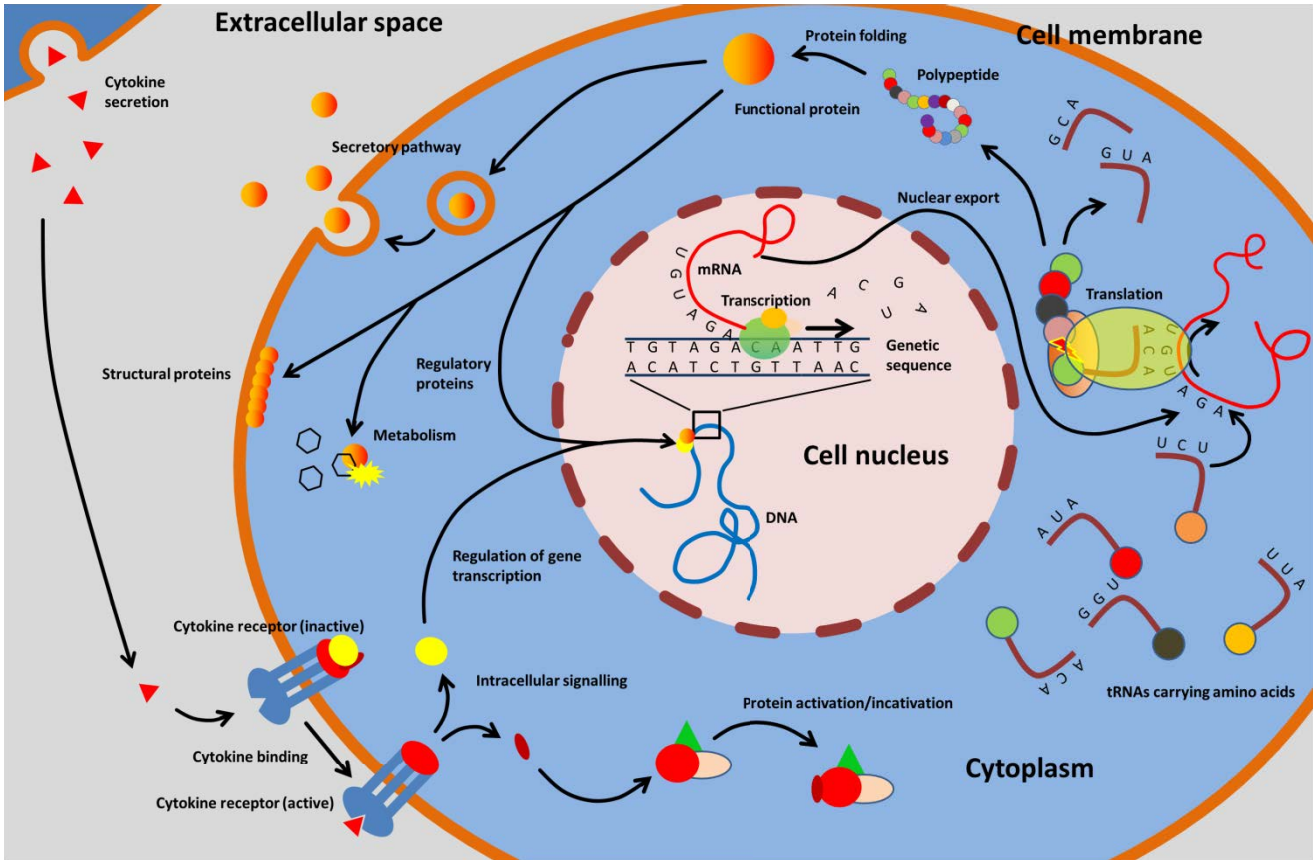
Biological background and term definitions

Cellular signaling, gene transcription and protein translation

A simplified scheme of cellular signalling as it occurs for example in course of pro-inflammatory stimulation: A cell (top left) secretes signal molecules, for example pro-inflammatory cytokines (red triangles) in response to certain stimulus. The cytokines diffuse through the surroundings and eventually bind to specific cytokine receptors on the surface of other cells. Cytokine binding activates the receptor which triggers an intracellular signal cascade, for example by stimulating the release of proteins from the intracellular domain of the receptor. Trancription factors (yellow sphere) are intracellular signal proteins that upon activation enter the cell nucleus where they bind to the genetic material. Binding of transcriptin factors regulates the expression of the genes they bind to, for example by activating their transcription. During the process of transcription, the genetic sequence stored in form of double stranded DNA is used by protein complexes as a template for the production of single stranded mRNAs (red string). mRNAs are exported to the cytosol, where they serve as templates for the synthesis of proteins, called translation. During translation, an mRNA sequence is translated into a sequence of amino acids, the building blocks of proteins. Amino acids are provided bound to transfer RNAs (tRNAs). tRNAs are identified by a codon, a sequence of three units, that identifies a given tRNA as the carrier of specific amino acid. tRNAs and mRNA bind to the protein synthesis machinery, where pairing of the codon on tRNAs with three units of the mRNA sequence assures that the correct tRNA has bound and that therefore the correct sequence of amino acids is built. The result of translation is a polypeptide, a chain of amino acids with a specific sequence. The functional proteins are formed by folding the polypeptides into three dimensional structures (orange sphere). The functional proteins are the molecular machines whose activity/function depends on their amino acid sequence. Among other things, proteins may enter the secretory pathway and serve for example as signal molecules like cytokines, they may have structural functions (building block of the cell), they may have metabolic activity and they may have regulatory functions, as shown here for example they may be transcription factors that regulate their own production.



AhR
The aryl hydrocarbon receptor is an intracellular protein that binds various xenobiotic compounds. Upon binding, the AhR translocates into the nucleus where it acts as a transcription factor (a protein that regulates gene transcription). The AhR controls the transcriptional activity of wide variety of genes whose gene products (the proteins they encode) are among other things involved in immunomodulation, anti-oxidant responses and the detoxification of xenobiotics.
The activation of AhR signaling indicates the presence of bioactive xenobiotics, (as for instance many exhaust components), that may affect a cell adversely and therefore trigger a battery of defence mechanisms. It is an early event in response to various xenobiotics and the final outcome of its activity depends on the environmental/biological context.

Ames-test
The Ames test is a standard approach for testing chemical and physical stimuli for their potential to damage DNA and hence act genotoxic. It relies on bacteria in which the his-operon, a group of genes needed for the biosynthesis of the amino acid histidine, was rendered nonfunctional by the insertion of point mutations. Since histidine is vital, his^- bacteria rely on the presence of histidine in the culture medium. When cultures of his^- bacteria are treated with a mutagen, mutations in the bacterial genome may occur, some of which may re-establish the functionality of the his-operon. The resulting revertant (his^+) bacteria are able to synthesize histidine, hence they will grow in histidine free culture medium. Culturing exposed and non-exposed bacteria separately in histidine free medium and comparing their growth rate yields a direct measure for the genotoxicity of the test-substance.
The Ames-test is a highly sensitive, robust and efficient tool for genotoxicity assessment and it is highly accepted among toxicologists. It is important to note however, that since the test relies on bacteria, which because of various reasons may not be equally susceptible to a given genotoxin as human cells, their relevance for human toxicology may be questioned. The decision to still use this test in the present study was based on the lack of alternative (human cell based) approaches that, given the limited availability of bio-lab equipment at the exhaust gas control station, would have been suitable.

