from situations with the smallest mass emissions (HVO + cat and CNG) displayed the strongest potency in MIP-2 production. The catalyst slightly decreased the PM-induced TNFα responses and somewhat increased the MIP-2 responses with HVO fuel. Emission PM with EN590 and with 30% HVO blended in EN590 induced the strongest genotoxic responses, which were significantly greater than those with EN590 + cat or 100% HVO. The emission PM sample from the CNG bus possessed the weakest genotoxic potency but had the strongest oxidative potency of all the fuel and catalyst combinations. The use of 100% HVO fuel had slightly weaker and 100% RME somewhat stronger emission PM induced ROS production, when compared to EN590.

Conclusions

The harmfulness of the exhaust emissions from vehicle engines cannot be determined merely on basis of the emitted PM mass. The study conditions and the engine type significantly affect the toxicity of the emitted particles. The selected fuels and DOC + POC catalyst affected the PM emission from the heavy EURO IV engine both qualitative and quantitative ways, which influenced their toxicological characteristics. The plain HVO fuel performed very well in emission reduction and in lowering the overall toxicity of emitted PM, but the 30% blend of HVO in EN590 was no better in this respect than the plain EN590. The HVO with a DOC + POC catalyst in the EURO IV engine, performed best with regard to changes in exhaust emissions. However some of the toxicological parameters were significantly increased even with these low emissions.

Keywords:
Biodiesel; Hydrotreated vegetable oil; Particulate matter; Emissions; In vitro toxicology; Compressed natural gas

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Traffic-related air pollution – the health effects scrutinized

Gezondheidseffecten en luchtverontreiniging – het kan verkeren
(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof.dr. G.J. van der Zwaan, ingevolge het besluit van het college voor promoties in het openbaar te verdedigen op dinsdag 2 juli 2013 des middags te 12.45 uur

door Miriam Elisabeth Nijland

geboren op 26 januari 1970
te Monnickendam

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Promotoren: Prof. dr. F.R. Cassee
Prof. dr. ir. B. Brunekreef

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Contents

Numerous studies have been published on the health effects associated with exposure to air pollution. Air pollution is acknowledged as a public health risk and air quality regulations are set for specific air pollutants to protect human health. A major pollutant, well known for its adverse health impact, is particulate matter (PM) of which road traffic is a major source. Therefore, the health effects of traffic-related air pollution have been under considerable scrutiny and the research described in this thesis contributes, at least in part to unravelling of the puzzle.

This thesis is based on the research of two European Commission funded projects and two projects funded by the Ministry of Infrastructure and the Environment and conducted at the National Institute for Public Health and the Environment. The overall objective of the conducted research is to examine the adverse health effects of inhalable PM and assess in experimental studies the toxicity of components and sources of PM emissions. The research presented in this thesis was also performed to strengthen the link between scientific evidence and interventional measures taken in order to limit the adverse effects of air pollution generated by road traffic.

The research described in Chapter 2 has been conducted at a time that the health effects of traffic-derived PM were less evident. As such, the research described in Chapter 2 has contributed to our knowledge on the role of road traffic emissions exerting biological responses and toxicity of PM. In the *in vivo* study, we observed a trend of enhanced capacity to trigger an inflammatory response of PM from high compared to low traffic environments (Chapter 2). At that time, ***the importance of road traffic as a source of harmful air pollutants*** supported the efforts of policymakers to reduce traffic emissions and exposure to traffic-derived air pollutants.

In addition, the results of our study suggested that other sources like wood combustion and wear emissions (e.g. from brake and tyre wear) could be of relevance based on the associations with specific elements like potassium, copper, barium and zinc (Chapter 2). ***Both engine exhaust and non-exhaust PM from wear emissions are of significance in terms of toxicity*** and the contribution of non-exhaust emission to traffic-related health effects is now beginning to gain recognition by policymakers. However, to what extent various sources of air pollution contribute to the health risks is still surrounded by uncertainty.

PM mass seems not the best metric to predict air-pollution-related health effects and research from our own group (Chapter 2 and 3) has emphasized the ***importance of particle size and composition as determinants of the health risks imposed by air pollution***. The *in vivo* studies examining the short-term detrimental effects of PM from different locations (Chapter 2 and 3), showed that ***coarse particles (2.5-10 µm) exert significant adverse toxicity***, which emerged the importance of coarse particles, in addition to fine (<2.5 µm) PM. This implies that coarse particles should not be ignored by air pollution regulation. In addition, the specific role of metals and polyaromatic hydrocarbon content (PAHs) has been examined in more depth *in vivo* using PM samples with significant contrast in those PM components (Chapter 3). ***Transition metals and PAHs are likely to be crucial factors affecting pulmonary toxicity*** as we observed increased adverse pulmonary responses in relation to these PM components and particle composition may be considered useful to establish a strategy for reduction of harmful PM levels.

As rather little is known about the impact of long-term exposure to road traffic PM from a toxicological point of view, we have examined the pulmonary and cardiovascular effects of prolonged exposure to roadside and diesel engine exhaust particles *in vivo* (Chapter 4). Contrary to what was expected ***no adverse cardiopulmonary effects occurred in healthy animals after prolonged exposure to PM generated from road traffic***. This might be explained by adaptive response mechanisms present in healthy animals or a lack in toxic potency of the pollution studied. Still, ***prolonged exposure to diesel engine exhaust resulted in the induction of pro-inflammatory markers in specific brain regions*** (Chapter 5), suggesting the susceptibility of the central nervous system to air pollutants and the impact of air pollution that may have been underestimated in the past.

The last research chapter of this thesis (Chapter 6) considers interventions that aim to decrease emissions from road vehicles e.g. introduction of a particle trap and/or a biodiesel blend as a fuel. Policies are already in place promoting the use of such approaches based on their expected PM mass emission reduction and expected benefits for human health. Our study revealed that ***significant reduction in PM emission by the use of 50% biodiesel blend as a fuel is not necessarily accompanied by reduced toxicity of the emitted PM*** as shown by increased cytotoxicity and the release of the pro-inflammatory marker IL-6 *in vitro* compared to diesel engine exhaust PM.

Yet, we observed that ***the application of a particulate filter on combustion engines has a beneficial effect on both PM mass emission and the hazard of PM***. To elucidate systematically the impact of road traffic-derived emissions, and technologies or fuels used to tackle them, an international harmonised methodology is needed.

Finally, the outcomes and implications of the conducted research described in this thesis are considered in Chapter 7. This research confirms the importance of road traffic exhaust as a source of harmful PM, and especially the significance of non-exhaust emissions. Despite regulation on air pollution, considering the sheer number of people exposed to road traffic-generated emissions globally, road traffic-specific health-based policy measures will remain significant for the near future, and the research in this thesis highlights the importance of integrating scientific research findings with that of regulatory policy.

BioToxDi/EngToxDi

Final Report, September 2013

Background and objective

Adverse health effects arising by diesel exhaust inhalation have been described in a large number of epidemiological and experimental studies, as reviewed for example by Donaldson *et al*. in 2005 [1]. The key findings indicate that the ultrafine fraction of diesel exhaust particles (DEPs), hydrocarbons (HC, in particular polyaromatic- and nitrated polyaromatic ones PAH/NPAH) and nitrogen oxides (NO_x) are key players in this regard. DEPs are assumed to be of particular importance because once inhaled, they persist in the respiratory tract for prolonged periods of time and via cellular uptake and translocation over the air-blood barrier may act intracellularly and systemically [2].
Consequentially, increasingly stringent exhaust emission legislations were introduced which, together with the demand for economically and ecologically more sustainable transportation, results in the development of numerous exhaust after-treatment systems, new fuel and lubrication oil types and fuel additives for diesel engines. The success is impressive: For instance new deNO_x and filter technologies are well able to cope with the most recently introduced emission limits for NO_x and DEPs [3, 4].
A detailed understanding of which exhaust properties are of major importance with regard to human health is currently missing and it is not possible to deduce exhaust toxicity from exhaust composition. It has been shown that exhaust after-treatment systems – even though resulting in a quantitative reduction of the emission of regulated pollutants - may even increase exhaust toxicity by the generation of secondary emissions, i.e. the formation of chemical species that are not present in the untreated exhaust [5]. Also, components of new fuels, fuel additives and lubrication oils may increase exhaust toxicity, either directly because of their intrinsic toxicity or because they promote the increased formation of toxic exhaust components [6, 7].
Newly developed diesel technologies therefore require not only evaluation in terms of engine performance, emission reduction and sustainability, but also in terms of their effect on exhaust toxicity. This relies on biological testing and because of the large number of developments in the diesel market requires efficient and reproducibly working biological test systems and exhaust exposure systems.

In the project BioToxDi/EngToxDi, the combination of a newly developed exhaust exposure system and a complex cellular model of the human airway epithelium was used to investigate how diesel exhaust toxicity is affected by the settings under which it is produced. The tested settings are listed in table 1 and included exhaust filtration by a diesel particle filter (DPF), the use of biodiesel, lubrication oils differing in their sulphated ash, phosphorus and sulphur (SAPS) content, increased NO_2 concentration (simulation of a diesel oxidation catalyst, DOC) and a fuel additive for particle filter regeneration (the fuel borne catalyst (FBC) Satacen®3). Experiments with biodiesel were performed with 20% rapeseed methyl-ester (RME) in fossil diesel (B20) and pure RME (B100). Lubrication oil experiments were performed by varying the oil used for lubrication (high SAPS under reference settings vs. low and zero SAPS) and by adding 2% by volume of lubrication oil into the fuel (the additive free DEA-oil and high SAPS oil were used). Satacen®3 (40 ppm in reference fuel) was tested with and without diesel particle filter.
The aims of the project were to obtain further insight into which exhaust parameters are relevant with regard to exhaust toxicity and whether the tested settings in- or decrease exhaust toxicity. In addition, the in course of the project generated large data set contributes to the validation of the experimental approach as a highly efficient and reliable tool for future exhaust toxicity assessment.

Experimental procedure

Exposure of human lung cell cultures: A three dimensional *in vitro* model of the human epithelial airway barrier as described in [8, 9] was used as a biological system. It is composed of i) a confluent epithelial cell layer of the

bronchial epithelial cell line 16HBE14o$^-$ cells, ii) human whole blood monocyte derived macrophages (MDMs) and iii) human whole blood monocyte derived dendritic cells (MDDCs) was used as a biological system. To further reflect the physiological conditions *in vivo* this triple cell co-culture system was cultured at the air-liquid interface, as previously described by Blank *et al*. [9].

Table 1: Project Matrix

work package 0: September-December 2010					
evaluation of the optimal experimental settings					
fuel	**lube oil**	**DPF**	**NO_2**	**FBC**	**ageing**
B0	high	---	---	---	---

work package 1: January-November 2011					
fuel	**lube oil**	**DPF**	**NO_2**	**FBC**	**ageing**
B0	high	yes	---	---	---
B20	high	---	---	---	---
B100	high	---	---	---	---
B0	low	---	---	---	---
B0	zero	---	---	---	---

work package 2: December 2011					
fuel	**lube oil**	**DPF**	**NO_2**	**FBC**	**ageing**
B0	high	---	yes	---	---

work package 3: January-December 2012					
Repetitions, cross-combinations, new tasks					
fuel	**lube oil**	**DPF**	**NO_2**	**FBC**	**ageing**
B20/ B100	high	-	-	-	-
B0	high	-	yes	-	-
B0	DEA 2%	-	-	-	-
B0	high 2%	-	-	-	-

work package 4: August-December 2012					
fuel	**lube oil**	**DPF**	**NO_2**	**FBC**	**ageing**
B0	high	---	---	---	yes
B0	high	---	---	yes	

Using a well-established exhaust exposure system [10], the triple cell co-cultures were exposed to freshly produced diesel exhaust. As a test vehicle, an Opel Astra X20DTL, running on a dynamometer at constant velocity of 35 km/h (engine speed 2180 rpm) was used.

Exhaust samples used for exposure experiments were taken directly downstream the tailpipe and diluted 1:10 with filtered ambient air. With a delay of about 1.5 seconds, the exhaust samples were brought to the cell exposure chamber, prior to which their temperature, relative humidity and CO_2 concentration were adjusted 37°C, 85% and 5% (reflecting the conditions in the human lung). The volume flow in the system was set to 2 l/min, the volume of the exposure chambers being 4 l.

Cells were exposed to diesel exhaust for 2 or 6 hours (except for ageing, where only 6 h was tested), reflecting a low and a high dose exposure respectively. In order to be able to differentiate between diesel exhaust mediated and diesel exhaust independent effects, a control set of cell cultures was exposed to filtered air at 37°C, 85% relative humidity and 5% CO_2 in parallel to the exhaust exposure experiments. Following the exposure period, cells and the cell culture medium were collected for subsequent biochemical analysis.

In parallel to the exposure experiments, a fraction of the diluted exhaust samples (taken directly downstream the dilution) was used for exhaust characterization. The concentrations of nitrogen monoxide, nitrogen oxides, carbon monoxide and hydrocarbons as well as the particle number-size distribution were measured.

In a first step, reference exposure condition was defined (no exhaust after-treatment, standard low sulfur diesel (Greenergy) and high SAPS lubrication oil (V10.237, Motorex)) and the resulting toxicity was measured. This reference toxicity served as a benchmark to which the toxicity of the exhausts produced under the various specified settings was compared. Reference exposures were repeated before each exposure in order to account for changes in exhaust toxicity that could be attributed to changes in engine equipment.

Biological endpoints

According to a widely accepted model on how particle-cell interaction result in adverse health effects [11], the direct consequences of particle-cell interactions may include cytotoxicity (resulting in necrotic cell death), genotoxicity (DNA damages, potentially resulting in mutations in the genetic code) and the formation of reactive oxygen species (ROS). ROS will result in the induction of oxidative stress, which at low levels activates cellular anti-oxidant responses, at higher levels however, additionally triggers pro-inflammatory responses. Persistent inflammation in the respiratory tract is assumed to be the starting point for a multitude of chronic respiratory disorders, systemic complications such as stroke, cardiovascular diseases and – by causing DNA damages and modulations of cell proliferation patterns - tumor formation. Under normal circumstances, tumor formation is avoided by apoptosis, a highly regulated process in which severely damaged cells undergo programmed cell death in order not affect the surrounding tissue (Figure 3). Based on this model, we selected necrotic cell death, the induction of oxidative stress and the according cellular response, pro-inflammatory responses and the induction of apoptosis as biological endpoints to be assessed in this study.

Cytotoxicity was assessed i) by fluorescent microscopy of cell cultures labeled for nuclear DNA and the actin cytoskeleton allowing for the detection of any morphological changes in the epithelial cell layer, and ii) by

quantification of extracellular lactate dehydrogenase (LDH). LDH is a cytosolic enzyme, the presence of which outside the cell is indicative for damaged cell membranes and hence for cytotoxicity.

The induction of oxidative stress was assessed by quantification of total reduced glutathione (GSH), a reactive oxygen species scavenger, the total amount of which in a cell culture is indicative for the level of oxidative stress the cells experience.

Cellular responses to oxidative stress were estimated by measuring the gene expression level of the two oxidative stress-responsive genes superoxide-dismutase 1 (*SOD1*) and heme-oxygenase 1 (*HMOX1*) by real-time reverse-transcriptase polymerase chain reaction (real-time RT-PCR).

The two pro-inflammatory signal molecules tumor necrosis factor (TNF)-α and interleukin (IL)-8 served as a measure for the induction of inflammatory responses. We quantified the release of the two cytokines into the culture medium by enzyme linked immunosorbent assay (ELISA) and measured the expression levels of their genes (*TNF* and *IL-8*) by real-time RT-PCR.

The induction of apoptotic responses was estimated by measuring the expression levels of the two pro-apoptotic genes caspase7 (*CASP7*) and TNF receptor superfamily member 6 (*FAS*) by real-time RT-PCR.

Results

Independently on the exhaust type or the exposure duration, no changes in cellular morphology were observed. Similarly, severe cytotoxic effects did not arise, even though in some cases extracellular LDH was increased more than 1.5-fold compared to the control air exposure (DEA (fuel), 6 h: 1.6-, FBC+DPF, 6 h: 1.7-, ageing 1.6-fold). Significant induction of pro-apoptotic responses were not induced either. This indicates that no exhaust over-doses were applied, that the cells were fully viable and that the measured responses reflect the behavior of cells in a normal regulatory and metabolic state.

In the following, the results obtained for reference exposures will briefly be presented, followed by a description on how the different tested settings changed the toxicity relative to the reference.

Reference settings	The results show a considerable level of GSH oxidation, indicative for the induction of severe oxidative stress. An increase in the transcriptional activity of oxidative stress-responsive genes could however, only be detected for *HMOX1*, but not for *SOD1*. Pro-inflammatory responses were observable for both assessed cytokines. *TNF* expression was increased upon high dose exposures only, whereas TNF-α secretion was increased by both doses, with a weak dose effect being detectable. Since the production and secretion of TNF-α is an event subsequent of *TNF*-transcription, it can be assumed that the genetic response to the low dose exposures was already terminated in the moment the cell cultures were harvested, whereas this was not yet detectable on the protein level. *IL-8* expression but not IL-8 secretion was increased, with no dose effect being detected. The absence of an increased IL-8 secretion by simultaneous gene expression indicates that the production/secretion of the protein had not reached a detectable level in the moment the cells were harvested.
DPF:	A non-catalyzed DPF reduces the oxidative and pro-inflammatory potential of diesel exhaust and thereby makes a valuable contribution to exhaust de-toxification. This finding is in-line with the literature and highly robust. Further research should focus on coated DPFs and on systems that combine catalytic systems and DPFs.
B20	B20-exhaust is of lower oxidative and pro-inflammatory potential than exhaust produced from reference diesel and can hence be considered superior from a toxicological point of view. This applies however, only for rapeseed methyl-ester and cannot be generalized. Future research should investigate biofuels produced form other prominent feedstocks and also other blending ratios should be tested
B100	B100-exhaust is of higher pro-inflammatory potential than exhaust produced from reference diesel. The use of pure biodiesel therefore appears not to be suitable with respect

	to exhaust toxicity. This applies however, only for rapeseed methyl-ester and needs to be investigated separately for biofuels produced from other feedstocks.
Lubrication oil	A decrease in the ash content of lubrication oils increases the oxidative potential of diesel exhaust and at very low levels may also increase its pro-inflammatory potential. This finding could however, not be confirmed by adding additive-free lubrication oil directly to the fuel, which puts the active role of oil additives into question and shows that further research in this direction is needed, especially with respect to the influence of oil additives on the elemental composition of the emitted particles.
NO_2	High concentrations of NO_2 in diesel exhaust appears not to increase the oxidative potential of the exhaust but may increase its pro-inflammatory properties. The obtained findings point towards the activation of complex cellular responses (e.g. strong activation of anti-oxidant and anti-inflammatory responses), which deserves more detailed investigation. Also, because only acute effects were covered in this study, it cannot be excluded that severe NO_2-related toxicity would appear only after prolonged exposures and/or post-incubation. Biological artifacts due to overdosing cannot be ruled out and the experiments should be repeated with lower and preferably several NO_2 concentrations.
Exhaust ageing	Photochemical ageing increases the cytotoxic and oxidative potential of diesel exhaust, but has no profound effect on pro-inflammatory properties. These results suffer from the very high levels of ozone formation in the exhaust ageing-chamber however, and hence do not necessarily reflect responses that are to be expected under real-world situations. Exposure-experiments with aged exhaust should be repeated with an improved exhaust-ageing chamber.
FBC	The iron-based fuel additive Satacen®3 reduces the oxidative potential of diesel exhaust independently on whether it is used with or without DPF. In absence of a DPF, it increases its pro-inflammatory potential of diesel exhaust, which must be attributed to the emitted particles originating from the additive.