Apoptosis
A highly regulated process during which cells eliminate themselves from the surrounding tissue. Apoptosis occurs in response to sever stress as for example irreparable damages to the cellular genetic material, but also during normal development when cells that are not needed any more have to be eliminated. Pro-apoptotic stimulation can be detected on the gene expression level, on the protein level and morphologically by the presence of condensed cell nuclei and condensed.

CYP1A1
Is the gene encoding for Cytochrome P450 1A1, a protein that is involved in the detoxification of xenobiotic compounds. The protein oxidizes non-polar molecules in order to render them more polar, hence more water soluble and thereby easier to handle for the cell. *CYP1A1* transcription is activated by the aryl-hydrocarbon receptor (AhR).

Cytokine
Cytokines are signal molecules (proteins), *i.e.* they serve as means of communication between cells. They are secreted by certain cells in response to specific stimuli and by binding to specific receptors on the surface of another cell they convey a defined signal that triggers according responses.

Dendritic cells
Immune cells that engulf, process and present foreign material such as for example bacteria. By presenting the processed material to other immune cells, they trigger responses of the adaptive immune system. Dendritic cells are present in high numbers beneath the epithelium of the respiratory tract.

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 highly 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.

GAPDH
Is the gene encoding for glyceraldehyde 3-phosphate dehydrogenase, a protein involved in sugar metabolisms. Because of its continuous and high expression levels in all cell types it is frequently used as an internal standard for semi-quantitative measurement of gene expression.

Gene
The genome of living organisms stores the information that is necessary for the production of proteins – the molecular machines performing (almost) all biochemical, regulatory and structural functions - in form of DNA. For each protein an organism is able to synthesize, there is a gene in its genome, a string of DNA (usually several thousands of base-pairs long), that encodes for the protein. Proteins consist of amino acids linked to a string in a highly specific sequence and the sequence in which the building blocks of DNA (the four nucleotides A, T, G and C) are arranged in a gene can be translated into the sequence of amino acids in a protein.

Genotoxicity
The property of a chemical or physical stimulus to damage a cell’s genetic material (DNA). Damages in a cell’s DNA can result in the introduction of mutations in the genetic sequence, with the possible result of cancer formation.

Glutathione
Glutathione (GSH) is an anti-oxidative molecule that is continuously produced by any cell. It serves as scavenger for reactive oxygen species, which are present in the environment and are continuously formed by the cellular metabolism. ROS may oxidize any cellular components such as DNA, proteins and lipids, thereby rendering them non-functional. Anti-oxidative molecules such as GSH provide a substrate for ROS and thereby protect cells from oxidative damage. Under circumstance of severe oxidative stress, a cell is not able to replenish its GSH pool, GSH quantification of GSH therefore gives an estimate on the level of oxidative stress a cell suffers from.

GSR
Is the gene encoding for the protein Glutathione reductase, a protein needed for the restoration of the reduced form of glutathione GSH.

HMOX1
Is the gene that encodes for heme-oxygenase 1, 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.

IDO-1
Is the gene encoding for the protein Indoleamine 2,3-dioxygenase 1, which catabolizes the amino acid tryptophan. Its biological functions are so far poorly described, but it has been shown that it modulates the properties of immune cells.

IL8
Is the gene encoding for the cytokine interleukine-8 (IL-8).

LDH
Lactate dehydrogenase (LDH) is a soluble protein that is frequently used as a marker for cytotoxicity. This use does not originate from its molecular function, which has no connection to cytotoxicity (it is involved in sugar metabolism), but relies on its high abundance in all cell types and its enzymatic activity that allows detecting it easily. Under normal circumstances, LDH is found exclusively inside a cell and its presence in the surroundings indicates leaky cell membranes. Membrane disintegration is a key marker of severe physical and/or chemical distortions of a cell.

Macrophages
Are immune cells, primarily responsible for engulfing and digesting foreign substances (e.g. bacteria, particles), and cellular debris. Upon discovering foreign material, they are involved in the onset of inflammatory responses by secreting pro-inflammatory cytokines. Macrophages are present in high numbers on the epithelium of the respiratory tract.

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-specific sequences. The blueprint of these sequences is stored in the cellular genome in form of DNA (deoxyribonucleic acid), which basically consists of a long sequence four different molecules (nucleotides).
For each protein an organism is able to produce, there is a gene present in the genome providing 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. In order to do so, in a process called gene transcription, cells produce copies of the genetic sequence in form of ribonucleic acid (RNA). These copies (called messenger RNAs, mRNAs) are then used as template for protein synthesis, a process called translation, in which three building blocks of an mRNA are translated into one building block (an amino acid) of a protein.

NFE2L2
Is the gene encoding for the transcription factor NFE2-related factor 2, a protein that (upon activation in the cytosol) binds to DNA and activates the transcription of a variety of genes. NFE2-related factor 2 signaling affects various processes, including the responses to oxidative stress, pro-inflammation, proteome maintenance and xenobiotic detoxification.

NQO1
Is the gene encoding for NAD(P)H dehydrogenase [quinone] 1, a protein that is involved in the detoxification of xenobiotics. The protein acts by conjugating molecular moieties to oxidized xenobiotics, which renders them more polar and easier to handle for the cell. It also has anti-oxidative functions. *NQO1* transcription is activated by the aryl-hydrocarbon receptor (AhR).

Nucleus
The structure in a cell where the genetic material (DNA) is stored.

Oxidative stress
An imbalance in the redox-equilibrium (the ratio of oxidized to reduced molecular moieties) in a cell, more precisely the presence of too high amounts of oxidizing species such as ROS. Oxidative stress is usually directly or indirectly induced by environmental conditions.

Pro-inflammation
Inflammation is a part of complex response of an organism to defend itself from harmful stimuli such as for instance pathogens. Pro-inflammation is the state of induction of inflammation and is characterized for example by the presence of pro-inflammatory cytokines.

Real-time RT-PCR
Reverse-transcriptase polymerase chain-reaction is a method for the analysis of gene expression, the process in which information encoded on genes is used to produce proteins. Real-time RT-PCR measures the abundance of specific mRNAs, intermediate molecules that provide the building plan of a protein. Information on gene/protein function (e.g. used against oxidative stress) and information about gene activity indicates cellular responses to certain stimuli.

SOD2
Is the gene encoding for superoxide dismutase 2, 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.

TNF
Is the gene encoding for the cytokine tumor necrosis factor alpha (TNFα).

UGT1A6
Is the gene encoding for the protein UDP-glucuronosyltransferase 1-6 that is involved in the detoxification of xenobiotics. The protein acts by conjugating molecular moieties to oxidized xenobiotics, rendering them more polar and easier to handle for the cell. *UGT1A6* transcription is activated by the aryl-hydrocarbon receptor (AhR).

Xenobiotic
Chemical compound that in its structure/composition cannot or only in very low amounts be found in living organisms (or ecosystems) under normal circumstances.

Programme PEAR : Résumé synthétique :

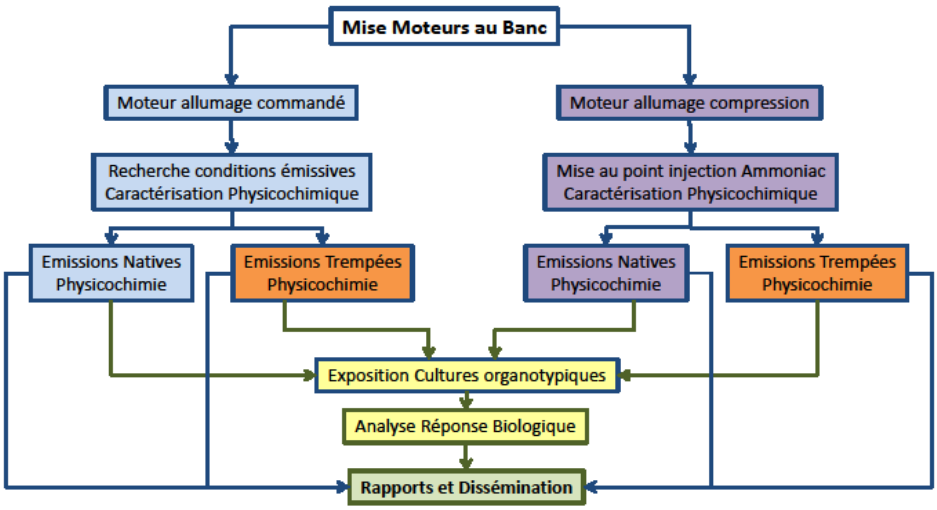
Dans ce programme nous avons étudié principalement les polluants dérivés de l'azote générés au cours des processus de combustion et de dépollution mis au service de la production d'énergie mécanique dans le domaine des transports. Parallèlement aux oxydes d'azote qui ont été très étudiés au cours de ces dernières années, deux polluants le NH_3 et le nitrate d'ammonium émergent avec la mise en œuvre de nouveaux systèmes catalytiques, que ce soit sur les moteurs à allumage commandé comme sur les moteurs à allumage par compression qui sont l'objet des taches suivantes.

Moteur à allumage commandé : Caractérisation des niveaux de NH_3 derrière la catalyse trois voies en fonction des conditions d'exploitation d'un moteur à allumage commandé au banc

Moteur à allumage par compression : Modélisation de dysfonctionnement SCR (Catalyse sélective à l'Urée) sur moteur diesel avec filtre à particules au banc par dopage à l'ammoniac

Simulation du phénomène de trempe à l'échappement pour caractériser les teneurs en nitrate d'ammonium produit dans un échappement Diesel

Métrologie toxicologique avant et après trempe des émissions de moteurs à essence et diesel



Organigramme Technique Projet PEAR

Les codes couleurs utilisés dans cet organigramme se réfèrent à ceux utilisés dans le diagramme de Gantt couleurs encadrement pour le partenaire P1 et P2 les couleurs de remplissage se rapportant aux tâches

En conclusion de ce travail, il apparait clairement que les émissions de NH_3 sont susceptibles de se produire à l'échappement de véhicules équipés de moteurs thermiques. Nous avons démontré que les conditions de richesse de combustion sur les moteurs à allumage commandé sont un facteur important déterminant les émissions de NH_3 par les moteurs à allumage commandé. Ces émissions peuvent atteindre des concentrations de plusieurs centaines de ppm de NH_3 sur des transitoires de richesse en cycle mais aussi en conditions stabilisées pour de très faibles élévations de moins de 0.01% par rapport à la stœchiométrie (richesse 1). Ce polluant qui n'est actuellement pas réglementé pour les émissions automobile est considéré actuellement comme ayant une origine essentiellement agricole, mais risque de devenir un polluant à surveiller car potentiellement en émergence en proximité du trafic automobile.

Nous savons par ailleurs que des émissions de NH_3 sont susceptibles de se produire lors d'un dysfonctionnement de la catalyse sélective à l'urée, pour des conditions d'injection excessive d'urée, ou lors d'une inhomogénéité d'arrosage du pain de catalyseur par l'urée injectée. Dans ces conditions, il y a un risque d'émission conjointe de NH_3 et d'oxydes d'azote dont le NO_2. Nous avons clairement montré à l'occasion de ce travail que le NH_3 était capable de réagir avec le NO_2 pour former un aérosol de nitrate d'ammonium.

La réactivité du NH_3, qu'il soit émis par les moteurs à allumage commandé ou par les moteurs diesel équipés d'un dispositif de SCR, avec le NO_2 conduit potentiellement à la formation d'un aérosol secondaire de nitrate d'ammonium qui dans certaines conditions d'hygrométrie (hygrométrie relative élevée) et de température (températures inférieures à 10-15°C) et de pressions partielles est susceptible de contribuer aux PM_{10} et $PM_{2.5}$ mesurées par TEOM/FDMS dans l'atmosphère. L'émergence d'émissions de NH_3 par le trafic automobile risque donc de contribuer significativement aux particules atmosphériques en proximité du trafic.

En matière de réponse toxicologique, le nitrate d'ammonium a été étudié en milieu professionnel et ne semble pas induire de pathologie spécifique. Cependant, par inhalation, le nitrate d'ammonium peut être un irritant des voies respiratoires. Le nitrate d'ammonium ne fait pas l'objet de VME ni de VLCT (source INRS).

Dans les conditions expérimentales que nous avons testées dans ce programme, Compte tenu des concentrations modérées (30 et 150 ppm en gaz bruts et au maximum une dilution de 10%, les concentrations maximales d'exposition ont été de 15 ppm de NH_3, la VLCT du NH_3 étant de 100 ppm de NH_3 pour 15 minutes chez l'homme (source INRS).

Nous avons cependant observé un faible impact du NH_3 sur la viabilité cellulaire, que le NH_3 soit utilisé en gaz pur, ou en mélange avec des émissions de moteurs à combustion.