Note that this summary only applies for the biological endpoints assessed in this study, i.e. it is not necessarily descriptive for the integral exhaust toxicity, and that only acute effects are covered.

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Cerium dioxide nanoparticles can interfere with the associated cellular mechanistic response to diesel exhaust exposure

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HIGHLIGHTS

- First study to assess dual effects of diesel exhaust and cerium dioxide NPs.
- Cerium dioxide NPs interfere with the secondary toxicity of diesel exhaust.
- Cerium dioxide NPs could be advantageous fuel borne catalysts.

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ABSTRACT

The aim of this study was to compare the biological response of a sophisticated *in vitro* 3D co-culture model of the epithelial airway barrier to a co-exposure of CeO_2 NPs and diesel exhaust using a realistic air–liquid exposure system. Independent of the individual effects of either diesel exhaust or CeO_2 NPs investigation observed that a combined exposure of CeO_2 NPs and diesel exhaust did not cause a significant cytotoxic effect or alter cellular morphology after exposure to diesel exhaust for 2 h at 20 μg/ml (low dose) or for 6 h at 60 μg/ml (high dose), and a subsequent 6 h exposure to an aerosolized solution of CeO_2 NPs at the same doses. A significant loss in the reduced intracellular glutathione level was recorded, although a significant increase in the oxidative marker *HMOX-1* was found after exposure to a low and high dose respectively. Both the gene expression and protein release of tumour necrosis factor-α were significantly elevated after a high dose exposure only. In conclusion, CeO_2 NPs, in combination with diesel exhaust, can significantly interfere with the cell machinery, indicating a specific, potentially adverse role of CeO_2 NPs in regards to the biological response of diesel exhaust exposure.

1. Introduction

Diesel engines are known to emit high amounts of diverse toxic compounds, such as nitrogen oxides (NO_x), polyaromatic hydrocarbons (PAHs) and diesel exhaust particles (DEPs) (Westerholm and Egeback, 1994). Between 1994 and 2010 the European automobile manufacturers' association (www.acea.be) reported an increase in the percentage of passenger cars containing a diesel engine from 23% to 51% within the European Union. This observed trend has been suggested to contribute to a higher tropospheric load of these compounds. The consequence of which therefore, is a continuously higher exposure to these volatile compounds towards a large fraction of the population, with a heightened risk of detrimental effects towards human health.

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Reduction in (pro-)inflammatory responses of lung cells exposed *in vitro* to diesel exhaust treated with a non-catalyzed diesel particle filter☆

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HIGHLIGHTS

- A non-catalyzed DPF decreases the oxidative potential of diesel exhaust.
- Inhalation of diesel exhaust induces severe oxidative stress *in vitro*.
- Unfiltered diesel exhaust induces acute pro-inflammatory responses *in vitro*.
- Filtered diesel exhaust does not induce acute pro-inflammation *in vitro*.
- Exhaust filtration alone is not sufficient to reduce *in vitro* diesel exhaust toxicity.

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ABSTRACT

Increasingly stringent regulation of particulate matter emissions from diesel vehicles has led to the widespread use of diesel particle filters (DPFs), the effect of which on exhaust toxicity is so far poorly understood. We exposed a cellular model of the human respiratory epithelium at the air-liquid interface to non-catalyzed wall-flow DPF-filtered diesel exhaust and compared the resulting biological responses to the ones observed upon exposure to unfiltered exhaust. Filtered diesel exhaust acted highly oxidative, even though to a lesser extent than unfiltered exhaust (quantification of total reduced glutathione), and both exhaust types triggered comparable responses to oxidative stress (measurement of heme-oxygenase 1 (*HMOX1*) and superoxide-dismutase (*SOD1*) gene expression). Further, diesel exhaust filtration significantly reduced pro-inflammatory responses (measurement of tumor necrosis factor (*TNF*) and interleukin-8 (*IL-8*) gene expression and quantification of the secretion of their gene products TNF-α and IL-8). Because inflammatory processes are central to the onset of adverse respiratory health effects caused by diesel exhaust inhalation, our results imply that DPFs may make a valuable contribution to the detoxification of diesel vehicle emissions. The induction of significant oxidative stress by filtered diesel exhaust however, also implies that the non-particulate exhaust components also need to be considered for lung cell risk assessment.

1. Introduction

The percentage of diesel cars in the total passenger car fleet in Western Europe grew from 13.8% in 1990 to 50.6% in 2010 (ACEA, European automobile manufacturers' association. Resulting in considerably higher traffic-related emissions of nitrogen oxides (NO_x), hydrocarbons (HC) and particulate matter (Chan et al., 2007). Of special interest is the particulate fraction of diesel exhaust, the

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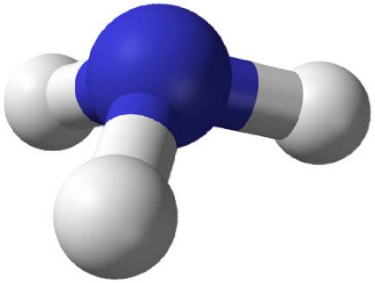
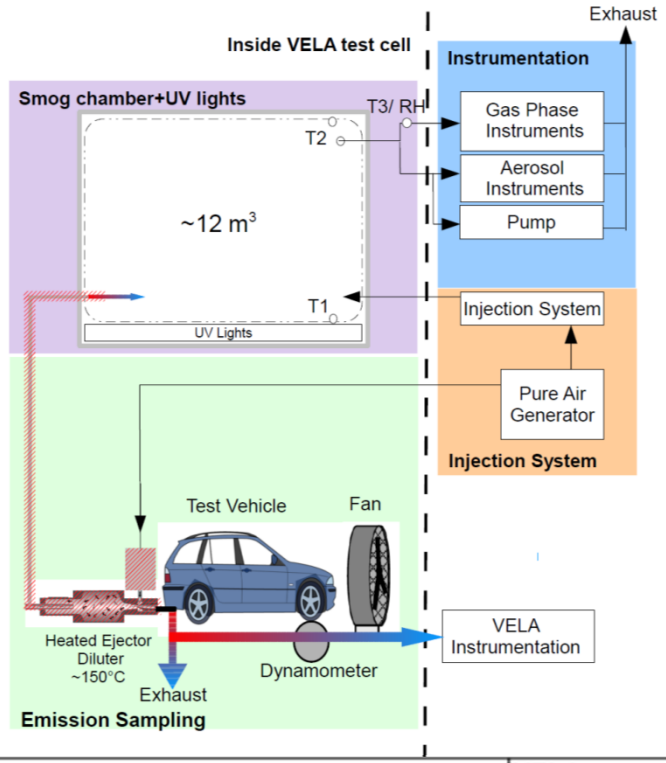
Secondary aerosol from road vehicles

S. M. Platt, I. El Haddad, S. M. Pieber, A.A. Zardini, M. Clairotte, R. Suarez-Bertoa, C. Astorga, L. Pfaffenberger, P. Barmet, R. Wolf, F. Bianchi, J. Dommen, R. Huang, J. G. Slowik, R. Chirico, S. J. Fuller, M. Kalberer, G. Mocnik, I. Jezek, L. Drinovec, S. Hellebust, B. Temime-roussel, N. Marchand, U. Baltensperger and A.S.H. Prévôt



Experimental II (Ispra)



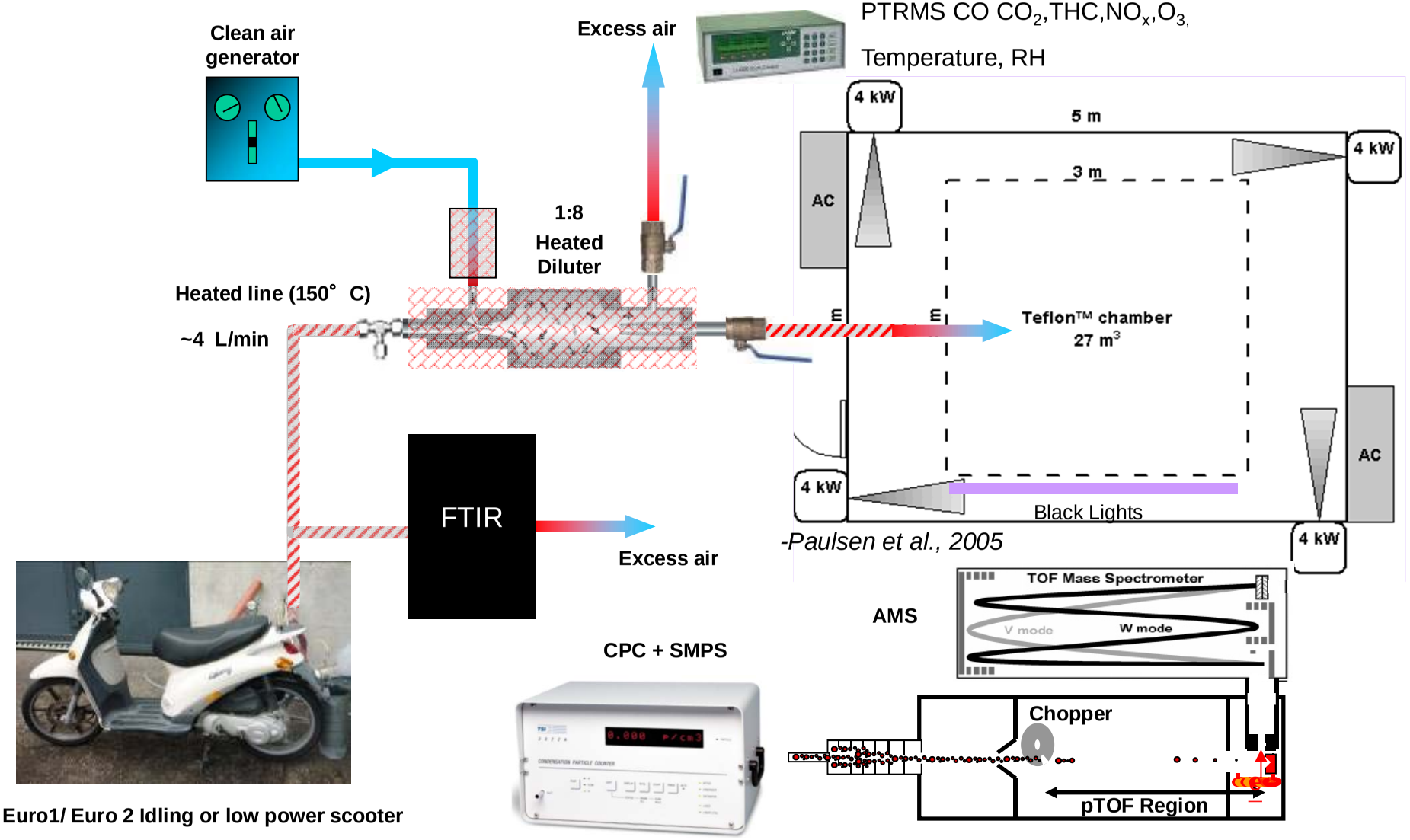
Chassis dynamometer+driving cycles into chamber

Scooters: additional NO, car addition of propene (2011)

Conditions such as humidity and temperature could be varied

Smog Chamber		VELA		
Aerosol	Gas Phase	Aerosol	Gas Phase	Other
HR-ToF-AMS ($PM_{2.5}$ lens)	THC Analyser	Filter Collection (CVS)	FID	On board diagnostics
MAAP	NOx high		FTIR	T Sensors
Aethalometer	NOx low		CO/CO_2	
Prototype Aethalometer	O_3		NOx	
CPC (3775)	CO_2 Licor		GC-FID	
SMPS (Calibration unit)	CO			
EC/OC Filters	Picarro (CH_4, H_2O CO, CO_2)			
	PTR-ToF-MS			
	T, RH Sensors			

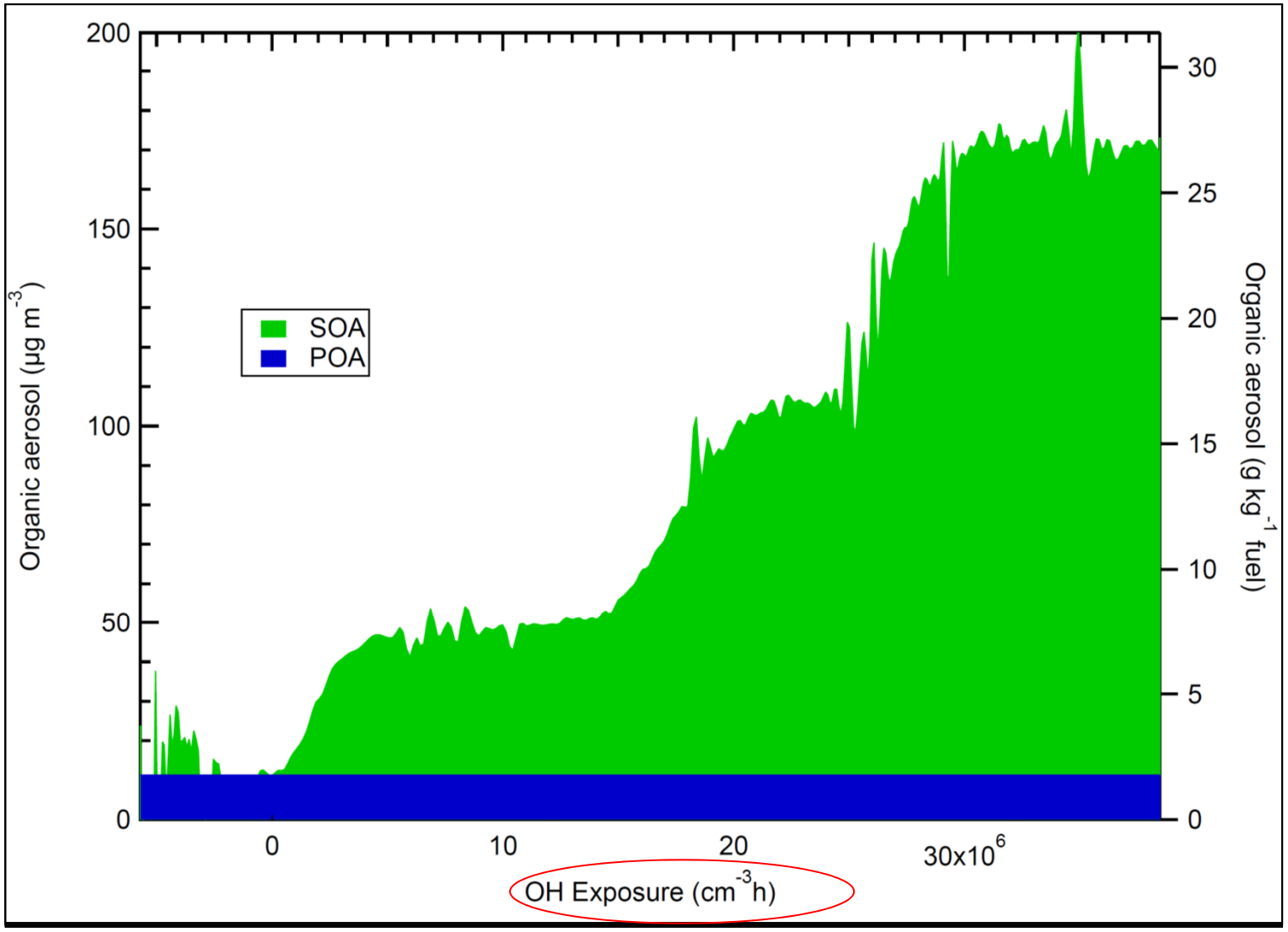
Experimental III (PSI 2010)



Euro1/ Euro 2 Idling or low power scooter

Data Analysis (e.g. PSI 2010)

AMS Organic Time trace (PSI 111019, Euro 2 Moped)



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Exposure of Highway Maintenance Workers to Fine Particulate Matter and Noise

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In this study, we assessed the mixed exposure of highway maintenance workers to airborne particles, noise, and gaseous co-pollutants. The aim was to provide a better understanding of the workers' exposure to facilitate the evaluation of short-term effects on cardiovascular health endpoints. To quantify the workers' exposure, we monitored 18 subjects during 50 non-consecutive work shifts. Exposure assessment was based on personal and work site measurements and included fine particulate matter ($PM_{2.5}$), particle number concentration (PNC), noise (Leq), and the gaseous co-pollutants: carbon monoxide, nitrogen dioxide, and ozone. Mean work shift $PM_{2.5}$ concentrations (gravimetric measurements) ranged from 20.3 to 321 µg m^{-3} (mean 62 µg m^{-3}) and PNC were between 1.6×10^4 and 4.1×10^5 particles cm^{-3} (8.9×10^4 particles cm^{-3}). Noise levels were generally high with Leq over work shifts from 73.3 to 96.0 dB(A); the averaged Leq over all work shifts was 87.2 dB(A). The highest exposure to fine and ultrafine particles was measured during grass mowing and lumbering when motorized brush cutters and chain saws were used. Highest noise levels, caused by pneumatic hammers, were measured during paving and guardrail repair. We found moderate Spearman correlations between PNC and $PM_{2.5}$ ($r = 0.56$); PNC, $PM_{2.5}$, and CO ($r = 0.60$ and $r = 0.50$) as well as PNC and noise ($r = 0.50$). Variability and correlation of parameters were influenced by work activities that included equipment causing combined air pollutant and noise emissions (e.g. brush cutters and chain saws). We conclude that highway maintenance workers are frequently exposed to elevated airborne particle and noise levels compared with the average population. This elevated exposure is a consequence of the permanent proximity to highway traffic with additional peak exposures caused by emissions of the work-related equipment.

Keywords: exposure assessment; highway; mixed exposure; noise; particle monitoring—ultrafines; respirable dust

INTRODUCTION

Highway maintenance workers spend most of their work time in traffic and are constantly exposed to traffic-related emissions that have been linked to myocardial infarction (Bigert *et al.*, 2003; Peters *et al.*, 2004) as well as increased cardiovascular morbidity and mortality (Hoek *et al.*, 2002; Beelen *et al.*, 2009). Traffic emissions are composed of a complex mixture of particulate and volatile air pollutants on one hand and noise on the other. Levels of particulate matter (PM), carbon monoxide (CO), nitrogen oxides as well as volatile compounds including aldehydes and hydrocarbons are significantly elevated in traffic environments (Roorda-Knape *et al.*, 1998; Zhu *et al.*, 2002; Riediker *et al.*, 2003; Kaur *et al.*,

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Environmental Science & Technology

Article

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Combustion of Hydrotreated Vegetable Oil and Jatropha Methyl Ester in a Heavy Duty Engine: Emissions and Bacterial Mutagenicity

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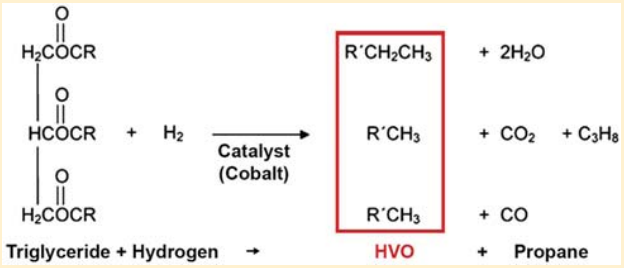
Supporting Information

ABSTRACT: Research on renewable fuels has to assess possible adverse health and ecological risks as well as conflicts with global food supply. This investigation compares the two newly developed biogenic diesel fuels hydrotreated vegetable oil (HVO) and jatropha methyl ester (JME) with fossil diesel fuel (DF) and rapeseed methyl ester (RME) for their emissions and bacterial mutagenic effects. Samples of exhaust constituents were compared after combustion in a Euro III heavy duty diesel engine. Regulated emissions were analyzed as well as particle size and number distributions, carbonyls, polycyclic aromatic hydrocarbons (PAHs), and bacterial mutagenicity of the exhausts. Combustion of RME and JME resulted in lower particulate matter (PM) compared to DF and HVO. Particle numbers were about 1 order of magnitude lower for RME and JME. However, nitrogen oxides (NO_X) of RME and JME exceeded the Euro III limit value of 5.0 g/kWh, while HVO combustion produced the smallest amount of NO_X. RME produced the lowest emissions of hydrocarbons (HC) and carbon monoxide (CO) followed by JME. Formaldehyde, acetaldehyde, acrolein, and several other carbonyls were found in the emissions of all investigated fuels. PAH emissions and mutagenicity of the exhausts were generally low, with HVO revealing the smallest number of mutations and lowest PAH emissions. Each fuel showed certain advantages or disadvantages. As proven before, both biodiesel fuels produced increased NO_X emissions compared to DF. HVO showed significant toxicological advantages over all other fuels. Since jatropha oil is nonedible and grows in arid regions, JME may help to avoid conflicts with the food supply worldwide. Hydrogenated jatropha oil should now be investigated if it combines the benefits of both new fuels.



INTRODUCTION

The limited fossil oil resources urge the research for renewable fuels for the transport sector. Biodiesel (fatty acid methyl esters, FAME) was introduced to the market in the 1980s as a suitable alternative and was supposed to be environmentally friendly. Compared to petrol diesel fuel, the combustion of biodiesel results in a reduction of greenhouse gas emissions.[1] Biodiesel is mainly produced by transesterification of rapeseed oil in Europe (rapeseed methyl ester, RME) and soybean oil in the USA (soy methyl ester, SME). In Asia, palm oil serves as the major source for biodiesel production (palm methyl ester, PME).

Increasing research activities are focused on use of nonedible plant oils for biodiesel production, since the extensive use of edible vegetable oils raises concern due to the competition between fuel and food production resulting in rising prices of vegetable oils.[1,2] *Jatropha curcas* has gained attention as a source for biodiesel production in tropical and subtropical countries and has spread beyond its center of origin, because of its hardiness, easy propagation, drought endurance, high oil content, rapid growth, adaptation to wide agro-climatic conditions, and multiple uses of the plant as a whole.[3] A recent study reported a general reduction in the global warming potential and the nonrenewable energy demand by use of *Jatropha curcas* biodiesel compared to fossil diesel. On the other hand, environmental impacts on acidification, ecotoxicity, eutrophication, and water depletion showed increases.[4] We included jatropha methyl ester (JME) in this study as an alternative to RME, SME, and PME.

Hydrotreated vegetable oil (HVO) was introduced to the market as a new alternative biogenic diesel fuel. HVO can be produced by the catalytic hydrogenation of plant oils. Its physicochemical properties are similar to petroleum derived diesel fuel (DF). Blends (mixtures) of DF and HVO did not result in elevated CO_2 emissions or fuel consumption.[5,6]

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Exhaust emissions and mutagenic effects of diesel fuel, biodiesel and biodiesel blends

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HIGHLIGHTS

- We run three diesel engines (Euro 0, III and IV) using biodiesel blends.
- The influence on the exhaust emissions and mutagenic effects were measured.
- Regulated exhaust emissions change approximately linearly with the blend.
- Blends with 20% biodiesel shows a maximum of mutagenic effects and change nonlinear with the blend.

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ABSTRACT

The replacement of petroleum-derived fuels by renewable biogenic fuels has become of worldwide interest with the environmental effects being scientifically investigated. Biodiesel has been proven to be a suitable alternative to petrodiesel and blending up to 20% biodiesel with petrodiesel is policy promoted in the USA and the EU.

To investigate the influence of blends on the exhaust emissions and possible health effects, we performed a series of studies with several engines (Euro 0, III and IV) using blends of rapeseed-derived biodiesel and petrodiesel. Regulated and non-regulated exhaust compounds were measured and their mutagenic effects were determined using the Bacterial Reverse Mutation Assay (Ames-Test) according to OECD Guideline 471.

Exhaust emissions of blends were approximately linearly dependent on the blend composition, particularly when considering regulated emissions. However, a negative effect of blends was observed with respect to mutagenicity of the exhaust emissions. In detail, an increase of the mutagenic potential was found for blends with the maximum observed for B20. From this point of view, B20 must be considered as a critical blend when petrodiesel and biodiesel are used as binary mixtures.

1. Introduction

Biodiesel can be used as a neat fuel (B100) or in any blend ratio with petrodiesel. The most popular biodiesel blend in the USA is B20 (20% biodiesel, 80% diesel fuel), which can be used for Energy Policy Act of 1992 (EPAct) compliance. In the European Union, the use of biofuel blends is recommended and was introduced by federal regulations in several countries. In Germany, biodiesel is currently blended as B7 (7% biodiesel). Actually, B7 plus three percent hydrotreated vegetable oil (HVO) as well is intended to become possible in Germany.

Biodiesel reduces most exhaust emissions when used in unmodified diesel engines [1–3]. The amount of reduction depends on the blend level. B100 produced from rapeseed oil or soybean oil reduces life cycle CO_2 emissions by 50–75% compared to petrodiesel. This effect is linear to the blend level, leading to life cycle CO_2 emissions reduced by 2.5–3.75% per 5% increase of biodiesel blending. Low-level blends induce small reductions in emissions of hydrocarbons, carbon monoxide, particulate matter, and air toxins as well. However, nitrogen oxides (NO_X) contributing to smog formation may increase slightly when biodiesel is used. Numbers vary, however B20 is believed to increase NO_X by 2–4%. Several biodiesel researchers are working on fuel additives to address this problem. For blend levels of $\leqslant$5%, the NO_X increase is negligible.

Long-term occupational diesel engine emissions (DEE) exposure was associated with the risk of lung cancer in a pooled analysis of

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REVIEW ARTICLE

Potential hazards associated with combustion of bio-derived versus petroleum-derived diesel fuel

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Abstract

Fuels from renewable resources have gained worldwide interest due to limited fossil oil sources and the possible reduction of atmospheric greenhouse gas. One of these fuels is so called biodiesel produced from vegetable oil by transesterification into fatty acid methyl esters (FAME). To get a first insight into changes of health hazards from diesel engine emissions (DEE) by use of biodiesel scientific studies were reviewed which compared the combustion of FAME with common diesel fuel (DF) for legally regulated and non-regulated emissions as well as for toxic effects. A total number of 62 publications on chemical analyses of DEE and 18 toxicological *in vitro* studies were identified meeting the criteria. In addition, a very small number of human studies and animal experiments were available. In most studies, combustion of biodiesel reduces legally regulated emissions of carbon monoxide, hydrocarbons, and particulate matter. Nitrogen oxides are regularly increased. Among the non-regulated emissions aldehydes are increased, while polycyclic aromatic hydrocarbons are lowered. Most biological *in vitro* assays show a stronger cytotoxicity of biodiesel exhaust and the animal experiments reveal stronger irritant effects. Both findings are possibly caused by the higher content of nitrogen oxides and aldehydes in biodiesel exhaust. The lower content of PAH is reflected by a weaker mutagenicity compared to DF exhaust. However, recent studies show a very low mutagenicity of DF exhaust as well, probably caused by elimination of sulfur in present DF qualities and the use of new technology diesel engines. Combustion of vegetable oil (VO) in common diesel engines causes a strongly enhanced mutagenicity of the exhaust despite nearly unchanged regulated emissions. The newly developed fuel "hydrotreated vegetable oil" (HVO) seems to be promising. HVO has physical and chemical advantages compared to FAME. Preliminary results show lower regulated and non-regulated emissions and a decreased mutagenicity.

Keywords: Biodiesel, hydrotreated vegetable oil, diesel engine exhaust, exposure, health hazard

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Diesel Soot Toxification

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Economic and environmental amenities of diesel engines such as high fuel efficiency led to a steady increase in popularity.[1] However, their major disadvantage with regard to environmental and health protection is the typically enhanced production of diesel particulate matter (DPM) comprising soot and unburned carbonaceous compounds. Stricter emission levels, for example, the Euro I to VI standards in the European Union, and tax incentives are imposed. One strategy to lower this burden is the optimization of fuel combustion in the engine as typically realized by a turbocharger. Here, the drastically lowered fuel droplet size negatively influences the DPM nanostructure. Smaller particles may penetrate more deeply into the respiratory tract, where their large surface-to-volume ratio could allow for more biological interaction. It could turn out to be ironical history that huge effort made in this direction is driven by the increasing awareness of the public on the toxicity of diesel soot.

What are the structural and chemical features of low-emission DPM on the nanoscale? The most evident change is in the size of primary soot particles as evidenced for common heavy-duty diesel engines fulfilling the Euro III, IV, and VI standard. Here, the average diameter steadily decreased from 30−40 nm down to 10−15 nm.[2] The second significant trend is the more defective bulk and surface structure as the result of alteration of the fuel combustion process. The enhanced localization of conjugated π-electrons on graphitic surfaces generates favored anchoring points for surface functional groups by reaction with water or oxygen. The consequence is an abundance of chemically reactive oxygen functional groups on the highly defective modern low-emission diesel soot.

The chemical activation of the DPM is astonishing. The carbon surface in its initial highly functionalized state shows an outstanding activity in heterogeneous catalysis. The oxidative dehydrogenation of ethylbenzene to styrene and the selective oxidation of acrolein to acrylic acid are large-scale chemical processes and are catalyzed by highly developed promoted (mixed) oxides with excess of steam, respectively. However, they were also proven to be successfully catalyzed by nanostructured carbon materials. We were surprised to see that the initial productivity of the untreated soot of a Euro IV test engine used as the catalyst in these reactions exceeds the data of well performing other carbonaceous materials.[1] Especially the curvature of the outermost carbon shells (Figure 1), which is more pronounced the smaller the spherical carbon particles are, promotes the activation of molecular oxygen.

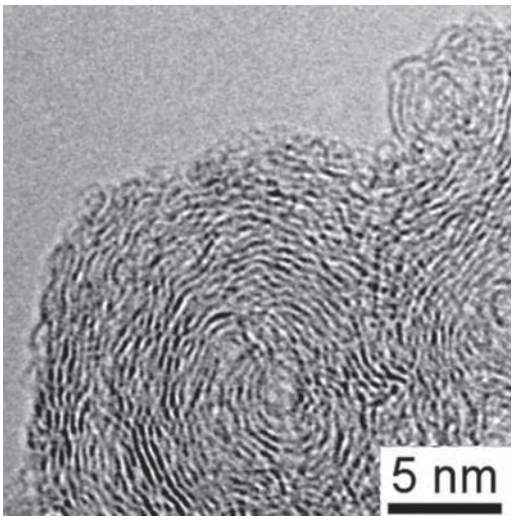

Figure 1. HRTEM micrograph of the defect-rich surface of Euro IV diesel soot particle. Reprinted with permission from ref 2, 2008, American Chemical Society.

DPM as a major constituent of air pollution is associated with respiratory and cardiovascular diseases as well as skin cell alterations. Like airway epithelial cells, the epidermal cells are among the first cell populations exposed to chemical pollutants and are an important source of pro-inflammatory mediators. Soot nanoparticles are spontaneously internalized by keratinocytes and distributed mostly around the cell nucleus.[3] Low-emission

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Euro IV soot particles exhibit a very high oxidative, pro-fibrotic, and toxic potential on these cell types. Further irrefutable environmental consequences and health effects are seen in an increased cytotoxicity and inflammatory potential of Euro IV soot toward human peripheral blood monocyted-derived macrophage cells (MDM). Euro IV soot exhibits a much higher toxic and inflammatory potential than Euro III particles.[2] The latter did not induce any significant signs of necrosis or apoptosis, whereas Euro IV DPM produces extensive damage of the cells.

In addition to these alarming effects of as-produced DPM a fatal postactivation can proceed. The heterogeneous reactions of aerosol particles with ozone, which is ubiquitously formed in traffic zones by UV radiation induced reactions of nitric oxide with molecular oxygen, are of central importance to air quality.[4] Reactive oxygen intermediates with a lifetime greater than 100 s can play a key role in the chemical transformations and adverse health effects of toxic and allergenic air-particulate matter, such as soot, polycyclic aromatic hydrocarbons, and proteins.

Thus, the major question arises whether the emissions of modern diesel engines are characterized by a potentially greater threat to human beings. The answer is not straightforward. The reduction of the emission rate of soot nanoparticulate does not automatically lead to a reduction of the toxic effects toward humans if, concurrently, the structure and functionality of the soot changes and therefore its biological, cytotoxic and inflammatory potential, increase. Clearly, on the qualitative basis, the low-emission soot imposes higher risks. On the other hand, its total amount is substantially lower than for the older generations of diesel engines. Within the past 20 years the DPM emission level for diesel engines was decreased by almost 2 orders of magnitude rendering an evaluation, which combines qualitative and quantitative aspects, difficult. Although the development of improved particle filters and novel methods for particulate removal in diesel cars is an ongoing task for industry the ultimate answer could be given by the future statistical analysis of mortality due to soot exposition. For 2003 in Germany, 10 000−19 000 diesel soot-related deaths, for example, due to lung cancer, were estimated as a result of long-term effects.[5] The future course of this death rate will be the final benchmark for the appraisal of past and current efforts to overcome the environmental and health burden of the diesel engine technology.

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Notes

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Cytotoxicity and Inflammatory Potential of Soot Particles of Low-Emission Diesel Engines

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We evaluated, in vitro, the inflammatory and cytotoxic potential of soot particles from current low-emission (Euro IV) diesel engines toward human peripheral blood monocyte-derived macrophage cells. The result is surprising. At the same mass concentration, soot particles produced under low-emission conditions exhibit a much higher toxic and inflammatory potential than particles from an old diesel engine operating under black smoke conditions. This effect is assigned to the defective surface structure of Euro IV diesel soot, rendering it highly active. Our findings indicate that the reduction of soot emission in terms of mass does not automatically lead to a reduction of the toxic effects toward humans when the structure and functionality of the soot is changed, and thereby the biological accessibility and inflammatory potential of soot is increased.

1. Introduction

Since the implementation of the 1970 Clean Air Act in the United States of America, progress has been made in the reduction of exhaust gas and soot emissions of light-duty and heavy-duty vehicles (passenger cars and trucks). Particulate standards for diesel engines were introduced in 1982 and were tightened in 1991, 1994, and 1998 (*1*). The European Union followed with emission standards for heavy-duty diesel engines in 1992 (Euro I), and in stiffer form in 1998 (Euro II), 2000 (Euro III), and in October 2005 (Euro IV) (*1*). All major automobile companies have developed low-emission engines as well as filters for soot particles. Research and development strategies have focused on the reduction of soot emission yet have neglected the question of how changes in soot quality may change its effect on human health. Hence, the question is: does the low-emission engine Euro IV soot pose the same health risk per unit mass as the soot produced from old engines?

The cytotoxicity and inflammatory potential of soot nanoparticles (NPs) can be assessed by in vitro studies. Macrophages constitute the primary cellular effectors of the immune response, playing a pivotal role in the detection of all foreign bodies. These cells are ubiquitously present in the mucosal and submucosal tissues (especially in the bronchial and alveolar membrane), and human macrophage primary cultures in vitro can provide a model of potential effects upon in vivo inhalation of the soot NPs. When these cells come in contact with particles or pathogens, they become activated and secrete a variety of chemical mediators of inflammation, very aggressive against foreign molecules or particles. Currently, the toxicity of NPs is a hot research topic because the increasing production of nanomaterials is likely to significantly enhance the exposure of humans to NPs (*2*–*4*). However, the research in the field of nanotoxicology is still at its infancy. The parameters that determine the toxicity of NPs are not known in any detail, as one can tell from the large number of review articles published recently on the topic (*5*). The parameter most frequently used as a measure of dose is the surface area. However, lung inflammation studies involving instillation of different types of carbon NPs in mice have revealed a much more complex situation: particles prepared by different techniques exhibit significant differences in surface toxicity (*5*).

The purpose of this study was to compare the cytotoxicity and the inflammatory response, in vitro, of human monocyte-derived macrophage cells (MDMs) to a Euro IV test heavy-duty diesel engine soot and to soot from an old diesel engine and to relate the results to the microstructure of these particles, previously determined in detail by means of high-resolution transmission electron microscopy and other methods of NP characterization.

2. Experimental Section

In the following, the soot from a Euro IV test heavy-duty diesel engine will be referred to as EuroIV soot; the soot from an old diesel engine operating at black smoke conditions will be referred to as BS soot. The methods of soot production and collection have been described elsewhere (*6*). Briefly, the EuroIV soot originated from a modified MAN D0836 LF-4V six cylinder engine (6.9 L displacement, 228 kW), with two-stage controlled turbocharging, an externally controlled cooled exhaust gas recirculation, and a common rail injection system. The engine was developed to fulfill the Euro IV emission standard. The engine was set for a NO_x emission of 3.3 g/kWh and a PM emission of 50 mg/kWh (European stationary cycle, ESC). The BS soot originated from a D2876 CR engine, operated at 30% load, extra-low rail pressure, and air throttling (blackening number 5). The emission rate of the BS engine is 200–600 mg/kWh. The diesel fuel used for both engines was a standard low-sulfur type, containing 78% paraffin and 22% aromatic hydrocarbons (European Norm 590). All samples were collected directly from the exhaust gas of the engine using a special particle collector that was heated to the exhaust gas temperature at the collection position (200 °C).

Transmission electron microscopy, energy-dispersive X-ray spectroscopy, and temperature programmed oxidation studies revealed that EuroIV soot contained about 10% ash from the combusted engine lubricant oil (*7*). This kind of ash was not found in BS soot. For the in vitro studies, the EuroIV and BS soot was sterilized by heating to 180 °C, washed three times in distilled water, then suspended in PBS at a stock concentration of 1 mg/mL and sonicated for 48 h before the use.

Human peripheral blood monocytes were isolated from buffy coats of healthy donors by density gradient centrifugation using lympholyte-H (Cederlane, Hornby, Ontario, Canada). The lymphocytic/monocytic fraction was then

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Research
Project F 2246

O. Creutzenberg

Toxic Effects of Various Modifications of a Nanoparticle Following Inhalation

Dortmund/Berlin/Dresden 2013

This publication is the final report of the project “Toxic Effects of Various Modifications of a Nanoparticle Following Inhalation” – Project F 2246 – on behalf of the Federal Institute for Occupational Safety and Health.
The responsibility for the contents of this publication lies with the author.

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5 Discussion and Conclusions

This project aimed at comparing the toxic effects of a triple of TiO_2 nanoparticles after a 28-day nose-only inhalation. TiO_2 UV TITAN M212, TiO_2 UV TITAN M262 and TiO_2 P25 coded in the European nanomaterial repository with NM-103, NM-104 and NM-105 differ in the crystal structure (rutile; rutile; 80% anatase/20% rutile) and in the surface properties (silicone → hydrophobic; glycerol → hydrophilic; untreated → hydrophilic, respectively) suggesting a different toxic potential after uptake in lungs. Exposure aerosols were generated with a dry dispersion technique mimicking an exposure scenario at workplaces. Wistar rats were exposed to aerosol concentrations of 3, 12 and 48 mg/m^3 for 28 days (6 hours/day, 5 days/week) while concurrent controls inhaled clean air. This dosing scheme induced a non-overload, partial overload and complete overload in lungs, respectively.
Endpoints investigated upon cessation of exposure after 3 days, 1.5 and 3 months of recovery were i.) a bronchoalveolar lavage fluid (BALF) analysis; ii.) histopathology; iii.) transmission electron microscopy (TEM) analysis; and iv.) chemical analysis of test item retention in lungs, liver and brain.