Le refroidissement rapide des émissions de moteur diesel lors du passage à travers l'échangeur thermique a permis une diminution de l'ordre de 150°C des émissions. Cette condition n'a montré que des effets d'ampleurs marginales. Il faut cependant noter que ce dispositif n'a pas permis d'atteindre la température ambiante température à laquelle la formation de particules de nitrate d'ammonium aurait été plus favorisée.

Recommandations

Les résultats de ce travail, montrant l'émergence du NH_3 et du nitrate d'ammonium comme polluants primaires du trafic automobile, doivent servir de point de départ à l'élaboration d'une stratégie de surveillance et de suivi des concentrations atmosphériques de NH_3 d'une part et de caractérisation de l'éventuelle formation de nitrate d'ammonium en proximité du trafic automobile par rapport au fond urbain, cela même en dehors des évènements majeurs de pics de pollution printaniers au cours desquels de fortes quantités de nitrate d'ammonium d'origine agricole sont importés.

La question de l'impact sanitaire du nitrate d'ammonium devrait faire l'objet de travaux approfondis. En effet, sa contribution potentiellement importante lors d'évènements de pollution particulaire et les faibles impacts toxicologiques connus pour cette substance doit faire poser la question de sa prise en compte dans la métrologie des PM_{10} et $PM_{2.5}$ pour les seuils d'information et d'alerte de la population. En effet, le nitrate d'ammonium n'était pas pris en compte par la métrologie TEOM avant 2009, ni par la mesure des fumées noires antérieures au TEOM, métrologie sur laquelle reposent essentiellement les travaux épidémiologiques sur l'impact sanitaire des particules. Il faut aussi rappeler que les seuils de concentrations particulaires (PM_{10} et $PM_{2.5}$) préconisés par l'OMS et la communauté européenne n'ont pas été revus en 2009 avec l'introduction de la métrologie par TEOM-FDMS qui inclut la fraction semi-volatile pour laquelle le nitrate d'ammonium représente un fort contributeur. Ces seuils ont de plus été sévérisés en 2011 sans prise en considération de ces évolutions métrologiques, ce qui pose une réelle question de pertinence en matière sanitaire de la non continuité de la métrique PM_{10} et $PM_{2.5}$.

Summary of the PHD-Thesis:

Diesel Engine Emissions and their Toxicity in Dependency of Engine Equipment

Sandro Steiner
Adolphe Merkle Institute
University of Fribourg

Abstract

In order to reduce diesel engine emissions and to increase engine efficiency, new exhaust after-treatment systems, fuels, fuel additives, lubrication oils and oil additives are continuously developed, which may result in profound changes in the final exhaust composition and hence exhaust toxicity. This bears the risk of implementing new technologies which, whilst reducing the overall emissions of diesel engines, increase their toxicity. In order to prevent this, toxicological testing of exhaust toxicity needs to be performed prior to putting new technologies on the market, which requires efficient and reliable toxicological screening tools.
In the research project EngToxDi, a novel approach for exhaust toxicity assessment was used for testing diesel engine emissions for their toxicity *in vitro*. The aims of the project were i) to gain insight into how the toxicity of diesel engine emissions is influenced by a selection of settings at the engine such as exhaust after-treatment systems, different fuel types, different lubrication oils and fuel additives and ii) to generate an extended data package that allows estimating the efficiency, reproducibility, sensitivity and specificity of the experimental approach.
The experimental approach was to compare the toxicity measured for exhaust produced under defined reference engine settings to the toxicity measured for exhaust that was generated by the same engine, but under different settings, *e.g.* by installing a diesel particle filter or by fuelling the engine with biodiesel.
The project focused on the toxicity of emissions produced in urban centers. Therefore, a passenger diesel car, representative for a large fraction of the current diesel vehicle fleet in Switzerland, was used as a test-vehicle. As a biological test system, an *in vitro* model of the human epithelial airway barrier was used, based on the fact that the respiratory tract is the main site of interaction between the human body and air pollutants such diesel engine emissions.
The results show that exhaust filtration by a non-catalyzed DPF, the use of particle filter additives, and the use of biodiesel may contribute to the reduction of exhaust toxicity. This does not apply however, if pure biodiesel is used and if the fuel additive is used without particle filter. Lubrication oil additives and NO_2 emissions appeared to have a minor effect on acute exhaust toxicity.

BioToxDi/EngToxDi was a collaboration between the Bern University of Applied Sciences (AFHB), the Adolphe Merkle Institute at the University of Fribourg (AMI), the Paul Scherrer Institute (PSI) Villigen and the Swiss Federal Laboratories for Materials Science and Technology (EMPA) in Dübendorf. As major

partners, AFHB provided technical know-how as well as the test-vehicle, the exposure system and the location for the exposure experiments and AMI provided the biological and toxicological know-how and the necessary biological laboratories. PSI and EMPA provided knowledge about exhaust chemistry and atmospheric chemistry and performed detailed chemical analyses of collected exhaust fractions. PSI further provided an exhaust aging chamber needed for experiments with aged exhaust samples.

Project outline

As depicted in Table 1, the project was made up of five basic work packages.
In work package 0, biological responses to a defined reference-setting (Ref) were measured in order to obtain a benchmark to which the results of all further tested settings could be compared.
In subsequent work packages (1, 2 and 4) a single parameter of the reference engine setting were changed. According to the original project matrix, this included:

a) The installation of a non-catalyzed diesel particle filter (DPF).
b) The use of alternative fuels. Two setups were tested: 100% rapeseed methyl-ester (RME, B100) and a blend of 20% RME in fossil (Ref) diesel (B20).
c) The reference oil (high SAPS) in the oil circuit was exchanged for low- and zero SAPS lubrication oils (Low SAPS, Zero SAPS).
d) The artificial addition of NO_2 (50ppm) to the exhaust (NO_2).
e) The use of a fuel borne catalyst (FBC). As catalytically active fuel additive Satace®3, developed by Innospec Inc. was used.
f) The aging of the exhaust in a mobile aging chamber (Ageing).

Work package 3 was reserved for repetitions, cross-combinations and new tasks and its contents depended on the outcome of previous experiments. Repetitions of the DPF, B100, B20 and NO_2-exposure experiments were included here. Additionally, two supplementary exposures were performed, in which lubrication oil was added to the fuel (2% v/v), simulating an engine with high oil consumption. An additive-free oil (DEA) and the oil used for the Ref-exposures (high SAPS) were used (DEA-fuel and High SAPS-fuel). For DEA, the oil in the oil circuit was exchanged for DEA oil. In a further set of experiments (not included in work package 3, but performed after FBC), FBC-exposures were repeated with inclusion of the DPF (FBC-DPF).
In parallel to each exposure experiment, diesel exhaust particles were collected on PallFlex filters for later detailed chemical analysis and/or for genotoxicity studies using particle suspensions or particle extracts (results not included in this report).