5.1 Bronchoalveolar Lavage (BAL)

Lung wet weights showed dose-dependent increases for all three TiO_2 (statistically significant in the mid and high dose groups; full recovery after 3 months in the mid dose groups).
Data of polymorphonuclear cells (PMN) as inflammation indicator allowed a differentiation of effects between the three TiO_2 test items in the low and mid dose groups. In the low dose groups for NM-105 in a level similar to clean air controls whereas NM-103 and NM-104 showed a slight inflammation reaching approx. 10% PMN. After 45 and 94 days of recovery in clean air NM-103 also returned to normalisation; in contrary, NM-104 remained still in the significant 5-8% range. In the mid and high dose groups, NM-105 showed statistically significant increases but was weaker in the inflammatory effect than NM-103 and NM-104. The same tendencies can be observed evaluating the differential cell count on the basis of absolute cell numbers.

5.2 Retention Analysis

Retention data reflect well the different grade of clearance retardation due to the various lung loads.
The soluble moiety of the test items (5.5%, 2.2% and 0.9% in the low, mid and high dose groups, respectively) suggests that solubility of the test item is limited by a given maximum under the conditions of the lung ambience.
In liver and brain the detected amounts of TiO_2 test items were generally below the limit of detection. In some individual rats considerable masses of particulate test items were detectable up to 206 µg/lung.

5.3 Histopathology

Deposition of particle-laden macrophages was observed in the compartments of the respiratory tract, in lungs mainly in the alveoli with a minor portion in the bronchus associated lymphoid tissue and in the interstitium. After 94 days of recovery, the interstitial portion of particle-laden macrophages was more prominent compared to recovery day 3 indicating a time-dependent translocation of particle-laden macrophages or particles. Furthermore, the distribution of the intraalveolar particle-laden macrophages changed over the time period slightly from an initially more disseminated distribution to a more multifocal distribution with a higher concentration of these cells in the vicinity of the alveolar ducts. The interstitial accumulation of particle-laden macrophages was accompanied in a dose-dependent manner by a very slight interstitial fibrosis and an interstitial mononuclear cell infiltration which increased slightly during the time period investigated. In addition, a very slight bronchiolo-alveolar hyperplasia was observed in the mid- and high-dose groups. This type of hyperplasia is considered to be non-preneoplastic and to represent an “attempt of the lung” to facilitate a more efficient removal of inhaled materials. Within the alveoli a lipoproteinosis and an infiltration of granulocytes were noticed in a dose-dependent way which was still observable after 94 days of recovery. The diagnosis of lipoproteinosis in this study included debris of degenerated macrophages. The detection of both mentioned lesions indicated an ongoing inflammatory stimulus in the higher dosed groups.
In all other organs than the respiratory tract treatment-related changes were not observed.
Comparing all three treatment groups (NM-103, NM-104 and NM-105) among themselves showed a similar dose-dependent character of changes. In addition, all three groups exhibited a similar way of deposition and distribution of particle in the respiratory tract. Though marginal differences in the degree of some changes exist, no obvious differences of the particles in terms of degree and character of induced lesions were observable.

5.4 TEM Analysis

The most prominent compartment in which particles were detected in the present study represent intraalveolar macrophages independent of the kind of particles (NM-103, NM-104 and NM-105) used. In the lower dosage groups (low- and mid-dose) the second important compartment are pneumocytes type I whereas intraalveolar free particles are the second important compartment in the high-dose groups followed by pneumocytes type I and macrophages in the interstitium. Other compartments such as cellular interstitial or free in the interstitium were of minor importance. In addition, using the described method no particles were found in the compartment pneumocyte type II and epithelium bronchiolar.

Differences between the different treatment groups (NM-103, NM-104 and NM-105) were not obvious in the low and mid-dose groups. However, in the high-dose groups differences were apparent in the intraalveolar macrophages between the NM-105 and the other groups (NM-103 and NM-104) with higher amount in the NM-105 treated animals.

A preliminary ranking on the basis of the induction of PMN influx and other endpoints is: NM-104 > NM-103 > NM-105.

5.5 Discussion of Kinetic Data Referring to the Paper of Pauluhn (2011)

Pauluhn (2011) has presented an approach how to predict NOAEL values for granular poorly soluble particles following inhalation. In terms of the volume-based cumulative dose in lungs, target volume loads in rat studies should be in the range of approx. 1 µl/lung (no adverse effects expected) to maximum 10 µl/lung, lest a lung clearance collapse with clearance half-times > 1 year should occur.

In Figure 5.1, key information on the novel approach is given. The lung overload threshold in rats is defined as 4.2 µl particulate matter/kg b.w. Up to this value adverse effects due to particle load will not be observed for low soluble dusts without special surface reactivity ("inert dusts"). Using this value of **4.2 µl/kg b.w.**, fractional doses/day on volume basis (unit: µl/day) can be derived, varying depending on study duration, to attain the steady state (for a **4-wk study: 17.5**; for a 3-mth study: 40). The NOAEL (unit: mg/m^3) can be calculated using the following equation:

$NOAEL_{pred.}$ (mg/m^3) = 1 µl x ρ (mg/µl) / 17.5 (AF) x 0.29 m^3 (MV) x PM_{resp}

pred. = predicted;
AF = accumulation factor;
MV = daily respiratory volume;
PM_{resp} = deposition fraction (%according to MPPD model)

Ultrafine Particles Cross Cellular Membranes by Nonphagocytic Mechanisms in Lungs and in Cultured Cells

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High concentrations of airborne particles have been associated with increased pulmonary and cardiovascular mortality, with indications of a specific toxicologic role for ultrafine particles (UFPs; particles < 0.1 μm). Within hours after the respiratory system is exposed to UFPs, the UFPs may appear in many compartments of the body, including the liver, heart, and nervous system. To date, the mechanisms by which UFPs penetrate boundary membranes and the distribution of UFPs within tissue compartments of their primary and secondary target organs are largely unknown. We combined different experimental approaches to study the distribution of UFPs in lungs and their uptake by cells. In the *in vivo* experiments, rats inhaled an ultrafine titanium dioxide aerosol of 22 nm count median diameter. The intrapulmonary distribution of particles was analyzed 1 hr or 24 hr after the end of exposure, using energy-filtering transmission electron microscopy for elemental microanalysis of individual particles. In an *in vitro* study, we exposed pulmonary macrophages and red blood cells to fluorescent polystyrene microspheres (1, 0.2, and 0.078 μm) and assessed particle uptake by confocal laser scanning microscopy. Inhaled ultrafine titanium dioxide particles were found on the luminal side of airways and alveoli, in all major lung tissue compartments and cells, and within capillaries. Particle uptake *in vitro* into cells did not occur by any of the expected endocytic processes, but rather by diffusion or adhesive interactions. Particles within cells are not membrane bound and hence have direct access to intracellular proteins, organelles, and DNA, which may greatly enhance their toxic potential. *Key words:* aerosol, erythrocytes, lungs, macrophages, microscopy, nanoparticles, rats, surfactant. *Environ Health Perspect* 113:1555–1560 (2005). doi:10.1289/ehp.8006 available via *http://dx.doi.org/* [Online 26 May 2005]

High concentrations of airborne particles have been associated with increased pulmonary and cardiovascular mortality, with indications of a specific toxicologic role for ultrafine particles (UFPs; particles with diameters < 0.1 μm) (Peters et al. 1997). UFPs may induce inflammatory and prothrombotic responses, promoting atherosclerosis, thrombogenesis, and the occurrence of other cardiovascular events (Schulz et al. 2005). Human data suggest that inhaled UFPs influence lung physiology (Pietropaoli et al. 2004). UPFs may also affect the autonomic nervous system or act directly on cells in various organs and induce mutations (Harder et al. 2005; Samet et al. 2004). After exposure of the respiratory system to UFPs, the UFPs may appear within hours in many compartments of the body, including the liver, heart, and nervous system (Brown et al. 2002; Kreyling et al. 2002; Oberdörster et al. 2004).

UFPs are formed by gas-to-particle conversion or by incomplete fuel combustion. Despite considerable efforts to reduce air pollution, the environmental burden by UFPs may have increased rather than decreased over time (Kreyling et al. 2003). Moreover, the fast-growing nanotechnology industry generates new UFPs daily, which may become aerosolized at some stage and may present additional health risks. UFPs possess increased toxicity compared with larger particles composed of the same materials (Ferin et al. 1992). Their environmental burden is characterized by high number concentrations but low mass concentrations. Thus, a relatively large surface area per unit mass facilitates adsorption of various organic compounds from the ambient air and enhances interaction with biological molecules within the organism.

Deposition of UFPs in the respiratory system is caused by diffusional displacement. Depending on particle size, deposition occurs efficiently in the nose, the conducting airways, and the alveoli. Although particles with diameters > 1 μm usually remain on the epithelial surface upon their deposition (Gehr et al. 1990; Geiser et al. 2003; Schürch et al. 1990) and are subjected to clearance by cough, mucociliary transport, and/or phagocytosis by macrophages, UFPs seem to penetrate the boundary membranes of the lungs rapidly—a unique feature for insoluble particles (Brown et al. 2002; Kreyling et al. 2002; Oberdörster et al. 2002). In addition, transport across the olfactory epithelium and accumulation in the brain were reported for various UFP types (Oberdörster 2004). *In vitro* experiments revealed penetration of UFPs into mitochondria of macrophages and epithelial cells that was associated with oxidative stress and mitochondrial damage (Li et al. 2003).

Because everyone on earth inevitably inhales thousands to millions of UFPs with each breath, it is important to assess health risks by UFP air pollution. The costs of actions to be taken to reduce ambient aerosol particles are high and will affect the economy greatly, presenting an urgent need to clarify the fate of inhaled UFPs. To date, the mechanisms by which UFPs penetrate boundary membranes and the distribution of UFPs within tissue compartments of their primary and secondary target organs are largely unknown.

This study is the first to investigate the distribution of inhaled UFPs within lungs at the individual particle level and combines different experimental approaches—an *in vivo* inhalation study in rats and an *in vitro* cell exposure study on pulmonary macrophages and red blood cells (RBCs).

In the *in vivo* experiments, rats inhaled an ultrafine titanium dioxide aerosol of 22 nm count median diameter (CMD) during 1 hr, resulting in a deposition of 4–5 μg TiO_2 per animal. The intrapulmonary distribution of deposited particles was analyzed immediately or 24 hr after the end of exposure, using energy-filtering transmission electron microscopy (EFTEM) to allow elemental microanalysis of individual particles (Kapp et al. 2004).

In the *in vitro* study, we exposed cultured porcine pulmonary macrophages and human RBCs to fluorescent polystyrene microspheres with diameters of 1, 0.2, and 0.078 μm and assessed particle uptake by confocal laser scanning microscopy (CLSM).

Materials and Methods

Animals. The animal experiments were conducted under federal guidelines for the use and

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The authors declare they have no competing financial interests.

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Effect of Ultrafine particles on whole blood and A549 Cells: Both Collected Dust and Engineering Material

Introduction

The adverse health effect of fine and ultrafine particles in the air has been noticed for the positive correlation between daily ambient particle concentration and emergency hospital admissions and death rate. Although the cause of death was usually cardiovascular disease in these cases, the main exposure route for those airborne particles is inhalation and cumulating evidence show that the effect on the inhalation system is also of importance.

Some occupational lung diseases caused by very high particle doses in the work environment, such as in miners and in cotton workers have been well studied. But how the low dose particles that everyone are exposed to and how new material produced by modern industrial processes affect our respiratory health is not fully understood.

A widely accepted theory is that ultrafine particles especially nano size particles will cause oxidative stress in the tissue that they attach to and even damage to the cells because of the large surface area of the particles. This theory explained why the same material in different sizes can have different toxicity and revealed the potential risk of engineering nano products. But when considering respiratory health effect specifically, there are still many questions, such as the deposition, translocation and clearance of particles in the airways; whether particle number or total surface area matters the most; how the oxidative stress in a restriction site leading to the clinic symptoms; evaluation the safety of new nano material that may be inhaled.

To understand the mechanisms underlying those observations, airway epithelial cells are focused on for multiple reasons: they are the barrier of airway and will contact to the inhaled particles; the concentration of particles is usually highest on the surface of epithelial in the airway; epithelial cells can secrete a series of cytokines and interact with immune cells such as dendritic cells (DCs) and macrophages; and they are involved in the translocation and clearance of particles.

In fig. 1 is summarized some pathways that have been proposed by recent reports considering how epithelium respond to different kinds of air pollutants. For inert ultrafine particles the dominant pathways are expected to be I and II which relate to oxidative stress and cell injury. But as we also have samples that may have active organic content the pathway III cannot be ignored.

This project is developed from the need of evaluating a series of ultrafine dust collected from indoor environment. A549 cell line can be used to test these and can provide information from particle uptake to cytokine secretion [17, 18, 19]. Moreover, resent studies suggested airway epithelium plays an essential role in the response to inhaled stimuli. With careful examination we not only can predict the possible response in vivo, but also reveal the pathway leading to the response. For the purpose of evaluating its own safety and comparing with collected dust which has complex chemical contents, pure engineering nano materials are included in the test too.

Like other cell lines, such as Hela cell, oxidative stress indicating by ROS generating and biomacromolecules oxidation products can be measured in A549 culture as well as cell death. However A549 cell line has its especial advantage in respiratory studies. It is a human airway epithelium cell line with the most similarity to the cells contact to inhaled particles in vivo. It has Toll-like receptors (TLRs) on its surface which can be activated by certain content of dust sample such as endotoxin. It can release IL-33 as an alarm during cell damage which is believed to contribute to the development of asthma. Particle uptake can be observed in A549 culture. These traits enable us to distinguish different effects of different dusts or engineering materials.

But as a single cell culture, test on A549 culture lack the crosstalk between immune system, thus usually cannot drew a hard conclusion from this test alone. In present project, whole blood assay (WBA) will work as an efficient complementary model. Combining the results of A549 test and WBA will give conclusions based on insights from two different compartments. This is specially interesting, since inhaled ultrafine particles can pass the barrier of airway epithelium and enter the circulatory system. It is an important aspect of health effect of ultrafine particles related to a systematic inflammation and cardiovascular disease.

Whole blood is an easily obtained material for testing the reaction of immune system. So we can use WBA to scan large number of samples. Its usage in investigation of occupational allergy and pathology is quite successful [14, 15, 16]. Because of the large sample number and working as a scan step to choose candidate for A549 culture test, we will only focus on the detecting of inflammatory and proinflammatory interleukins in the WBA.
Most of the samples were already collected, my work in this project will start from the physical and chemical analysis step. The aim of this project is to test the main hypothesis by testing multiple particle samples and combining the results of A549 culture and WBA. Meanwhile the safety of those engineering materials and the indoor environments from which the dusts collected will be evaluated. Thus provide information for further studies such as animal and human exposure study which would be too much to include in this three-year PhD project.

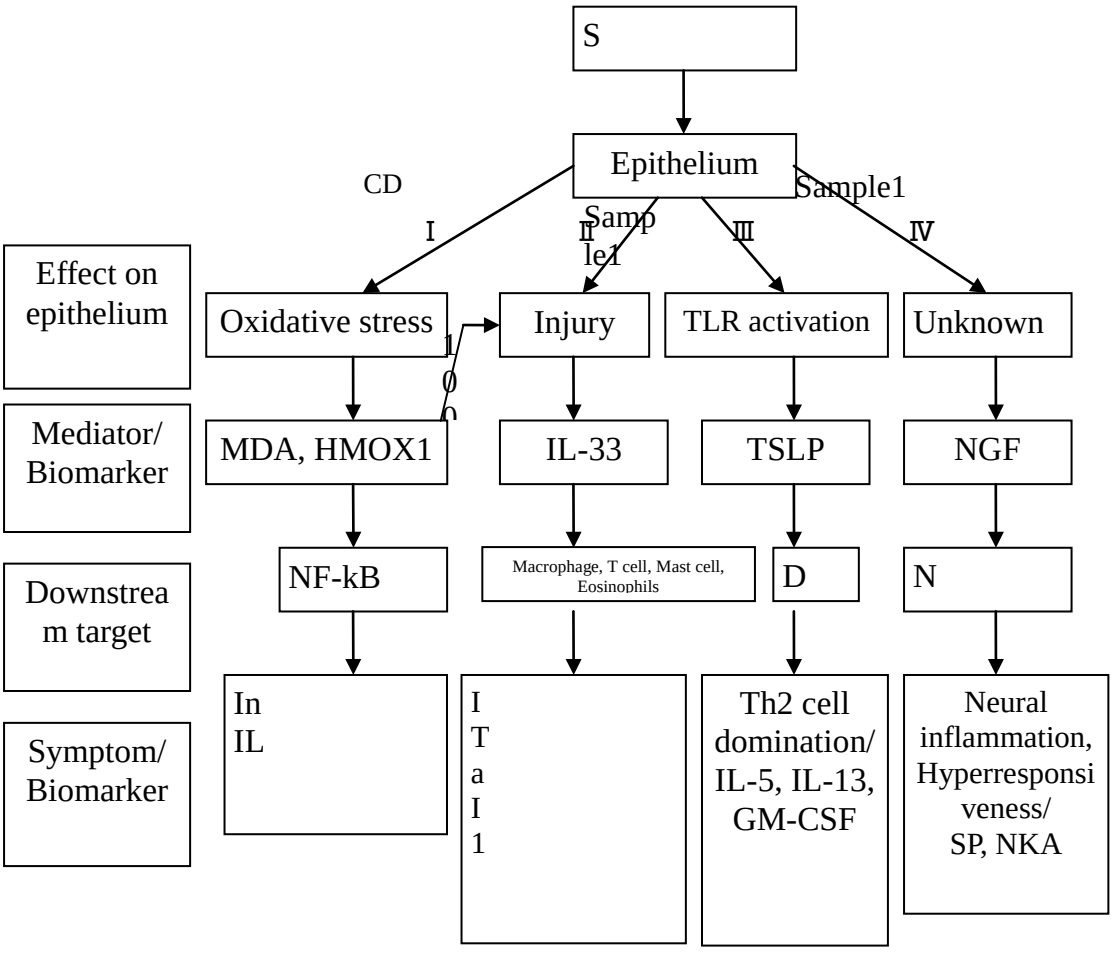

Figure 1. Epithelium plays an essential role in the response to stimuli.

Main Hypothesis

1. Inert ultrafine particles affect epithelial cells mainly through generating oxidative stress on the cells, and the ability of inducing oxidative stress is negatively correlated to particle size.
2. Epithelia cells play an essential role in the response to inhaled particles. The inflammation inducing ability of particles evaluated by WBA and A549 will be consistent with each other.

Material and Methods

Sample collection
Dust collected (CD) by filter from suspicious indoor enviroments.

Engineered nanomaterial (EM) purchased or produced by iNANO-center.
Carbon Black (CB) purchased.

Scanning electron microscopy (SEM) [1, 2, 3]
Provide physical information of particles including size, structure and shape. We focus on ultrafine and nano size particles in this study. So it is essential to know the particle size. For pure engineering product we can also calculate its surface/number ratio.
After adding particles to A549 culture, we can observe the location of particles on cell surface as well as inside the cells.

Chemical composition analyses
Metal, organic carbon, sulfate and nitrate contents [20].
Endotoxin content by Limulus amebocyte lysate test (LAL) [21, 22].

Plasmid Scission Assay [4, 5, 6, 7, 8]
It is a cell free method to detect particle surface-generated free radicals by measuring the ability of particles to break strands of supercoiled DNA plasmid. Free radical damage to the plasmid is expressed as depletion of the supercoiled plasmid band intensity relative to a control.

The whole blood assay (WBA) [9, 10]
The inflammatory and proinflammatory interleukins IL-1, IL-6 and IL-8 are chosen in this study to monitoring the triggering of inflammation.
Peripheral blood will be provided by healthy volunteers. EDTA will be used as anticoagulant. Blood from one volunteer will be divided into four parts equally to incubate with four different samples. Each sample will be test in two final concentrations: 10μg/ml and 100μg/ml. The incubation condition will be: 18 hours at 37℃ in humidity air with 5% CO2. Each concentration will be tested three times and the result will be measured in duplicate as illustrated in fig. 2.

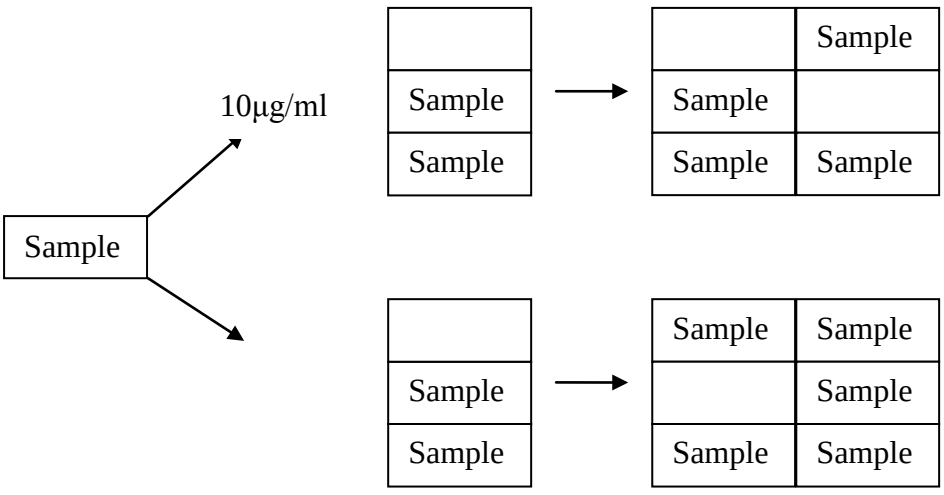

Figure 2. WBA procedure.

The A549 cell culture
In addition to the cytokines we measured in WBA, IL-33 and thymic stromal lymphopoietin (TSLP) are also our interests for their role in pathway II and III.
IL-33 is a novel mediator working as an “alarm”. It is released from cell nucleus in the process of cell death. IL-33 targets multiple cell types, among which, mast cells, macrophages, T cells and eosinophils are closely related to allergy and/or lung inflammation. [11, 12].
TSLP can direct dendritic cells (DCs) towards a Th2 response. It is presumed that TSLP production can be induced in airway epithelial cells by ligands that activate TLR2 and TLR3. [13]

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Editorial

Particulate Matter and Nanoparticles Toxicology

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Humans have been exposed for thousands of years to particulate matter (PM) from natural and anthropogenic sources. Since the first third of the twentieth century, health problems related to dust exposure in miners have been documented [1]. Early epidemiological data have shown evidence of the relation between inhalation of PM and several lung diseases, including lung fibrosis and lung cancer [2]. The Meuse valley fog of 1930 [3], the Donora smog incident of 1948 [4], and the London great smog event of 1952 [5] were the foundation to create a legislation regarding to air pollutants. In the US, the Clean Air Act was enacted in 1972. Before 1970, the main efforts in this field were aimed at measuring the PM suspended in the air and its relation to death increases associated to lung diseases. During the 1970s and 1980s, the measurements of environmental particles improved, and efforts to quantify particles with different aerodynamic sizes gave a view on how the particles could be related to different diseases. These methods helped to identify sources of different particles, leading to specific actions to control the emission of PM. During the 1990s and the first decade of this century, a great effort has been done to determine the cellular and molecular mechanisms related to the particle toxicity, and many studies have shown that particle size and composition play central roles in the biological effects. During the last 20 years, a great concern has grown regarding the use of nanoparticles (NPs) and its possible impact on workers and final users. To present, it is evident that inhaled particles may have local and systemic effects, and that the size, the composition, and the physicochemical characteristics of these airborne particles play a central role in their toxicity.

Despite all the clinical, epidemiological, and toxicological evidence, we are far from understanding the toxicology of particles, in part by the combined effects and interactions of various substances mixed within the particles. In addition, the lack of evidence of a threshold value makes it difficult to set safe limit values. Therefore, a constant growth in the total number of publications related to urban PM and NP is easy to observe when a simple search is done on PUBMED. When the words "particles" and "air pollution" are searched, 101 publications are found from 1900 to 1970. The number rises to 149 from 1971 to 1980. During the 1980s, the first efforts were done to evaluate the effects of particles with different aerodynamical sizes but the number of publications remained in 150. Later on, during the 1990s, the evaluation of particles with different aerodynamic sizes (PM_{10}, $PM_{2.5}$, and ultrafine particles) made the number of publications to grow up to more than 600. The continuous evaluation of urban particles and the arising use of nanomaterials led to almost 2000 publications during the first decade of this century. If the rate of publications on these fields keeps the same rhythm

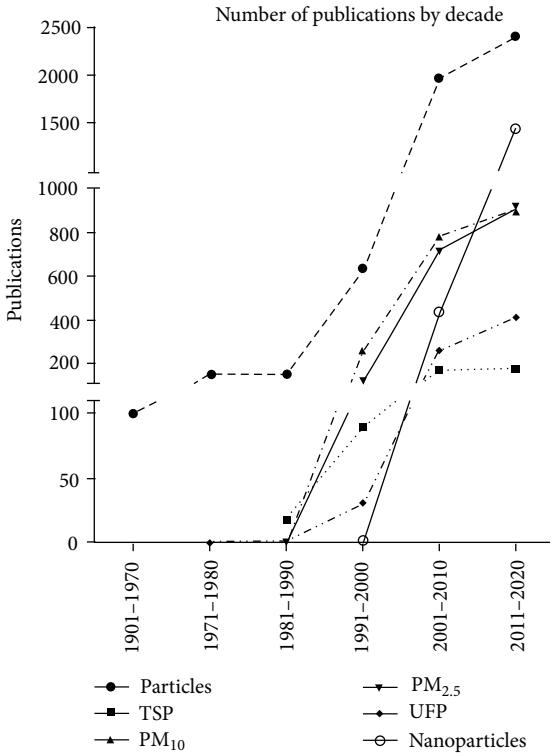

Figure 1

as the first two years of the present decade, we will find about 2400 publications for the 2011–2020 decade (Figure 1).

The previously-mentioned numbers gave a clear idea of why a special issue on particulate matter and nanoparticles toxicology is important for this field. In this special issue, we are publishing a selection of studies dealing with particle sampling and characterization, *in vitro* toxic effects characterization using traditional and novel models, *in vivo* effects of PM, and *in vivo* and *in vitro* effects of different types of NP.

We also include three reviews, discussing the cellular effects of diesel particles, another one discussing the evidence relating the exposure to particles and other inhaled pollutants to the increased risk of Alzheimer and Parkinson's diseases. Finally, a review of the state of the art in the *in vivo* and *in vitro* toxicological characterization of particles was prepared for this issue by the guest editors, where we discuss the latest evidence of local and systemic effects induced by inhaled particles.

Great efforts have been done during the last 50 years to understand the toxicology of particulate matter, and much information is available helping to understand the risks of exposure to different types of particles. Nevertheless, there is much to do in the field, and the efforts presented here will be of great value to push further the frontiers of our knowledge on the particulate matter toxicology field.

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Czech Republic: activities on engine exhaust toxicology

Worldwide efforts aimed at the establishment of cooperation among engine and toxicology research groups and assessment of the effects of new fuels and engine and exhaust aftertreatment technologies have resulted in the formation of a national group within the EU LIFE+ project MEDETOX (Innovative Methods of Monitoring of Diesel Engine Exhaust Toxicity in Real Urban Traffic, LIFE10 ENV/CZ/651, www.medetox.cz), which has started in late 2011.

Assessment of severe congestion on particle emissions and their toxicity has been the main focus of the MEDETOX project. Three trucks were tested on the road, with emissions measured online using a portable, on-board emissions monitoring system and also sampled using a portable, on-board proportional sampling system. In addition, extended low-load operation has been simulated on an engine dynamometer in a laboratory. Approximately 1 m3 of diluted exhaust per minute has been sampled with an in-house made dilution and sampling system utilizing two common atmospheric high-volume samplers. Results of the laboratory tests have been presented at the ETH Conference on Combustion Generated Nanoparticles and suggest that the emissions of PM mass, carcinogenic PAH (cPAH), US EPA 16 priority PAH and benzo(a)pyrene were an order of magnitude higher (a) during extended operation at 2% load, as compared to operation at 2% load immediately after higher load, and (b) during operation at 100% load immediately after extended low-load operation, as compared to stabilized operation at 100% load, on both diesel fuel and neat biodiesel.

A new engine test facility, part of the Center for Vehicles and Sustainable Mobility at the Czech Technical University of Prague, has been opened at Science and Technology Park in Roztoky, near Prague. The laboratory houses four transient engine dynamometers and one all-wheel-drive light duty chassis dynamometer, and a full-flow dilution tunnel with EU-legislation compliant emissions monitoring system, with online particle classifiers and FTIR spectrometer being added this year, and various additional emissions measurement and sampling systems being brought in on a project-by-project basis.

A collaboration has been established between the newly build engine testing laboratories and the Laboratory of Genetic Ecotoxicology at the Institute of Experimental Medicine of the Czech Academy of Sciences, within the project BIOTOX (Mechanisms of toxicity of biofuel particulate emissions) funded by the Czech Science Foundation. The use of biofuels in vehicles introduces the need for better understanding of the biological impacts of exposure to particulate matter emitted by the engines, a major urban health issue. Major aim of this project is to get more insight into the mechanism of toxicity of respirable particles from emissions of biofuels in comparison with classic fuels. The project will use the model of human lung cells (HEL, A549) to partly solve this problem. Development of the methodology for sampling of high quantities of particulate matter has paved the way for ongoing sampling of particles from diesel and gasoline engines running on conventional and alternative fuels (ethanol, biodiesel, hydrotreated vegetable oil, and their blends with traditional petroleum fuels). Respirable particles (PM2.5) from engine emissions produced by selected commonly used biofuels and, for comparison, by

classic diesel fuel and gasoline, will be collected from various engines during various test cycles. The chemical analysis of extracts from sampled PM2.5 will be performed and selected toxicity markers will be analyzed in cell cultures. Genome-wide transcriptomics and selected protein expression will be employed to get more insight into the mechanisms of biological effects of engine emissions. Combined use of chemical analysis with genomics will enable to identify major toxic emission components as well as biological pathways involved. Project will contribute substantially to direct comparison of mechanisms of the toxicity of biofuels with classic fuels and may be used for a risk assessment.

A novel compact on-board particle measurement system has been developed and presented. Common low-cost ionization type smoke detectors, produced in mass quantities for fire detection in buildings, have been tested and suggested as a qualitative tool for on-road, online measurement of total particle length concentration, a measure believed to have closer correlation to lung-deposited surface area than total particle number or total particulate matter mass. Laboratory results suggest that the ionization chamber signal correlates best to total particle length, with correlation to number and mass dependent on particle size distribution. Particle filter regeneration events were clearly discerned from the ionization chamber readings. With detection limits on the order of 0.1 mg/m^3 and one million of particles per cm^3 in raw exhaust, the method appears to be sufficiently sensitive for inspection of vehicles equipped with particle filters and for preliminary measurements of particle emissions from modern engines, as traditional smoke opacity measurement, performed on diesel engines during regular emissions inspections, sensitive primarily to larger particles of elemental carbon, is very little sensitive to nanoparticles and to semi-volatile „organic carbon“ particles. (Vojtisek-Lom, Society of Automotive Engineers Technical Paper 2013-24-0168). On-board measurements of particulate matter, including particle length concentrations, have been carried within MEDETOX and BIOTOX projects on automobile and motorcycle gasoline engines in real-world operation.

Additional off-line analysis and characterization of particulate matter collected within the mentioned projects was done by dr. Popovicheva’s group at the Moscow State University, in order to compare particles originating out of different fuels and engine operating modes. Preliminary results have been submitted for publication, with additional tests being planned.

The experiences gained so far suggest that a working interdisciplinary engine-toxicology group has been successfully formed in the Czech Republic over the last several years.

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Characterization of a multiculture in-vitro cell exposure chamber for assessing the biological impact of diesel engine exhaust

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Abstract. In order to study the various health influencing parameters related to particulate as well as to gas-phase pollutants emitted by Diesel engine exhaust, there is an urgent need for appropriate sampling devices and methods for cell exposure studies and associated biological and toxicological tests. In a previous paper [1], a specific concept for a cell culture exposure chamber was introduced to allow the uniform exposure of cell cultures to diesel aerosols. In the present work, this cell culture exposure chamber is evaluated and characterized with state-of-the-art nanoparticles measurement instrumentation to assess the local deposition of soot aggregates on the cell cultures and any losses due to particle deposition on the cell culture exposure chamber walls, and in addition an upgraded Multiculture Exposure Chamber (MEC) for in vitro continuous flow cell exposure tests is introduced with improved, compared to the previous version, features. Analysis and design of the MEC employs CFD and true to geometry representations of soot particle aggregates.

1. Introduction

Primary health concerns from airborne pollutants include lung carcinogenicity and non-malignant respiratory effects such as irritation, inflammation, and exacerbation or initiation of allergic hypersensitivity. The latter especially is an emerging area of concern [2]. As the prevalence of asthma and other allergic diseases has increased throughout the industrialized world in recent decades, air pollution, including exhaust emissions, especially in urban areas has been suggested as one possible cause. Therefore, the environmental and health related impacts of Diesel exhaust emissions continue to attract the attention of researchers, industry and legislative bodies. The Diesel engine effluent is a complex mixture of particles and gases with hundreds of chemicals, including many organics, present both in the gaseous and condensed phase. Even though advanced technology Diesel engines and emission control devices have been developed to improve their environmental performance and therefore to reduce the expected emissions, the currently prevailing approach does not guarantee that a decrease in regulated emissions will not generate compounds that might have more deleterious health

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Development of a dose-controlled multiculture cell exposure chamber for efficient delivery of airborne and engineered nanoparticles

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Abstract. In order to study the various health influencing parameters related to engineered nanoparticles as well as to soot emitted by Diesel engines, there is an urgent need for appropriate sampling devices and methods for cell exposure studies that simulate the respiratory system and facilitate associated biological and toxicological tests. The objective of the present work was the further advancement of a Multiculture Exposure Chamber (MEC) into a dose-controlled system for efficient delivery of nanoparticles to cells. It was validated with various types of nanoparticles (Diesel engine soot aggregates, engineered nanoparticles for various applications) and with state-of-the-art nanoparticle measurement instrumentation to assess the local deposition of nanoparticles on the cell cultures. The dose of nanoparticles to which cell cultures are being exposed was evaluated in the normal operation of the *in vitro* cell culture exposure chamber based on measurements of the size specific nanoparticle collection efficiency of a cell free device. The average efficiency in delivering nanoparticles in the MEC was approximately 82%. The nanoparticle deposition was demonstrated by Transmission Electron Microscopy (TEM). Analysis and design of the MEC employs Computational Fluid Dynamics (CFD) and true to geometry representations of nanoparticles with the aim to assess the uniformity of nanoparticle deposition among the culture wells. Final testing of the dose-controlled cell exposure system was performed by exposing A549 lung cell cultures to fluorescently labeled nanoparticles. Delivery of aerosolized nanoparticles was demonstrated by visualization of the nanoparticle fluorescence in the cell cultures following exposure. Also monitored was the potential of the aerosolized nanoparticles to generate reactive oxygen species (ROS) (e.g. free radicals and peroxides generation), thus expressing the oxidative stress of the cells which can cause extensive cellular damage or damage on DNA.

1. Introduction

Research on airborne nanoparticles has already proven that they can have adverse impacts on health, affecting primarily the respiratory and the cardiovascular systems [1-3]. This body of research naturally has led to increased concerns on the potential of Engineered Nanoparticles (ENP) to also cause adverse health effects and occupational safety impacts, since increasing use of ENP is caused by the growing nanotechnology market. As any adverse public or ethical reaction to nanotechnology-

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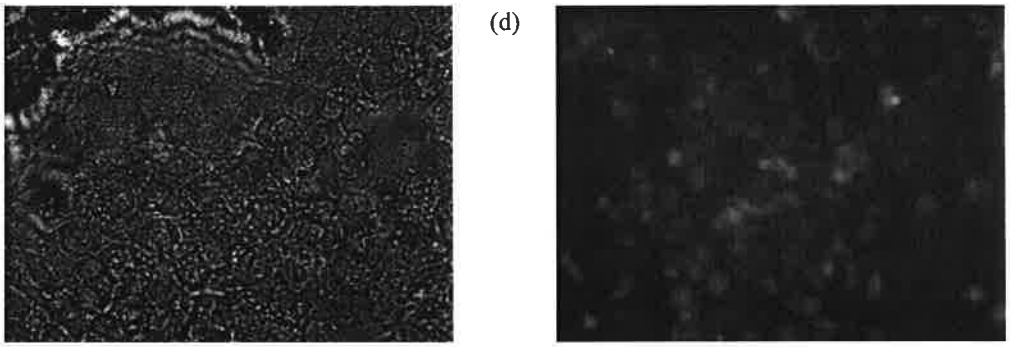

Figure 9. Microscope images of A549 cells at positions A1 (a & b) and B5 (c & d) exposed to Fluoprobe532 labeled SiO_2 nanoparticles. Magnification = 200x (a & b) 400x (c & d), Blue = nuclei (DAPI), Green = ROS (DCF), Red = nanoparticles (Fluoprobe532).

4. Conclusions

The advancement of an upgraded Multiculture Exposure Chamber (MEC) into a dose-controlled system for efficient delivery of nanoparticles to cells and its validation with various types of nanoparticles (Diesel engine soot aggregates, SiO_2 nanoparticles) is presented. The average efficiency in delivering nanoparticles in the MEC is approximately 82%, while the cell-specific deposition efficiency is roughly half of the inner deposition efficiency, i.e. 35%, which is much greater than the collection efficiencies reported in the literature. A high degree of flow homogeneity has been observed with flow visualization tests, while a slight non-uniformity in the velocity (uniformity metric = 1.25) was attributed to defects in the inlet channel, which, however is easily eliminated by design modification in future work. Nanoparticle deposition was demonstrated by TEM and final validation of the dose-controlled cell exposure system was performed with exposure tests of A549 cell cultures to SiO_2 nanoparticles. The efficient delivery of nanoparticles as well as the homogeneity in particle deposition among the 6-well plate inserts was demonstrated by fluorescence microscopy.