Table 1: Project outline. List and description of the engine settings tested for their effect on exhaust toxicity compared to the reference-setting (Ref).

Work package	Fuel[1]	Lubrication oil[2]	Exhaust after treatment	Other
0	Ref	Ref	---	---
1	Ref	Ref	**Silicium carbide DPF (non-catalyzed)**	---
	B20	Ref	---	---
	B100	Ref	---	---
	Ref	**low SAPS[3]**	---	---
	Ref	**zero SAPS[3]**	---	---
2	Ref	Ref	---	**50ppm NO_2**
3	**B20/B100**	---	---	---
		---	---	**50ppm NO_2**
	Ref + 2% v/v DEA oil	**DEA oil[4]**	---	---
	Ref + 2% v/v Ref oil	Ref	---	---
4	Ref	Ref	---	**Exhaust ageing**
	Ref + 40ppm Satacen®3[5]	Ref	---	---
	Ref + 40ppm Satacen®3[5]	Ref	**Silicium carbide DPF (non-catalyzed)**	---

1) Throughout the project, standard low sulfur petrodiesel (Greenergy, <10 ppm sulfur, according to the Swiss standard SN-EN 590) from the same stock barrel was used as reference fuel.
2) Reference oil: Motorex, V10.237
3) SAPS: Sulfated ash, phosphorous, sulfur
4) DEA-oil: An additive free test oil, purchased by the Deutsche Erdöl AG
5) Satacen®3: A fuel borne catalyst, developed by Innospec Inc.

Atmospheric Environment 81 (2013) 380–388

Contents lists available at ScienceDirect

Atmospheric Environment

journal homepage: www.elsevier.com/locate/atmosenv

Comparison of the toxicity of diesel exhaust produced by bio- and fossil diesel combustion in human lung cells *in vitro*☆

Sandro Steiner [a, *], Jan Czerwinski [b], Pierre Comte [b], Olga Popovicheva [c], Elena Kireeva [c], Loretta Müller [d], Norbert Heeb [e], Andreas Mayer [f], Alke Fink [a], Barbara Rothen-Rutishauser [a, d]

[a] *Adolphe Merkle Institute, University of Fribourg, Route de l' ancienne Papeterie, 1723 Marly, Switzerland*
[b] *Bern University for Applied Sciences, Switzerland*
[c] *Institute of Nuclear Physics, Moscow State University, Russia*
[d] *University of Bern, Department of Clinical Research, Switzerland*
[e] *EMPA, Swiss Federal Laboratories for Materials Testing and Research, Switzerland*
[f] *TTM, Technik Thermischer Maschinen, Switzerland*

HIGHLIGHTS

- Biodiesel (rapeseed methyl-ester) affects particle emissions by diesel engines.
- The blending ratio (bio-/fossil diesel) influences this effect.
- Quantitative effects on the gas-phase exhaust composition are weak.
- The pro-inflammatory potential of the exhaust is influenced by the changes.
- Strong changes in particle emissions translate into weak changes in exhaust toxicity.

ARTICLE INFO

Article history:
Received 10 June 2013
Received in revised form
26 August 2013
Accepted 28 August 2013

Keywords:
Rapeseed methyl-ester
Biodiesel
Diesel exhaust
Inhalation toxicity
Lung cell cultures

ABSTRACT

Alternative fuels are increasingly combusted in diesel- and gasoline engines and the contribution of such exhausts to the overall air pollution is on the rise. Recent findings on the possible adverse effects of biodiesel exhaust are contradictive, at least partly resulting from the various fuel qualities, engine types and different operation conditions that were tested. However, most of the studies are biased by undesired interactions between the exhaust samples and biological culture media. We here report how complete, freshly produced exhausts from fossil diesel (B0), from a blend of 20% rapeseed-methyl ester (RME) and 80% fossil diesel (B20) and from pure rapeseed methyl ester (B100) affect a complex 3D cellular model of the human airway epithelium *in vitro* by exposing the cells at the air–liquid interface. The induction of pro-apoptotic and necrotic cell death, cellular morphology, oxidative stress, and pro-inflammatory responses were assessed. Compared to B0 exhaust, B20 exhaust decreased oxidative stress and pro-inflammatory responses, whereas B100 exhaust, depending on exposure duration, decreased oxidative stress but increased pro-inflammatory responses. The effects are only very weak and given the compared to fossil diesel higher ecological sustainability of biodiesel, it appears that – at least RME – can be considered a valuable alternative to pure fossil diesel.

1. Introduction

The International Energy Agency reports that in the period from 2005 to 2011, the global consumption of alternative fuels in road transport has doubled from approximately 70–140 million tons of oil equivalents, which corresponds to approximately 8.8% of the total road transport consumption. The dominant alternative fuels are ethanol and natural gas, but with an estimated contribution of 3.5%, biodiesel is important as well (IEA advanced motor fuels, annual report 2011). Biodiesel is defined as the monoalkyl esters of vegetable oils or animal fat (American Society for Testing and Materials (ASTM) Standard D6751) and can be produced from various sources such as algae, used frying oil, soy beans, palm kernels, or rapeseed. The choice of feedstock thereby strongly

* Corresponding author. Tel.: +41 26 300 95 15; fax: +41 26 300 96 24.
E-mail address: sandro.steiner@bfh.ch (S. Steiner).

1352-2310/$ – see front matter © 2013 The Authors. Published by Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.atmosenv.2013.08.059

Environmental Science & Technology

Article

pubs.acs.org/est

Test-Methods on the Test-Bench: A Comparison of Complete Exhaust and Exhaust Particle Extracts for Genotoxicity/Mutagenicity Assessment

Sandro Steiner,[†] Norbert V. Heeb,[‡] Jan Czerwinski,[§] Pierre Comte,[§] Andreas Mayer,[∥] Alke Petri-Fink,[†] and Barbara Rothen-Rutishauser*,[†]

[†]Adolphe Merkle Institute, University of Fribourg, 1700 Fribourg, Switzerland

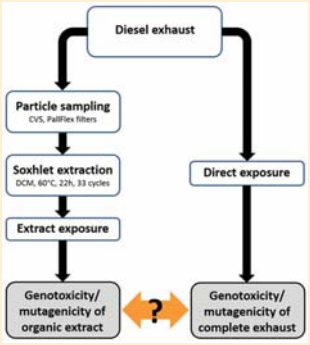
[‡]EMPA, Swiss Federal Laboratories for Materials Testing and Research, 8600 Dubendorf, Switzerland

[§]Bern University of Applied Sciences, 2560 Nidau, Switzerland

[∥]TTM, Technik Thermische Maschinen, 5443 Niederrohrdorf, Switzerland

Supporting Information

ABSTRACT: With the growing number of new exhaust after-treatment systems, fuels and fuel additives for internal combustion engines, efficient and reliable methods for detecting exhaust genotoxicity and mutagenicity are needed to avoid the widespread application of technologies with undesirable effects toward public health. In a commonly used approach, organic extracts of particulates rather than complete exhaust is used for genotoxicity/mutagenicity assessment, which may reduce the reliability of the results. In the present study, we assessed the mutagenicity and the genotoxicity of complete diesel exhaust compared to an organic exhaust particle extract from the same diesel exhaust in a bacterial and a eukaryotic system, that is, a complex human lung cell model. Both, complete exhaust and organic extract were found to act mutagenic/genotoxic, but the amplitudes of the effects differed considerably. Furthermore, our data indicate that the nature of the mutagenicity may not be identical for complete exhaust and particle extracts. Because in addition, differences between the responses of the different biological systems were found, we suggest that a comprehensive assessment of exhaust toxicity is preferably performed with complete exhaust and with biological systems representative for the organisms and organs of interest (i.e., human lungs) and not only with the Ames test.



INTRODUCTION

In June 2012, the International Agency for Research on Cancer (IARC) classified diesel engine exhaust as a group 1 carcinogen to humans (IARC press release 213, June 12th, 2012). The IARC stated that with this new classification, "governments and other decision-makers have a valuable evidence-base on which to consider environmental standards for diesel exhaust emissions and to continue to work with the engine and fuel manufacturers toward those goals". A large number of experimental studies in this field clearly supports the classification of diesel exhaust as carcinogen: Diesel exhaust or parts of diesel exhaust induce DNA damages or act mutagenic in cell cultures,[1] cell-free systems,[2] bacteria,[3−5] or in vivo.[6] But, as the IARC also stated: "it is not yet clear how the quantitative and qualitative changes" in exhaust composition "may translate into altered health effects" and that "research into this direction is needed".

Diesel exhaust is a very complex mixture, consisting of gaseous, condensed (liquid), and solid particulate fractions, all of which contribute to genotoxicity,[7] the potential of a substance to damage genetic material and hence to act mutagenic and cancerogenic (mutagenicity is a possible consequence of genotoxicity and is characterized by the changes in the genetic material being permanent and hereditary). The gas phase consists of inorganic gases, such as carbon monoxide, carbon dioxide, nitrogen oxides, and a variety of volatile and semivolatile organic compounds.

Among those, most notably heterocyclic aromatic compounds (HACs) and polyaromatic hydrocarbons and their nitrated forms (PAHs/NPAHs) may directly interact with DNA by intercalation between the stacked bases, DNA adduct formation or the formation of abasic sites.[8,9] The ultimate result is the loss of bases or the incorporation of additional or wrong bases during DNA replication. Furthermore, reactive oxygen and nitrogen species (ROS/RNS), such as nitric oxide (NO), nitrogen dioxide (NO_2), hydrogen peroxide (H_2O_2), or the hydroxyl radical ($^{\bullet}OH$), which are present in the exhaust or can be formed intracellularly by the chemical activity or metabolism of organic components, may cause DNA strand breaks and the formation of DNA adducts.[10−13]

Received: December 16, 2013
Revised: March 17, 2014
Accepted: April 3, 2014
Published: April 3, 2014

dx.doi.org/10.1021/es4056033 | Environ. Sci. Technol. 2014, 48, 5237−5244

Anal Bioanal Chem
DOI 10.1007/s00216-014-7878-5

RESEARCH PAPER

Effects of an iron-based fuel-borne catalyst and a diesel particle filter on exhaust toxicity in lung cells in vitro

Sandro Steiner · Jan Czerwinski · Pierre Comte · Norbert V. Heeb · Andreas Mayer · Alke Petri-Fink · Barbara Rothen-Rutishauser

Received: 14 January 2014 / Revised: 28 April 2014 / Accepted: 6 May 2014

Abstract Metal-containing fuel additives catalyzing soot combustion in diesel particle filters are used in a widespread manner, and with the growing popularity of diesel vehicles, their application is expected to increase in the near future. Detailed investigation into how such additives affect exhaust toxicity is therefore necessary and has to be performed before epidemiological evidence points towards adverse effects of their application. The present study investigates how the addition of an iron-based fuel additive (Satacen®3, 40 ppm Fe) to low-sulfur diesel affects the in vitro cytotoxic, oxidative, (pro-)inflammatory, and mutagenic activity of the exhaust of a passenger car operated under constant, low-load conditions by exposing a three-dimensional model of the human airway epithelium to complete exhaust at the air–liquid interface. We could show that the use of the iron catalyst without and with filter technology has positive as well as negative effects on exhaust toxicity compared to exhaust with no additives: it decreases the oxidative and, compared to a non-catalyzed diesel particle filter, the mutagenic potential of diesel exhaust, but increases (pro-)inflammatory effects. The presence of a diesel particle filter also influences the impact of Satacen®3 on exhaust toxicity, and the proper choice of the filter type to be used is of importance with regards to exhaust toxicity.

Keywords Exhaust exposures · Iron catalyst · Diesel particle filter · 3D lung cell model · Air–liquid interface

Published in the topical collection *Aerosols and Health* with guest editor Ralf Zimmermann.

Electronic supplementary material The online version of this article (doi:10.1007/s00216-014-7878-5) contains supplementary material, which is available to authorized users.

S. Steiner · A. Petri-Fink · B. Rothen-Rutishauser (✉)
Adolphe Merkle Institute, University of Fribourg, Route de l'Ancienne Papeterie, P.O. Box 209, 1723 Fribourg, Switzerland
e-mail: barbara.rothen@unifr.ch

J. Czerwinski · P. Comte
Laboratory for IC-Engines and Exhaust Gas Control, Bern University of Applied Sciences, Gwerdtstrasse 5, CH-2560 Nidau, Switzerland

N. V. Heeb
EMPA, Swiss Federal Laboratories for Materials Testing and Research, Uberlandstrasse 129, 8600 Dubendorf, Switzerland

A. Mayer
TTM, Technik Thermische Maschinen, Fohrholzlistrasse 14b, 5443 Niederrohrdorf, Switzerland

B. Rothen-Rutishauser
Respiratory Medicine, Bern University Hospital, Bern, Switzerland

Introduction

The popularity of diesel vehicles is continuously growing. For instance, ExxonMobil estimates that between now and 2040, diesel will account for 70 % of the growth in global transportation energy consumption (The outlook for energy: a view to 2040, ExxonMobil 2013). Partly, this trend can be assumed to be due to the high fuel efficiency and the robustness of diesel engines, both resulting in considerable economic advantages over gasoline-fueled engines.