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Rissler, J.; Swietlicki, E.; Bengtsson, A.; Boman, Ch.; Pagels, J.; Sandström, Th.;
Blomberg, A.; Löndahl, J.:

Experimental determination of deposition of diesel exhaust particles in the human respiratory tract

Abstract:

Diesel emissions are a major contributor to combustion-generated airborne ambient particles. To understand the role of diesel particulate emissions on health effects, it is important to predict the actual particulate dose deposited in the human respiratory tract, with respect to number, surface area and mass. This is complicated by the agglomerate nature of some of these particles. In this study the respiratory tract deposition fraction in the size range 10-500 nm, was determined for 10 healthy volunteers during both idling and transient engine running conditions of a heavy duty diesel engine. The aerosol was characterized with respect to both chemical and physical properties including size resolved particle effective density. The dominating part of the emitted particles had an agglomerate structure. For those formed during transient running conditions, the relationship between particle mass and mobility diameter could be described by a power law function. This was not the case during idling, most likely because of volatile compounds condensing on the agglomerates. The respiratory tract particle deposition revealed large intra-subject variability with some subjects receiving a dose that was twice as high as that of others, when exposed to the same particle concentration. Associations were found between total deposited fractions (TDF), and breathing pattern. There was a difference between the idling and transient cycle with TDF being higher with respect to number during idling. The measured size-dependent deposition fraction of the agglomerated exhaust particles from both running conditions was nearly identical and closely resembled that of spherical hydrophobic particles, if plotted as a function of mobility diameter. Thus, for the size range covered, the mobility diameter could well describe the diameter-dependent particle respiratory tract deposition probability, regardless of the agglomeration state of the particles. Whilst mobility diameter well describes the deposition fraction, more information about particle characteristics is needed to convert this to volume equivalent diameter or estimate dose with respect to surface area or mass. A methodology is presented and applied to calculate deposited dose by surface area and mass of agglomerated particles. The methodology may be useful in similar studies estimating dose to the lung, deposition onto cell cultures and in animal studies.

Langrish, J. P.; Bosson, J.; Unosson, J.; Muala, A.; Newby, D. E.; Mills, N. L.; Blomberg, A.;
Sandström, Th.:

Cardiovascular effects of particulate air pollution exposure: time course and underlying mechanisms

Abstract:

Objective Air pollution is now recognized as an important independent risk factor for cardiovascular morbidity and mortality and may be responsible for up to 3 similar to million premature deaths each year worldwide. The mechanisms underlying the observed effects are poorly understood but are likely to be multifactorial. Here, we review the acute and chronic effects of air pollution exposure on the cardiovascular system and discuss how these effects may explain the observed increases in cardiovascular morbidity and mortality.

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PARTICLE AND FIBRE TOXICOLOGY

RESEARCH Open Access

Exposure to wood smoke increases arterial stiffness and decreases heart rate variability in humans

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Abstract

Background: Emissions from biomass combustion are a major source of indoor and outdoor air pollution, and are estimated to cause millions of premature deaths worldwide annually. Whilst adverse respiratory health effects of biomass exposure are well established, less is known about its effects on the cardiovascular system. In this study we assessed the effect of exposure to wood smoke on heart rate, blood pressure, central arterial stiffness and heart rate variability in otherwise healthy persons.

Methods: Fourteen healthy non-smoking subjects participated in a randomized, double-blind crossover study. Subjects were exposed to dilute wood smoke (mean particle concentration of 314±38 μg/m^3) or filtered air for three hours during intermittent exercise. Heart rate, blood pressure, central arterial stiffness and heart rate variability were measured at baseline and for one hour post-exposure.

Results: Central arterial stiffness, measured as augmentation index, augmentation pressure and pulse wave velocity, was higher after wood smoke exposure as compared to filtered air ($p < 0.01$ for all), and heart rate was increased ($p < 0.01$) although there was no effect on blood pressure. Heart rate variability (SDNN, RMSSD and pNN50; $p = 0.003$, $p < 0.001$ and $p < 0.001$ respectively) was decreased one hour following exposure to wood smoke compared to filtered air.

Conclusions: Acute exposure to wood smoke as a model of exposure to biomass combustion is associated with an immediate increase in central arterial stiffness and a simultaneous reduction in heart rate variability. As biomass is used for cooking and heating by a large fraction of the global population and is currently advocated as a sustainable alternative energy source, further studies are required to establish its likely impact on cardiovascular disease.

Trial registration: ClinicalTrials.gov, NCT01488500

Keywords: Biomass, Air pollution, Arterial stiffness, Blood pressure, Heart rate variability, Cardiovascular

Background

Exposure to fine and ultrafine combustion-derived particulate air pollution ($PM_{2.5}$, particulate matter with a mean aerodynamic diameter <2.5 μm) is increasingly recognized as a short and long-term risk factor for cardiovascular disease and has been linked to the triggering of myocardial infarction within hours of exposure [1-5]. Controlled exposure studies have demonstrated that acute exposure to diesel exhaust and concentrated ambient particles, as models of urban particulate air pollution, generate acute vasoconstriction, increases in blood pressure and arterial stiffness [1,6,7], vascular endothelial dysfunction [8], myocardial ischemia [8,9] and changes in cardiac autonomic control [7,10]. This may in turn explain the observed increase in cardiovascular events [11].

Smoke from biomass combustion is the world's oldest anthropogenic air pollution and contributes significantly to ambient levels of free radicals, polycyclic aromatic hydrocarbons, aldehydes, partially oxidised organic chemicals and particulate matter [12]. Currently more than half the world's population relies on indoor burning of biomass for heating and cooking [13], and

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Cardiopulmonary Health Effects of Semi-Volatile and Non-Volatile Components of PM

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Overview

Heart disease is the leading cause of death in the U.S., and exposure to particulate matter (PM) air pollution may contribute to both disease and death among populations living in polluted environments. The observed associations between PM exposure and human heart disease and death may be related to PM-induced oxidative stress and/or inflammation in the body; however, the specific mechanisms by which PM exposure worsens heart function and cardiovascular disease are not well understood.

PM is composed of solid, liquid, and semi-volatile organic components. Preliminary studies demonstrated that if most of the organic components were removed from the particles, the particles would become much less chemically reactive. We therefore tested the hypothesis that removal of organic constituents of PM would reduce the particle's ability to induce or accelerate atherosclerosis. In atherosclerosis, plaques composed of fat, cholesterol, and other components build up inside arteries and cause them to narrow over time, potentially leading to heart attack or stroke.

For this study, an atherosclerotic mouse model was used. Mice were exposed by using a particle concentrator-thermal denuder system that removed semi-volatile organic components from ambient ultrafine PM. Changes in atherosclerotic plaque formation were measured, along with heart rate, heart rate variability, and levels of lipid peroxidation. Detailed chemical and physical characterizations of thermally denuded particles were also conducted. This study showed that a novel methodology could be employed to deliver intact ultrafine ambient PM, denuded ultrafine PM, and the organic constituents alone to an atherosclerotic mouse model.

Using this methodology a major finding was that exposures to intact PM and to the isolated organic constituents of the particles accelerated the development of atherosclerotic plaques and induced decreases in heart rate variability compared to controls, as well as promoting serum lipid peroxidation. These changes were not observed when mice were exposed to denuded particles free of the organic component. These results suggest that the organic constituents of ultrafine PM, rather than the denuded particle itself, may play an important role in the progression of heart disease.

Speaker Biography

Michael T. Kleinman, Ph.D., is a Professor and Co-Director of the Air Pollution Health Effects Laboratory in the Department of Medicine at the University of California, Irvine. Prior to joining the faculty at UCI in 1982, he directed the Aerosol Exposure and Analytical Laboratory at Rancho Los Amigos Hospital in Downey, California. Dr. Kleinman's current research has focused on toxicological studies of airborne contaminants using laboratory animals. Professor Kleinman has published more than 100 articles in peer-reviewed journals dealing with the uptake and dosimetry of inhaled pollutants, cardiopulmonary and immunological responses associated with inhalation of PM2.5, health effects of acidic and non-acidic aerosols, and studies of the effects of mixtures of particles with other pollutants such as ozone, formaldehyde, sulfur dioxide, and nitrogen dioxide. Dr. Kleinman's previous studies examined cardiopulmonary effects of concentrated ambient ultrafine, fine and coarse particles using animal models of susceptible human populations. His current studies address the role of organic and inorganic constituents of air pollution mixtures in the development or exacerbation of heart disease. Dr. Kleinman has been studying the health effects of exposures to particles and gases found in ambient air for more than 30 years.

Dr. Kleinman is a consultant to the U.S. EPA Science Advisory Board, a member of the Board of Scientific Counselors for the Center of Disease Control and Prevention (CDC-ATSDR/NCEH). Professor Kleinman has recently been appointed Chairman of the State of California's Scientific Review Panel on Toxic Air Contaminants. In addition Dr. Kleinman also serves as Chairman of the Air Quality Advisory Committee, which reviews California's air quality criteria documents. Professor Kleinman holds a M.S. in Chemistry from the Polytechnic Institute of Brooklyn and a Ph.D. in Environmental Health Sciences from New York University.

Nonequilibrium atmospheric secondary organic aerosol formation and growth

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Airborne particles play critical roles in air quality, health effects, visibility, and climate. Secondary organic aerosols (SOA) formed from oxidation of organic gases such as α-pinene account for a significant portion of total airborne particle mass. Current atmospheric models typically incorporate the assumption that SOA mass is a liquid into which semivolatile organic compounds undergo instantaneous equilibrium partitioning to grow the particles into the size range important for light scattering and cloud condensation nuclei activity. We report studies of particles from the oxidation of α-pinene by ozone and NO_3 radicals at room temperature. SOA is primarily formed from low-volatility ozonolysis products, with a small contribution from higher volatility organic nitrates from the NO_3 reaction. Contrary to expectations, the particulate nitrate concentration is not consistent with equilibrium partitioning between the gas phase and a liquid particle. Rather the fraction of organic nitrates in the particles is only explained by irreversible, kinetically determined uptake of the nitrates on existing particles, with an uptake coefficient that is 1.6% of that for the ozonolysis products. If the nonequilibrium particle formation and growth observed in this atmospherically important system is a general phenomenon in the atmosphere, aerosol models may need to be reformulated. The reformulation of aerosol models could impact the predicted evolution of SOA in the atmosphere both outdoors and indoors, its role in heterogeneous chemistry, its projected impacts on air quality, visibility, and climate, and hence the development of reliable control strategies.

atmospheric aerosol | nitrate radical | kinetic growth mechanism | condensation mechanism

Airborne particles are well-known to negatively affect human health (1) and to contribute to "haze" associated with urban and regional pollution, leading to a reduction in visibility (2). On a global scale, airborne particles scatter solar radiation and can act as cloud condensation (CCN) and ice nuclei (IN), influencing the radiative balance of the atmosphere (3, 4). Currently these effects represent the largest uncertainty in calculations of climate change (5). A major component of atmospheric particles is secondary organic aerosol (SOA) formed via the oxidation of gaseous anthropogenic and biogenic precursor compounds. The SOA material is formed from low-volatility oxidation products (3, 4). However, the processes and species leading to SOA formation and growth are not fully understood, which precludes reliable quantitative predictions of their impacts on climate, visibility, and human health.

Regional and global chemical models have generally underpredicted SOA concentrations compared to those from field measurements (6–9). Inclusion of a number of additional factors such as new SOA precursors, condensed phase chemistry, updated gas-phase chemistry and SOA yields, new primary semivolatile and intermediate volatility species, and improved emissions inventories of both gases and primary organic aerosols have lessened the magnitude of the disagreement (10–18). However, there is still significant uncertainty in predicting ambient SOA levels, and model-measurement discrepancies of a factor of two or more remain common.

One possible source of this uncertainty is that current models typically assume instantaneous equilibrium partitioning of semivolatile organic compounds (SVOCs) between existing liquid airborne particles and the gas phase using the theory of absorptive, activity coefficient-corrected, gas/liquid partitioning described in detail by Pankow (19, 20). Equilibrium partitioning is justified for particles with viscosities in the range of 0.01–100 Pa · s and diffusion coefficients ranging from 10^{-5} to 10^{-9} $cm^2\,s^{-1}$ (21). A volatility basis set approach has recently been developed for representing the partitioning of SVOCs, which also depends on saturation mass concentration of liquid particles (22).

In this paper, we present laboratory studies of particles formed in the simultaneous oxidation of α-pinene by ozone and NO_3 radicals using an aerosol flow system (23). Quantification of the organic nitrate contributions to the SOA provides unique insight into the mechanisms by which particles form and grow, which has important implications for model formulations of SOA, both outdoors and indoors, and the associated impacts predicted based on the model outputs.

Results and Discussion

Oxidants (O_3 and NO_2) are well mixed in the upstream end of an aerosol flow tube (8.5 m in length, 0.5 m in diameter) described in detail elsewhere (23). The reaction of NO_2 with O_3 forms NO_3 radicals which are mainly "stored" as N_2O_5 (see *SI Text*): $NO_2 + NO_3 \rightleftharpoons N_2O_5$. After approximately 4 min reaction time, α-pinene is injected. Experiments are performed at relative humidity (RH) < 3% without preexisting seed particles. Reactants, gas-phase products, and SOA then flow into the sampling section of the flow tube, which is equipped with five equally spaced ports (port 1 to port 5). Port 1 corresponds to a reaction time between α-pinene and the $NO_2/O_3/N_2O_5$ mixture of approximately 13 min, whereas port 5 corresponds to a reaction time of approximately 52 min. Most of the experimental data presented hereafter are taken at port 5; no major differences are observed in the SOA measurements between port 1 and port 5, establishing that the chemistry is essentially complete by port 1 and the particles travel along the length of the flow tube to port 5 without further significant modification or "aging." Integration of the rate equations for a simplified mechanism for this system (see *SI Text*) shows that the NO_3 chemistry is the dominant oxidation process for α-pinene under all experimental conditions, and is sufficiently rapid

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The authors declare no conflict of interest.

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Conditional Sampling for Source-Oriented Toxicological Studies Using a Single Particle Mass Spectrometer

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Current particulate matter regulations control the mass concentration of particles in the atmosphere regardless of composition, but some primary and/or secondary particulate matter components are no doubt more or less toxic than others. Testing direct emissions of pollutants from different sources neglects atmospheric transformations that may increase or decrease their toxicity. This work describes a system that conditionally samples particles from the atmosphere depending on the sources or source combinations that predominate at the sampling site at a given time. A single particle mass spectrometer (RSMS-II), operating in the 70−150 nm particle diameter range, continuously provides the chemical composition of individual particles. The mass spectra indicate which sources are currently affecting the site. Ten ChemVol samplers are each assigned one source or source combination, and the RSMS-II controls which one operates depending on the sources or source combinations observed. By running this system for weeks at a time, sufficient sample is collected by the ChemVols for comparative toxicological studies. This paper describes the instrument and algorithmic design, implementation, and first results from operating this system in Fresno, CA, during summer 2008 and winter 2009.

1. Introduction

Although air pollution regulations save the US economy billions of dollars per year, these same regulations are costly to industry and consumers (*1*). One reason for the high cost is that all sources of particulate matter (PM) are subject to controls regardless of their relative toxicity. Epidemiological studies have implicated PM in increased morbidity and mortality in many cities (*2*), which motivates the regulations. Although these types of studies have also shown correlation between certain health effects and particular sources (*3*), in many cases the literature lacks toxicological support. Efforts to understand the relative toxicity of different sources of air pollution have been performed on direct emissions from select sources, whereby the animals breathe the gases and particles, individually or together (*4*). By design, these studies do not include inevitable atmospheric processing and multisource effects that may alter the toxicity of the inhaled mixture. The relative toxicity of different sources may arise out of their atmospheric processing, possibly in the form of secondary components or in other ways not yet understood. Unfortunately, once the emissions have entered the atmosphere where they mix, react, and form secondary compounds, their source may be obscured, so separating them from each other is problematic. Single particle measurements in several US cities, however, have indicated that on sufficiently short time scales, parcels of air pollutants associated with different sources are potentially separable (*5*−*11*).

Single particle mass spectrometers practical for the atmospheric science community were introduced nearly 20 years ago (*12, 13*). These instruments analyze the chemical composition and size of particles one-by-one in real time. The rapid single-ultrafine-particle mass spectrometer family (RSMS-II and RSMS-III) analyze particles in the size range from 30 nm to 1 μm (*14*) and were deployed at the Atlanta, Houston, Baltimore, and Pittsburgh EPA Supersites (*6, 8, 9, 11*). Measurements from all four cities revealed that at most three different particle types, indicating three different sources, are observed at the same time. Layered on these source-oriented particles are secondary components that add to their chemical complexity, but relative amounts of such secondary material can also be discerned by single particle analysis.

The technique described here uses RSMS-II to control a bank of ChemVols (*15*). By taking advantage of the temporal patterns of PM observed in urban air sheds, each ChemVol samples when particles from a unique source or source combination affect the site, such that a sufficiently large sample is collected over the course of weeks that it can be bulk analyzed and used for toxicological studies. This work presents the experimental design and how it was implemented in summer 2008 and winter 2009 to collect milligrams of source-oriented PM in Fresno, CA.

2. Experimental Section

In this section, the basic constructs used to define the compositional state of the atmosphere and to identify transitions between states are set forth, followed by a brief description of the ChemVol sampling train. For the former, particle composition was the sole metric employed and the output of RSMS-II was translated into a snapshot of the mixing state of the atmosphere using a multitiered cluster analysis. A brief description of RSMS-II, which has been described elsewhere (*9, 14*), and details of how system components were interfaced to achieve conditional sampling, including sampling algorithms, runtime parameters, and real-time data flow, are provided in Supporting Information.

Data Constructs. Data clustering is common to many fields of study and has found widespread use in single particle mass spectrometry due mainly to the size of the data sets and complexity of the data (*6, 16*). The fundamental goal of clustering is to partition data sets into *particle classes*, or subpopulations, based on the distribution of mass spectral peaks with the understanding that different classes correspond to different particle compositions. Particle composition is the most revealing signature of the source and history of particles (*5, 6, 8*). In this sense, a particle class can be thought of as being synonymous with a source and/or process. This is essential to any real-time estimate of the mixing state of the atmosphere, in terms of source input and

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OBSTETRICS

Maternal engineered nanomaterial exposure and fetal microvascular function: does the Barker hypothesis apply?

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OBJECTIVE: The continued development and use of engineered nanomaterials (ENM) has given rise to concerns over the potential for human health effects. Although the understanding of cardiovascular ENM toxicity is improving, one of the most complex and acutely demanding "special" circulations is the enhanced maternal system to support fetal development. The Barker hypothesis proposes that fetal development within a hostile gestational environment may predispose/program future sensitivity. Therefore, the objective of this study was 2-fold: (1) to determine whether maternal ENM exposure alters uterine and/or fetal microvascular function and (2) test the Barker hypothesis at the microvascular level.

STUDY DESIGN: Pregnant (gestation day 10) Sprague-Dawley rats were exposed to nano-titanium dioxide aerosols (11.3 $\pm$ 0.039 mg/m^3/hr, 5 hr/d, 8.2 $\pm$ 0.85 days) to evaluate the maternal and fetal microvascular consequences of maternal exposure. Microvascular tissue isolation (gestation day 20) and arteriolar reactivity studies (<150 μm passive diameter) of the uterine premyometrial and fetal tail arteries were conducted.

RESULTS: ENM exposures led to significant maternal and fetal microvascular dysfunction, which was seen as robustly compromised endothelium-dependent and -independent reactivity to pharmacologic and mechanical stimuli. Isolated maternal uterine arteriolar reactivity was consistent with a metabolically impaired profile and hostile gestational environment that impacted fetal weight. The fetal microvessels that were isolated from exposed dams demonstrated significant impairments to signals of vasodilation specific to mechanistic signaling and shear stress.

CONCLUSION: To our knowledge, this is the first report to provide evidence that maternal ENM inhalation is capable of influencing fetal health and that the Barker hypothesis is applicable at the microvascular level.