Together with the high fuel efficiency, however, comes the production of large numbers of exhaust particles, mostly consisting of elemental carbon and adsorbed hydrocarbons [1]. Adverse health effects caused by these diesel exhaust particles (DEPs) have been described extensively in numerous in vitro, in vivo, and epidemiological studies [2–4], and consequently, emission legislations on diesel engine emissions including DEPs became more stringent. In order to cope with these regulations, a number of exhaust aftertreatment systems have been developed, among the most common ones being diesel particle filters, and among these, the most notable ones are the wall-flow filters [5, 6]. By forcing the exhaust stream

Published online: 01 June 2014

INFRAS Consulting Group for Policy Analysis and Implementation

Technik Thermische Maschinen (TTM)

Carcinogenic and non-carcinogenic effects of diesel exhaust components (incl. PAH and nitro-PAH) using different particulate trap technologies

final report

Peter de Haan, Mario Keller

03 October 2001 / B7048b1-02 / B7048b1-02 final report v00.doc

INFRAS **ISO 9001/14001 certified**
Mühlemattstrasse 45, CH-3007 Bern, Tel. +41 31 370 19 19, Fax +41 31 370 19 10, E-Mail bern@infras.ch
Gerechtigkeitsgasse 20, Postfach, CH-8039 Zurich, Tel. +41 1 205 95 95, Fax +41 1 205 95 99, E-Mail zuerich@infras.ch

Table of contents

5. Summary and conclusions

In this study, two different sets of weighting factors that are widely being used for the assessment of the human health impacts of exhaust gases have been extended with new factors to take into account individual PAH and nitro-PAH compounds. These new weighting factors aim at taking into account the carcinogenic effects of these pollutants. Data on their direct human carcinogenicity was not available. The carcinogenic effects of 20 PAH compounds and of 20 nitro-PAH compounds has been therefore been estimated using their mutagenicity as measured in bioassays with human cells (PAH) and Salmonella (nitro-PAH). These figures can only provide a rough estimate of their corresponding, but currently unknown, human carcinogenicity, however.

An assessment of the over-all effects of diesel exhaust on human health has been conducted using measurements from different configurations of a diesel engine with different fuel additives and/or particulate trap technology, which were performed at EMPA. Along with some regulated pollutants (T.HC, NO_x, CO), dioxins and the total sum of PAHs, five nitro-PAH compounds could be measured during the EMPA (2001) measurements and were above detection limits. Of these five compounds, 1-nitropyrene is clearly the compound with the highest expected carcinogenic effects.

The EMPA (2001) report clearly states that the measurements of the 1-nitropyrene concentration were difficult, and that in several cases it could be observed that part of the 1-nitropyrene formation took place not in the exhaust pipe, but in the laboratory. Moreover, the reported 1-nitropyrene emission factors appear to be somewhat inconclusive: if both using chlorinated fuel and the use of a fiber trap lead to higher 1-nitropyrene emissions (compared to the use of a platinum/cerium additive), the fact that no 1-nitropyrene could be detected when using both a chlorinated fuel and a fiber trap, is counter-intuitive. Nevertheless, the present figures suggest that 1-nitropyrene is a matter of concern with regard to carcinogenic effects on human health. In two configurations, the carcinogenicity of the 1-nitropyrene emissions clearly superseded those from their precursors, the sum of PAHs.

It therefore seems necessary to (i) measure more nitro-PAH compounds more accurately; (ii) improve the carcinogenicity weighting factors, for example by using laboratory animal studies which have been conducted for some of the more mutagenic PAHs, in order to confirm or reject the conclusion of the present report that 1-nitropyrene clearly seems to be the most dangerous nitro-PAH occurring in diesel exhaust gases.

HICE › Aerosols and Health
Helmholtz Virtual Institute of Complex Molecular Systems in Environmental Health

Helmholtz Virtual Institute of Complex Molecular Systems in Environmental Health - HICE

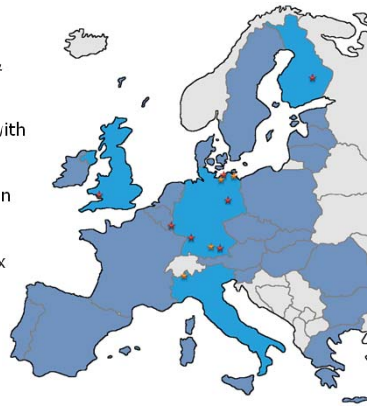
Prof. Dr. Ralf Zimmermann
Spokesperson

Place, TT/MM/JJ

HelmholtzZentrum münchen
German Research Center for Environmental Health

Description

- Helmholtz Virtual Institute in Aerosol & Health Research
- Funded by the Helmholtz Association with 3.000.000 Euros
- Led by the Helmholtz Zentrum München and the University of Rostock
- Cooperation with eight partners and six associated partners
- Started in January 2012 as a five-year project
- www.hice-vi.eu



HelmholtzZentrum münchen German Research Center for Environmental Health | Universität Rostock Traditio et Innovatio | HICE › Aerosols and Health

Partners

HelmholtzZentrum münchen
German Research Center for Environmental Health

Universität Rostock
Traditio et Innovatio

Technische Universität München

CARDIFF UNIVERSITY
PRIFYSGOL CAERDYDD

ASG
Analytik-Service Gesellschaft

KIT
Karlsruher Institut für Technologie

uni.lu
UNIVERSITÉ DU LUXEMBOURG

DECODON

MDC
MAX DELBRÜCK CENTER FOR MOLECULAR MEDICINE BERLIN-BUCH

iow

photonion

UNIVERSITY OF EASTERN FINLAND

JRC
EUROPEAN COMMISSION

VITROCELL

HelmholtzZentrum münchen German Research Center for Environmental Health | Universität Rostock Traditio et Innovatio | HICE › Aerosols and Health

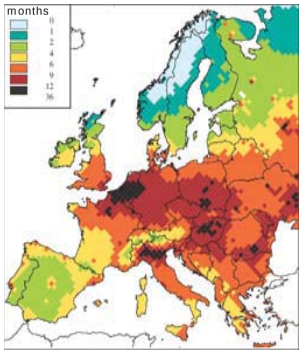
Motivation

"Exposure to particulate matter (PM) decreases the life expectancy of every person by an average of almost one year, mostly due to increased risk of cardiovascular and respiratory diseases, and lung cancer." *(WHO Europe)*

HICE addresses the question:

Which aerosol component or property is responsible for the observed health effects?