Key words: Barker hypothesis, engineered nanomaterials (ENM), microvascular

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Anthropogenic engineered nanomaterials (ENMs) are manufactured specifically for their unique properties at the nanometer scale (<100 nm in 1 dimension).[1,2] Although their applicability may appear infinite, significant resources have been committed to focus ENM development on engineering and biomedical applications.[3] ENMs have already impacted public health through diverse daily uses (eg, surface coatings, cosmetics, food, drug delivery systems, and implantable medical devices). In many of these applications, adult toxicities have been observed; however, the fetal consequences of maternal exposure to ENM are essentially unknown.

Fetal toxicity and the genetic basis of adult disease are an initiative within the National Institute of Environmental Health and Safety.[4] The general understanding of adult cardiovascular ENM toxicity is modest to good[5]; yet, the maternal and fetal consequences of maternal ENM exposures during gestation are unknown. The "Barker hypothesis" proposes that the association between retarded growth and cardiovascular disease is due to chronic physiologic and metabolic effects that are imposed on a fetus by a hostile gestational environment.[6,7] Limited animal and in vitro studies suggest that maternal ENM exposure has direct consequences on the uterus, placenta, and fetus.[8-11] Nanomaterial influence on any of these tissues can have dire consequences on maternal and/or fetal health. Long et al[8] evaluated the influence that direct nano-sized titanium dioxide (TiO_2) exposure would have on rat neuronal cell cultures and revealed rapid damage to neurons at low concentrations. Blum et al[9] found cadmium oxide within the uterine and placental tissue after maternal inhalation

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Video Article

Whole-Body Nanoparticle Aerosol Inhalation Exposures

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Keywords: Medicine, Issue 75, Physiology, Anatomy, Chemistry, Biomedical Engineering, Pharmacology, Titanium dioxide, engineered nanomaterials, nanoparticle, toxicology, inhalation exposure, aerosols, dry powder, animal model

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Abstract

Inhalation is the most likely exposure route for individuals working with aerosolizable engineered nano-materials (ENM). To properly perform nanoparticle inhalation toxicology studies, the aerosols in a chamber housing the experimental animals must have: 1) a steady concentration maintained at a desired level for the entire exposure period; 2) a homogenous composition free of contaminants; and 3) a stable size distribution with a geometric mean diameter < 200 nm and a geometric standard deviation $\sigma_g < 2.5$ [5]. The generation of aerosols containing nanoparticles is quite challenging because nanoparticles easily agglomerate. This is largely due to very strong inter-particle forces and the formation of large fractal structures in tens or hundreds of microns in size [6], which are difficult to be broken up. Several common aerosol generators, including nebulizers, fluidized beds, Venturi aspirators and the Wright dust feed, were tested; however, none were able to produce nanoparticle aerosols which satisfy all criteria [5].

A whole-body nanoparticle aerosol inhalation exposure system was fabricated, validated and utilized for nano-TiO_2 inhalation toxicology studies. Critical components: 1) novel nano-TiO_2 aerosol generator; 2) 0.5 m^3 whole-body inhalation exposure chamber; and 3) monitor and control system. Nano-TiO_2 aerosols generated from bulk dry nano-TiO_2 powders (primary diameter of 21 nm, bulk density of 3.8 g/cm^3) were delivered into the exposure chamber at a flow rate of 90 LPM (10.8 air changes/hr). Particle size distribution and mass concentration profiles were measured continuously with a scanning mobility particle sizer (SMPS), and an electric low pressure impactor (ELPI). The aerosol mass concentration (*C*) was verified gravimetrically (mg/m^3). The mass (*M*) of the collected particles was determined as $M = (M_{post}-M_{pre})$, *where* M_{pre} and M_{post} are masses of the filter before and after sampling (*mg*). The mass concentration was calculated as $C = M/(Q*t)$, where *Q* is sampling flowrate (m^3/min), and *t* is the sampling time (*minute*). The chamber pressure, temperature, relative humidity (RH), O_2 and CO_2 concentrations were monitored and controlled continuously. Nano-TiO_2 aerosols collected on Nuclepore filters were analyzed with a scanning electron microscope (SEM) and energy dispersive X-ray (EDX) analysis.

In summary, we report that the nano-particle aerosols generated and delivered to our exposure chamber have: 1) steady mass concentration; 2) homogenous composition free of contaminants; 3) stable particle size distributions with a count-median aerodynamic diameter of 157 nm during aerosol generation. This system reliably and repeatedly creates test atmospheres that simulate occupational, environmental or domestic ENM aerosol exposures.

Video Link

The video component of this article can be found at http://www.jove.com/video/50263/

Protocol

The whole-body nanoparticle inhalation exposure step-by-step operating procedures are described as follows.

Note: 1) steps 1 and 3 should be performed in a fume hood; 2) operators must wear appropriate personal protective equipment (respirators, goggles and rubber gloves).

1. Conditioning TiO_2 Nanoparticle Dry Powders

1. Place nano-TiO_2 powders in a nontransparent container.
2. Leave the container lid open.

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Pulmonary Cerium Dioxide Nanoparticle Exposure Differentially Impairs Coronary and Mesenteric Arteriolar Reactivity

Valerie C. Minarchick · Phoebe A. Stapleton · Dale W. Porter · Michael G. Wolfarth · Engin Çiftyürek · Mark Barger · Edward M. Sabolsky · Timothy R. Nurkiewicz

Abstract Cerium dioxide nanoparticles (CeO_2 NPs) are an engineered nanomaterial (ENM) that possesses unique catalytic, oxidative, and reductive properties. Currently, CeO_2 NPs are being used as a fuel catalyst but these properties are also utilized in the development of potential drug treatments for radiation and stroke protection. These uses of CeO_2 NPs present a risk for human exposure; however, to date, no studies have investigated the effects of CeO_2 NPs on the microcirculation following pulmonary exposure. Previous studies in our laboratory with other nanomaterials have shown impairments in normal microvascular function after pulmonary exposures. Therefore, we predicted that CeO_2 NP exposure would cause microvascular dysfunction that is dependent on the tissue bed and dose. Twenty-four-hour post-exposure to CeO_2 NPs (0–400 μg), mesenteric, and coronary arterioles was isolated and microvascular function was assessed. Our results provided evidence that pulmonary CeO_2 NP exposure impairs endothelium-dependent and endothelium-independent arteriolar dilation in a dose-dependent manner. The CeO_2 NP exposure dose which causes a 50 % impairment in arteriolar function (EC_{50}) was calculated and ranged from 15 to 100 μg depending on the chemical agonist and microvascular bed. Microvascular assessments with acetylcholine revealed a 33–75 % reduction in function following exposure. Additionally, there was a greater sensitivity to CeO_2 NP exposure in the mesenteric microvasculature due to the 40 % decrease in the calculated EC_{50} compared to the coronary microvasculature EC_{50}. CeO_2 NP exposure increased mean arterial pressure in some groups. Taken together, these observed microvascular changes may likely have detrimental effects on local blood flow regulation and contribute to cardiovascular dysfunction associated with particle exposure.

Keywords Cerium dioxide · Mesentery · Coronary · Arteriole · Microcirculation · Engineered nanomaterial

Disclaimer The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health.

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Introduction

Engineered nanomaterials (ENMs) have recently emerged both as integral components in manufacturing and as innovative materials to address challenges in therapeutics and diagnostics, ranging from drug delivery to imaging. However, answers to questions regarding ENM toxicity remain far behind production. ENMs are defined as a homogenous mixture of particles that are less than 100 nm in size in at least one direction and are engineered to take

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Nanoparticle inhalation alters systemic arteriolar vasoreactivity through sympathetic and cyclooxygenase-mediated pathways

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Abstract

The widespread increase in the production and use of nanomaterials has increased the potential for nanoparticle exposure; however, the biological effects of nanoparticle inhalation are poorly understood. Rats were exposed to nanosized titanium dioxide aerosols (10 μg lung burden); at 24 h post-exposure, the spinotrapezius muscle was prepared for intravital microscopy. Nanoparticle exposure did not alter perivascular nerve stimulation (PVNS)-induced arteriolar constriction under normal conditions; however, adrenergic receptor inhibition revealed a more robust effect. Nanoparticle inhalation reduced arteriolar dilation in response to active hyperaemia (AH). In both PVNS and AH experiments, nitric oxide synthase (NOS) inhibition affected only controls. Whereas cyclooxygenase (COX) inhibition only attenuated AH-induced arteriolar dilation in nanoparticle-exposed animals. This group displayed an enhanced U46619 constriction and attenuated iloprost-induced dilation. Collectively, these studies indicate that nanoparticle exposure reduces microvascular NO bioavailability and alters COX-mediated vasoreactivity. Furthermore, the enhanced adrenergic receptor sensitivity suggests an augmented sympathetic responsiveness.

Keywords: Microvascular, sympathetic nervous system, whole-body inhalation, nitric oxide, titanium dioxide

Introduction

Cardiovascular toxicity following inhalation of particulate matter (PM) is well established in the literature. General toxicity associated with PM exposure is enhanced as particle size decreases. As such, ultrafine particles and nanoparticles have been suggested to have an augmented toxicity compared with their larger counterparts (Stone et al. 2007). Nanotechnology is a burgeoning industry that is incorporated into our daily lives. Nanoparticles are classified as particles with a diameter of <100 nm in one dimension (Aitken et al. 2006) and have diverse applications in medical imaging, targeted drug delivery, anti-cancer therapy, as well as properties that are useful in the manufacturing of goods such as surface coatings, UV protectorates, among others (Aitken et al. 2006). However, without first properly identifying their potential for biological effects, the ubiquitous inclusion of these materials in everyday products significantly raises the risk of personal and occupational exposure that may be intentional or accidental. Nanotechnology has tremendous potential to contribute positively to society; however, for this to fully occur, the health effects of these materials must first be defined (Maynard et al. 2006).

Nano-TiO_2 is used in products such as photocatalysts (Sun et al. 2004), antibacterial surface coatings (Shieh et al. 2006), as well as in cosmetics and sunscreens (Aitken et al. 2006). These particles induce relatively minor levels of inflammation following pulmonary deposition, but nevertheless the biological effect is dose dependent (Nurkiewicz et al. 2008). Generally, inhalation of nano-TiO_2 results in diffuse alveolitis with some increases in phagocytic cell recruitment and oedema (Nurkiewicz et al. 2008). However, the pulmonary effects are relatively minor compared with other occupationally relevant particles such as residual oil fly ash (Dreher et al. 1997).

Based on a vast epidemiological, clinical and animal toxicology literature, three major hypotheses have been promoted to explain the remote cardiovascular effects following inhalation of particles. These are (1) particle translocation into the systemic circulation and direct particle–tissue interactions (Oberdorster et al. 2004), (2) systemic

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Nanoparticle Inhalation Impairs Coronary Microvascular Reactivity via a Local Reactive Oxygen Species-Dependent Mechanism

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Abstract We have shown that nanoparticle inhalation impairs endothelium-dependent vasodilation in coronary arterioles. It is unknown whether local reactive oxygen species (ROS) contribute to this effect. Rats were exposed to TiO_2 nanoparticles via inhalation to produce a pulmonary deposition of 10 μg. Coronary arterioles were isolated from the left anterior descending artery distribution, and responses to acetylcholine, arachidonic acid, and U46619 were assessed. Contributions of nitric oxide synthase and prostaglandin were assessed via competitive inhibition with N^G-Monomethyl-L-Arginine (L-NMMA) and indomethacin. Microvascular wall ROS were quantified via dihydroethidium (DHE) fluorescence. Coronary arterioles from rats exposed to nano-TiO_2 exhibited an attenuated vasodilator response to ACh, and this coincided with a 45% increase in DHE fluorescence. Coincubation with 2,2,6,6-tetramethylpiperidine-N-oxyl and catalase ameliorated impairments in ACh-induced vasodilation from nanoparticle exposed rats. Incubation with either L-NMMA or indomethacin significantly attenuated ACh-induced vasodilation in sham-control rats, but had no effect in rats exposed to nano-TiO_2. Arachidonic acid induced vasoconstriction in coronary arterioles from rats exposed to nano-TiO_2, but dilated arterioles from sham-control rats. These results suggest that nanoparticle exposure significantly impairs endothelium-dependent vasoreactivity in coronary arterioles, and this may be due in large part to increases in microvascular ROS. Furthermore, altered prostanoid formation may also contribute to this dysfunction. Such disturbances in coronary microvascular function may contribute to the cardiac events associated with exposure to particles in this size range.

KeyWords Microcirculation · Nanoparticle · Coronary · Arteriole · Vasodilation · Titanium dioxide · Inhalation · Reactive oxygen species

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Introduction

Pulmonary exposures to nanoparticle aerosols are currently most notable in occupational and industrial settings. However, given the rate at which nanotechnology is permeating modern society, it is reasonable to expect that the likelihood of these exposures occurring in personal, domestic, and environmental settings will increase in the near future. Therefore, it is critical to identify such health effects now, prior to the establishment of widespread, public exposures as has happened for example with asbestos [1].

We have previously shown that nanoparticle inhalation impairs endothelium-dependent vasodilation in coronary arterioles [2]. The titanium dioxide particles used in our

Research

Chronic Exposure to Fine Particles and Mortality: An Extended Follow-up of the Harvard Six Cities Study from 1974 to 2009

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Background: Epidemiologic studies have reported associations between fine particles (aerodynamic diameter ≤ 2.5 μm; $PM_{2.5}$) and mortality. However, concerns have been raised regarding the sensitivity of the results to model specifications, lower exposures, and averaging time.

Objective: We addressed these issues using 11 additional years of follow-up of the Harvard Six Cities study, incorporating recent lower exposures.

Methods: We replicated the previously applied Cox regression, and examined different time lags, the shape of the concentration–response relationship using penalized splines, and changes in the slope of the relation over time. We then conducted Poisson survival analysis with time-varying effects for smoking, sex, and education.

Results: Since 2001, average $PM_{2.5}$ levels, for all six cities, were < 18 μg/m^3. Each increase in $PM_{2.5}$ (10 μg/m^3) was associated with an adjusted increased risk of all-cause mortality ($PM_{2.5}$ average on previous year) of 14% [95% confidence interval (CI): 7, 22], and with 26% (95% CI: 14, 40) and 37% (95% CI: 7, 75) increases in cardiovascular and lung-cancer mortality ($PM_{2.5}$ average of three previous years), respectively. The concentration–response relationship was linear down to $PM_{2.5}$ concentrations of 8 μg/m^3. Mortality rate ratios for $PM_{2.5}$ fluctuated over time, but without clear trends despite a substantial drop in the sulfate fraction. Poisson models produced similar results.

Conclusions: These results suggest that further public policy efforts that reduce fine particulate matter air pollution are likely to have continuing public health benefits.

Key words: air pollution, cohort studies, concentration–response, follow-up studies, lag, lung cancer, mortality, particles, $PM_{2.5}$, threshold. *Environ Health Perspect* 120:965–970 (2012). http://dx.doi.org/10.1289/ehp.1104660 [Online 28 March 2012]

All-cause, cardiopulmonary, cardiovascular, and lung-cancer mortality have been associated with chronic air pollution exposure in prospective studies that controlled for individual covariates (Abbey et al. 1999; Beelen et al. 2008b; Beeson et al. 1998; Cao et al. 2011; Dockery et al. 1993; Eftim et al. 2008; Filleul et al. 2005; Gehring et al. 2006; Katanoda et al. 2011; Laden et al. 2006; Miller et al. 2007; Nafstad et al. 2004; Ostro et al. 2010; Pope et al. 2002; Puett et al. 2009; Yorifuji et al. 2011). The studies that specifically considered lung-cancer mortality associations with fine particles (aerodynamic diameter < 2.5 μm; $PM_{2.5}$), all found positive associations (Beelen et al. 2008b; Dockery et al. 1993; Laden et al. 2006; McDonnell et al. 2000), although this association was only statistically significant ($p < 0.05$) in the American Cancer Society study (ACS) (Pope et al. 2002; Turner et al. 2011).

Although compelling evidence supports the harmful effects of $PM_{2.5}$ on longevity, concerns have been raised regarding the sensitivity of the results to model specifications. In particular, Moolgavkar (2005, 2007) suggested that covariates may not be proportional and hence were not controlled for properly in proportional hazards models; that the concentration–response relation may not be linear; and that there are few observations at levels as low as or below the current World Health Organization and U.S. Environmental Protection Agency (EPA) air quality standards. In addition, the relative toxicity of particle elements is still controversial, and most of the recent reduction in $PM_{2.5}$ concentrations in the United States has come from sulfate control. Hence it is of interest whether the concentration–response curve has changed over time as particle composition has changed. Health impact assessments in the United States assume that health benefits of reducing particles are only fully realized after 20 years (U.S. EPA 2010), so examination of the lag between exposure and mortality is also relevant for consideration of changes in the standard.

Our goal was to test the robustness of the association between chronic exposure to $PM_{2.5}$ and mortality observed in the original study (Dockery et al. 1993), and the first extended follow-up of the Harvard Six Cities study (Laden et al. 2006) by replicating the analyses using 11 additional years of follow-up with exposures well below the U.S. annual standard (15 μg/m^3) (U.S. EPA 1997). We examined different lags of exposure, tested the shape of the $PM_{2.5}$ concentration–mortality relationship, tested for changes in this slope over time, and relaxed the proportion assumption by allowing the effects of covariates to vary each year. We reexamined the association of $PM_{2.5}$ with specific causes of death such as lung cancer and examined the effects of $PM_{2.5}$ depending on participants' chronic conditions and smoking status.

Methods

Study population. The Harvard Six Cities study population has been previously described (Dockery et al. 1993). Briefly, adults were randomly sampled from six cities in the eastern and midwestern United States between 1974 and 1977: in 1974, Watertown, Massachusetts; in 1975, Kingston and Harriman, Tennessee, and specific census tracts of St. Louis, Missouri; in 1976, Steubenville, Ohio, and Portage, Wyocena, and Pardeeville, Wisconsin; and in 1977, Topeka, Kansas. Information on age, sex, weight, height, educational level, smoking history, hypertension, and diabetes was collected by questionnaire at enrollment. All participants underwent spirometry tests at enrollment (Dockery et al. 1985) and chronic obstructive pulmonary disease (COPD) was defined as having

$$(FEV_1 \div FVC) < 70\%,$$

where FEV_1 is forced expiratory volume in 1 sec, and FVC is forced vital capacity. This analysis, as in the previous analyses, was restricted to 8,096 white participants with acceptable pulmonary function measurements. The study was approved by the Harvard School of Public Health Human Subjects Committee and all participants signed an informed consent before participation.

Mortality follow-up. Vital status and cause of death were determined by searching the National Death Index (NDI) for calendar years 1979–2009. Deaths before the NDI started in 1979 were identified by next of kin and Social Security records, and the cause of death was determined by a certified nosologist

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Nitrogen Dioxide and Ultrafine Particles Dominate the Biological Effects of Inhaled Diesel Exhaust Treated by a Catalyzed Diesel Particulate Filter

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Toxicological Sciences

Abstract:

We studied the impact of a catalyzed diesel particulate filter (DPF) on the toxicity of diesel exhaust. Rats inhaled exhaust from a Cummins ISM heavy-duty diesel engine, with and without DPF after-treatment, or HEPA-filtered air for 4h, on one day (single exposure) and 3 days (repeated exposures). Biological effects were assessed after 2h (single exposure) and 20h (single and repeated exposures) recovery in clean air. Concentrations of pollutants were: 1) untreated exhaust (-DPF), NO, 43ppm; NO_2, 4ppm; CO, 6ppm; hydrocarbons, 11ppm; particles, $3.2x10^5/cm^3$, 60-70nm mode, $269\mu g/m^3$; 2) treated exhaust (+DPF), NO, 20ppm; NO_2, 16ppm; CO, 1ppm; hydrocarbons, 3ppm; and particles, $4.4x10^5/cm^3$, 7-8nm mode, $2\mu g/m^3$. Single exposures to diesel exhaust (-DPF) resulted in increased neutrophils, total protein and the cytokines, GRO/KC, MIP1-α, and MCP-1 in lung lavage fluid, as well as increased gene expression of IL-6, PTGS2, MT2A,TNF-α, iNOS, GSTA1, HO-1, SOD2, ET-1, and ECE-1, in the lung, and ET-1 in the heart. Ratio of bigET-1 to ET-1 peptide increased in plasma in conjunction with a decrease in eNOS gene expression in the lungs after exposure to diesel exhaust, suggesting endothelial dysfunction. Rather than reducing toxicity, +DPF exhaust resulted in heightened injury and inflammation, consistent with the 4-fold increase in NO_2 concentration. The ratio of bigET-1 to ET-1 was similarly elevated after -DPF and +DPF exhaust exposures. Endothelial dysfunction thus appeared related to particle number deposited, rather than particle mass or NO_2 concentration. The potential benefits of particulate matter reduction using a catalyzed DPF may be confounded by increase in NO_2 emission and release of reactive ultrafine particles.

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• Original Article

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Exposure and Toxicity Assessment of Ultrafine Particles from Nearby Traffic in Urban Air in Seoul, Korea

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Objectives We investigated the particle mass size distribution and chemical properties of air pollution particulate matter (PM) in the urban area and its capacity to induce cytotoxicity in human bronchial epithelial (BEAS-2B) cells.

Methods To characterize the mass size distributions and chemical concentrations associated with urban PM, PM samples were collected by a 10-stage Micro-Orifice Uniform Deposit Impactor close to nearby traffic in an urban area from December 2007 to December 2009. PM samples for in vitro cytotoxicity testing were collected by a mini-volume air sampler with PM_{10} and $PM_{2.5}$ inlets.

Results The PM size distributions were bi-modal, peaking at 0.18 to 0.32 and 1.8 to 3.2 μm. The mass concentrations of the metals in fine particles (0.1 to 1.8 μm) accounted for 45.6 to 80.4% of the mass concentrations of metals in PM10. The mass proportions of fine particles of the pollutants related to traffic emission, lead (80.4%), cadmium (69.0%), and chromium (63.8%) were higher than those of other metals. Iron was the dominant transition metal in the particles, accounting for 64.3% of the PM_{10} mass in all the samples. We observed PM concentration-dependent cytotoxic effects on BEAS-2B cells.

Conclusions We found that exposure to $PM_{2.5}$ and PM_{10} from a nearby traffic area induced significant increases in protein expression of inflammatory cytokines (IL-6 and IL-8). The cell death rate and release of cytokines in response to the $PM_{2.5}$ treatment were higher than those with PM_{10}. The combined results support the hypothesis that ultrafine particles from vehicular sources can induce inflammatory responses related to environmental respiratory injury.

Keywords Cell toxicity, Transition metals, Ultrafine particles, Vehicle source

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Introduction

Environmental particulate air pollution is measured by a global sampling convention called particulate matter 10 (PM_{10}) that measures the mass of particles collected with a 50% efficiency for particles with an aerodynamic diameter of 10 μm. Attention has focused on PM_{10} in cities because that is where most deaths occur, where pollution is routinely monitored and hence any associations are best seen. Typical urban PM_{10} is comprised of up to 50% by mass of combustion-derived, ultrafine carbon-centered particles with associated metals including transition metals. Other major components include ammonium salts of nitrogen, sulfur and chlorine plus geological dust and organic matter [1].

During the last decade or so, there has been growing concern about the influence of particulate air pollution on human health

Professional drivers and lung cancer: a systematic review and meta-analysis

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► Additional materials are published online only. To view these files please visit the journal online (http://dx.doi.org/10.1136/oemed-2012-100666).

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ABSTRACT

The objective of this study was to conduct a systematic review to provide summarised evidence on the association between professional drivers and lung cancer in the last decade after taking into consideration the potential confounding effect of cigarette smoking. We systematically searched all published cohort and case–control studies in English from Medline and Embase, from January 1996 to January 2011. We extracted the risk estimate (ie, RR, OR, and standardised mortality ratio) from each individual study, and meta-analysis was used to combine the RR of individual studies. The methodological quality of each study was assessed using a standard approach proposed by Downs and Black. Nineteen studies were included in the meta-analysis. A significantly increased risk of lung cancer (pooled smoking-adjusted RR 1.18, 95% CI 1.05 to 1.33) among professional drivers was observed after combining four cohort studies and nine case–control studies. A higher pooled RR was observed among smoking-adjusted studies reporting 10 years or more of employment (RR 1.19, 95% CI 1.06 to 1.34) as compared with the study having a shorter duration of employment (6 years) (RR 1.00, 95% CI 0.92 to 1.09). This systematic review revealed that a 18% excess risk of lung cancer was linked to professional drivers who are potentially exposed to diesel exhaust, after adjustment for the confounding effect of smoking. There is a tendency for a positive lung cancer gradient with increasing years of employment as a professional driver.

INTRODUCTION

In both developed and developing countries, many vehicles, including trucks, buses and taxis, are powered by diesel engines because diesel is more adaptable and cheaper than petrol.[1] Professional drivers are therefore exposed to a large volume of diesel exhaust, which is generally accepted as hazardous to human health.[2] In 2008, the working group of the International Agency for Research on Cancer declared diesel exhaust to be a group 2A carcinogen because of the limited evidence of carcinogenicity in humans.[3] Diesel exhaust is a complex mixture of chemical gaseous compounds and particulates containing carbon monoxide, carbon dioxide, nitrogen oxides, sulphur oxides, polycyclic aromatic hydrocarbons, and other toxic substances, many of which are confirmed or suspected human lung carcinogens.[2] Several case–control and cohort studies have revealed a positive association between occupational exposure to diesel exhaust and the risk of lung cancer,[4 5] but the risk estimates of many individual studies were not statistically significant due to limited power. Two meta-analyses were published 10 years ago in which pooled smoking-adjusted risk estimates of 1.35 (95% CI 1.20 to 1.52, 1957–1993)[6] and 1.47 (95% CI 1.29 to 1.67, 1975–1995)[7] were observed, respectively, for the association between diesel exhaust and lung cancer after combining case–control and cohort studies. However, misclassification of diesel exposure in several individual studies (especially case–control studies) could not be ruled out when occupations were converted into specific exposure to diesel exhaust (eg, via a job exposure matrix) because of a lack of direct measurement of the exposure. In addition, some individual studies were criticised for inadequate control for smoking.[8] Professional driving potentially exposed to diesel exhaust will become a crucial public health issue if a positive association between diesel exhaust and lung cancer is shown, given the large number of professional drivers in the general population, especially in developing countries.[9] This study aimed to conduct a systematic review to provide summarised evidence of the association between professional drivers (potentially exposed to diesel engine exhaust) and lung cancer in the last decade, by including studies published in 1996–2011 and taking into consideration the potential confounding effect of cigarette smoking.

METHOD

Eligibility criteria and search strategy

We performed a systematic literature search in Medline and Embase to identify all published case–control and cohort studies (January 1996 and January 2011) that investigated the association between lung cancer and professional drivers, by using the search strategy described in online supplement 1. We carefully checked the references of each publication and retrieved all relevant studies. If different study updates were published in more than one journal, the latest update was chosen. We included individual papers with a particular type of driver or combined professional drivers. Studies consisting of mixed workers were also included if the proportion of drivers among the subjects was known. This review only included papers in English.

Types of outcome measures

We extracted the number of incident cases of, or deaths from, lung cancer, the effect measures and the 95% CI from each included study. Effect measure included the RR, OR and standardised mortality ratio (SMR) for the association between professional drivers and lung cancer risk. If a study included measures for several different kinds of drivers, the measure for the type most likely exposed to diesel

China Study Shows PM1 Air Pollution Most Harmful – A recent study led by Chinese scientists shows a strong link between smaller air pollution particles and a range of serious health conditions. Scientists said the smaller the airborne particles, the more likely they are to cause illness, suggesting the need for monitoring particulate matter of 1 micron or less in diameter, a category of pollution rarely monitored. In recent years, many locations across China have been blanketed with heavy air pollution, raising public health concerns.

In the study, published in the public health journal *Environmental Health Perspectives*, researchers from the School of Public Health at Fudan University in Shanghai have demonstrated correlations between PM2.5 pollution and the incidence of particular illnesses. Researchers spent about two years collecting data in a medium-sized city in northern China, measuring levels of PM in 23 size categories ranging from 0.25 microns to 10 microns. They then plotted the health conditions of residents in the city against the concentrations of particles of different sizes found in their locations.

Among the key findings was that those areas with larger concentrations of smaller particles showed higher incidences of particular illnesses, such as cardiovascular diseases. The fine particles measuring between 0.25 to 0.5 microns in diameter accounted for about 90% of the total number of particles found in the air during the study. Kan Haidong, a professor at the School of Public Health at Fudan University, said the smaller the particle, the higher the concentration in any given volume of air and so the greater the number of particles coming into contact with tissues inside the body. Kan said the smaller particles can also pass through the blood-air barrier in the lungs, entering the blood as toxins, and causing cardiovascular disease. He also said that smaller particles in the body can harm the regulation of the human nervous system.

More information on this is available at: usa.chinadaily.com.cn/china/2013-10/28/content_17061997.htm.

World Health Organization
REGIONAL OFFICE FOR Europe

Review of evidence on health aspects of air pollution – REVIHAAP Project

Technical Report

This publication arises from the project REVIHAAP and has received funding from the European Union.

The WHO European Centre for Environment and Health, Bonn, WHO Regional Office for Europe, coordinated the development of this publication.

ABSTRACT

This document presents answers to 24 questions relevant to reviewing European policies on air pollution and to addressing health aspects of these policies. The answers were developed by a large group of scientists engaged in the WHO project "Review of evidence on health aspects of air pollution – REVIHAAP". The experts reviewed and discussed the newly accumulated scientific evidence on the adverse effects on health of air pollution, formulating science-based answers to the 24 questions. Extensive rationales for the answers, including the list of key references, are provided. The review concludes that a considerable amount of new scientific information on the adverse effects on health of particulate matter, ozone and nitrogen dioxide, observed at levels commonly present in Europe, has been published in recent years. This new evidence supports the scientific conclusions of the *WHO air quality guidelines*, last updated in 2005, and indicates that the effects in some cases occur at air pollution concentrations lower than those serving to establish these guidelines. It also provides scientific arguments for taking decisive actions to improve air quality and reduce the burden of disease associated with air pollution in Europe.

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Keywords

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IARC SCIENTIFIC PUBLICATION NO. 161: AIR POLLUTION AND CANCER

IARC Scientific Publication No. 161
Air Pollution and Cancer
Editors: Kurt Straif, Aaron Cohen, and Jonathan Samet

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ABOUT THIS BOOK

Emissions from motor vehicles, industrial processes, power generation, the household combustion of solid fuel, and other sources pollute the ambient air across the globe. The precise chemical and physical features of ambient air pollution, which comprises a myriad of individual chemical constituents, vary around the world due to differences in the sources of pollution, climate, and meteorology, but the mixtures of ambient air pollution invariably contain specific chemicals known to be carcinogenic to humans.

Recent estimates suggest that the disease burden due to air pollution is substantial. Exposure to ambient fine particles was recently estimated to have contributed 3.2 million premature deaths worldwide in 2010, due largely to cardiovascular disease, and 223 000 deaths from lung cancer. More than half of the lung cancer deaths attributable to ambient fine particles were projected to have been in China and other East Asian countries.

The IARC Monographs Programme convened a multidisciplinary Advisory Group that included epidemiologists, toxicologists, atmospheric scientists, cancer biologists, and regulators to make recommendations for the development of a series of Monographs on air pollution. This book provides the updated state-of-the-art overviews from this Advisory Group on topics related to exposure characterization, atmospheric and engineering sciences, epidemiological studies on cancer, results of pertinent cancer bioassays, and data elucidating potential mechanisms of carcinogenicity of compounds related to air pollution.

International Agency for Research on Cancer

World Health Organization

PRESS RELEASE
N° 221

17 October 2013

IARC: Outdoor air pollution a leading environmental cause of cancer deaths

Lyon/Geneva, 17 October 2013 – The specialized cancer agency of the World Health Organization, the International Agency for Research on Cancer (IARC), announced today that it has classified outdoor air pollution as *carcinogenic to humans* (Group 1). [1]

After thoroughly reviewing the latest available scientific literature, the world's leading experts convened by the IARC Monographs Programme concluded that there is *sufficient evidence* that exposure to outdoor air pollution causes lung cancer (Group 1). They also noted a positive association with an increased risk of bladder cancer.

Particulate matter, a major component of outdoor air pollution, was evaluated separately and was also classified as *carcinogenic to humans* (Group 1).

The IARC evaluation showed an increasing risk of lung cancer with increasing levels of exposure to particulate matter and air pollution. Although the composition of air pollution and levels of exposure can vary dramatically between locations, the conclusions of the Working Group apply to all regions of the world.

A major environmental health problem
Air pollution is already known to increase risks for a wide range of diseases, such as respiratory and heart diseases. Studies indicate that in recent years exposure levels have increased significantly in some parts of the world, particularly in rapidly industrializing countries with large populations. The most recent data indicate that in 2010, 223 000 deaths from lung cancer worldwide resulted from air pollution. [2]

The most widespread environmental carcinogen
"The air we breathe has become polluted with a mixture of cancer-causing substances," says Dr Kurt Straif, Head of the IARC Monographs Section. "We now know that outdoor air pollution is not only a major risk to health in general, but also a leading environmental cause of cancer deaths."

The IARC Monographs Programme, dubbed the "encyclopaedia of carcinogens", provides an authoritative source of scientific evidence on cancer-causing substances and exposures. In the past, the Programme evaluated many individual chemicals and specific mixtures that occur in outdoor air pollution. These included diesel engine exhaust, solvents, metals, and dusts. But this is the first time that experts have classified outdoor air pollution as a cause of cancer.

"Our task was to evaluate the air everyone breathes rather than focus on specific air pollutants," explains Dr Dana Loomis, Deputy Head of the Monographs Section. "The results from the reviewed studies point in the same direction: the risk of developing lung cancer is significantly increased in people exposed to air pollution."

IARC Monographs evaluations
Volume 109 of the IARC Monographs is based on the independent review of more than 1000 scientific papers from studies on five continents. The reviewed studies analyse the carcinogenicity of various pollutants present in outdoor air pollution, especially particulate matter and transportation-related pollution. The evaluation is driven by findings from large epidemiologic studies that included millions of people living in Europe, North and South America, and Asia.

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[1] Please note that the summary evaluation will be published by The Lancet Oncology online on Thursday 24 October 2013
[2] http://www.iarc.fr/en/publications/books/sp161/index.php

IARC: Outdoor air pollution a leading environmental cause of cancer deaths

The predominant sources of outdoor air pollution are transportation, stationary power generation, industrial and agricultural emissions, and residential heating and cooking. Some air pollutants have natural sources, as well.

"Classifying outdoor air pollution as carcinogenic to humans is an important step," stresses IARC Director Dr Christopher Wild. "There are effective ways to reduce air pollution and, given the scale of the exposure affecting people worldwide, this report should send a strong signal to the international community to take action without further delay."

For more information, please contact

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or Dr Nicolas Gaudin, IARC Communications

The International Agency for Research on Cancer (IARC) is part of the World Health Organization. Its mission is to coordinate and conduct research on the causes of human cancer, the mechanisms of carcinogenesis, and to develop scientific strategies for cancer control. The Agency is involved in both epidemiological and laboratory research and disseminates scientific information through publications, meetings, courses, and fellowships. If you wish your name to be removed from our press release e-mailing list, please write to com@iarc.fr.

Wednesday, June 26th, 2013

Session 6b: Health Effects **08.00 –09.30** Chair: P. Gehr
Gehr P. / University of Bern, Switzerland *Introduction*
Müller L. / University of Bern, Switzerland *Diesel Exhaust Particles Modify Natural Killer Cell Function and Cytokine Release*
Vojtisek-Lom M. / University of Liberec, Czech Republik *PM and PAH-Emissions of Trucks under Severe Congestion Conditions: Part 1*
Topinka J. / IEM Prague, Czech Republik *Relative Toxicity of Particulate Matter Organic Extracts under Severe Congest.Cond.: Part 2*
Pinkerton K. / CHE UC Davis, USA *Cardiopulmonary Toxicity of Urban Source-Oriented Ultrafine and Submicron Fine PM*

***Poster Award* Ceremony** **09.30**

COFFEE BREAK
POSTER SESSION and POSTER VOTING **15.40 – 16 .40**

Session 6a: Health Effects **16.40 – 18.30** Chair: B. Rothen-Rutishauser
Rothen-Rutishauser B./ University of Fribourg, Switzerland *Introduction*
Tsai M. / Swiss TPH. Basel, Switzerland *Bridging the Gap: Bringing Measurements Closer to Health Effects*
Ducret-Stich R. / Swiss TPH, Basel, Switzerland *Modeling of Short-Term Ultrafine Particle Number Concentrations in a Swiss City*
Moshammer H. / Medical University of Vienna, Austria *How Health-Predictive are Routine Air Monitoring Data?*
Gerlofs-Nijland M.E. / RIVM Bilthoven, The Netherlands *Influence of NO_2 on Pulmonary Toxicity in Mice Exposed to Diluted Diesel Engine Exhaust*
Paulson, S. / University of California, L.A. *Factors controlling Spatial Variations of Combustion related Pollutants in Urban Areas*

DINNER PARTY invited by Sponsors **19.00** ***Dinner Speaker:*** **Claus-Detlef Schegk**

FOCUS-Event

How to Regulate Solid Nanoparticles in Ambient Air

Presentations	**14.00 – 15.30**
Chair: P. Gehr	
Gehr P. / University of Bern, Switzerland *Welcome Address*	
Künzli N. / Swiss Tropical and Public Health Institute, Basel, Switzerland *Introduction: Setting the Stage - Linking Policy with Science*	
Dockery D. / Harvard School of Public Health, Boston, USA *Lessons from Epidemiologic Studies of Ambient Fine and Ultrafine Particles*	
Frey U. / Childrens Hospital, University of Basel, Switzerland *Effect of Air Pollution on Newborns*	
Hüglin Ch./ EMPA, Dübendorf, Switzerland *Real Time Mapping of Air Quality in Cities for Improved Exposure Estimation*	
Baldauf R./ US Environmental Protection Agency, Durham NC, USA *Ambient Air Mitigation Strategies for Red. Expos. to Mobile Source Nanoparticle Emissions*	

COFFEE BREAK **15.30 – 16.00**

Pannel Discussion	**16.00 – 17.00**
Chair: P. Gehr	
Panellists: N. Künzli / Swiss TPH, Basel, Switzerland D. Dockery / Harvard-University, Boston / University U. Frey / Childrens Hospital University of Basel, Switzerland Ch. Hüglin / EMPA, Switzerland R. Baldauf / US-EPA , USA T. Reichert / EFCA, The Netherlands K. Pinkerton / University of California / USA M. Berger / University of Amsterdam / The Netherlands	

Concluding Remarks: K.Boulouchos

End of the 17th ETH-NPC ***17.00***

European Research on Environment & Health Funded by the FP7 – projects about the NP-exposure and health effects

Report FP 7

http://ec.europa.eu/research/environment/pdf/fp7_catalogue_eh.pdf#view=fit&pagemode=none

***G. Nanoparticles: Exposure and health impacts* 215**