Reduction of life expectancy due to particulate matter (PM) exposure



Source: EU- Clean Air For Europe (CAFE, 2005)

HelmholtzZentrum münchen German Research Center for Environmental Health | Universität Rostock Traditio et Innovatio | HICE › Aerosols and Health

Research Goals

1) Elucidation of the molecular mechanisms and agents in combustion aerosols relevant for the observed health effects

2) Identification of biomarkers for exposure and health effects

3) Evaluation of the relative toxicological potential of different anthropogenic aerosol sources

Scientific Hypotheses

1) Reactive chemical compounds in ambient aerosols, particles and gas are relevant for observed health effects

2) Health effects are related to synergistic mechanisms between compounds in gas and particulate phase

3) Expected increase of biofuel & biomass ("Energiewende") will change the composition and properties of aerosols and may lead to more or other aerosol-related health effect implications

Technical / Methodological Hypotheses

1) Health effect mechanisms can be studied by exposing human lung cells to freshly emitted aerosols → Determination of the biological response combined with a comprehensive chemical and physical analysis of the aerosol and a joint statistical data analysis

2) Application and improvement of highly sensitive stable isotope-labeling methods for the detection of biological responses and elucidation of the underlying mechanisms

3) State of the art exposure of human lung cell models at the Air-Liquid Interface (ALI) provides a realistic model for aerosols inhaled into the lung by simulating the relevant primary effects in the lung tissue and can replace animal tests

Results

Measurement campaign at University of Rostock (2012):
ship engine with Heavy Fuel Oil (HFO) and Diesel Fuel (DF):

- HFO: emits more aromatic species and other organic compounds as well as more transition metals (e.g. V, Ni)
- DF: emits more black carbon ("soot")
- Cellular response revealed biological effects especially in inflammatory, oxidative stress and protein synthesis pathways
- The adverse biological effects of DF-emission particles on lung cells were of similar strength or even slightly stronger than those of HFO-emission particles at comparable doses

Future results

Ongoing data analyses from

1) Measurement campaign at Joint Research Centre in Ispra (2013):
   passenger cars with gasoline and ethanol
2) Measurement campaign at the University of Eastern Finland (2013):
   wood combustion – beech and birch logwood, soft wood pellets

Publications of Universities: Copenhagen, Aarhus & Danish Technological Institute

Recent publications on engine toxicity:

Raaschou-Nielsen O, Sørensen M, Ketzel M, Hertel O, Loft S, Tjønneland A, Overvad K, Andersen ZJ. Long-term exposure to traffic air pollution and diabetes-related mortality: a cohort study. Diabetologia 56: 36-46, 2013

Møller P, Danielsen PH, Jantzen K, Roursgaard M, Loft S. Oxidatively damaged DNA in animals exposed to particles. Crit Rev Toxicol 43: 96-118, 2013

Vesterdal LK, Danielsen PH, Folkmann JK, Jespersen L, Aguilar-Pelaez K, Roursgaard M, Loft S, Møller P. Accumulation of lipids and oxidatively damaged DNA in hepatocytes exposed to particles. Toxicol Appl Pharmacol 274: 350-60, 2014

Vesterdal LK, Jantzen K, Sheykhzade M, Roursgaard M, Folkmann JK, Loft S, Møller P. Pulmonary exposure to particles from diesel exhaust, urban dust or single-walled carbon nanotubes and oxidatively damaged DNA and vascular function in apoE-/-mice. Nanotoxicology 8: 61-71, 2014

Rissler J, Nordin EZ, Eriksson AC, Nilsson PT, Frosch M, Sporre MK, Wierzbicka A, Svenningsson B, Löndahl J, Messing ME, Sjogren S, Hemmingsen JG, Loft S, Pagels JH, Swietlicki E. Effective density and mixing state of aerosol particles in a near-traffic urban environment. Environ Sci Technol

Recent publications related to engine toxicity:

Wichmann J, Folke F, Torp-Pedersen C, Lippert F, Ketzel M, Ellermannd T, Loft S. Out-of-hospital cardiac arrests and outdoor air pollution exposure in Copenhagen, Denmark. PLoS One 8: e53684, 2013,

Hertel O, Jensen SS, Ketzel M, Becker T, Peel RG, Ørby PV, Skjøth CA, Ellerman T, Raaschou-Nielsen O, Sørensen M, Brauner EV, Andersen ZJ, Loft S, Bønløkke JH, Sigsgaard T. Utilizing Monitoring Data and Spatial Analysis Tools for Exposure Assessment of Atmospheric Pollutants in Denmark, pp 95-122 in Occurrence, Fate and Impact of Atmospheric Pollutants on Environmental and Human Health. Eds. McConnell LL, Dachs J, Hapeman CJ. American Chemical Society Symposium Series 2013. DOI: 10.1021/bk-2013-1149

Beko G, Weschler CJ, Wierzbicka A, Karottki DG, Toftum J, Loft S, Clausen G Ultrafine particles: Exposure and source apportionment in 56 Danish homes. Environ Sci Technol 47: 10240-8, 2013

Karottki DG, Spilak M, Frederiksen M, Gunnarsen L, Brauner EV, Kolarik B, Andersen ZJ, Sigsgaard T, Barregard L, Strandberg B, Sallsten G, Møller P, Loft S. An indoor air filtration study in homes of elderly: cardiovascular and respiratory effects of exposure to particulate matter. Environ Health 12:116, 2013

Spilak M, Karottki DG, Frederiksen M, Kolarik B, Loft S Gunnarsen L. Evaluation of building characteristics in 27 dwellings in Denmark and effect of using particle filtration units on PM2.5 concentrations. Building Environ 73: 55-63, 2014

4th Information Report for IEA Implementing Agreement AMF, Annex XLII, international activities 2014 - Report from WNRI (Norway) on toxicity of exhaust emissions

Written by Otto Andersen, 24 June 2014
Contact: otto.andersen@vestforsk.no

Activities

WNRI has through the whole year 2013 been a partner in the EEA project "Influence of bioethanol fuels treatment for operational performance, ecological properties and GHG emissions of spark ignition engine" (BIOTRETH). WNRI has had the responsibility for the task "Toxicology assessment of emissions from bioethanol fuel blends".

WNRI has conducted a review of toxicology aspects of emissions from bioethanol fuel blends. The resutls of this work have been submitted in a manuscript titled "A review of emission products from bioethanol and its blends with gasoline. Background for new guidelines for emission control" that has been submitted to the international scientific journal *Fuel*.

An abstract and title "Exhaust emission components from bioethanol-based fuels: A review of toxicity of nanoparticles and aerosol compounds" has passed suitability check for submission to the "Nanotoxicology and Lung Diseases" special issue of *International Journal of Molecular Sciences*.

A software package for *in-silico* for early warning, providing input to the design of epidemiological studies, and prediction of toxicological impacts from the blending of bioethanol into gasoline has been aquired. Molecular dynamics simulations (MDS) on supercomputers are used for this task. From the knowledge that fossil fuel exhaust has significant presence of polycyclic aromatic hydrocarbons (PAHs) and bioethanol exhaust contain high levels of acetaldehyde, we investigate interaction between these molecules to predict the formation of new emission compounds. The other participants are Fjordforsk Environmental Services AS, Oil and Gas Institute Krakow (Poland), and University of Applied Sciences in Biel (Switzerland).

Publications/presentations

The toxicology results from the project "Influence of bio-components content in fuel on emission of diesel engines and engine oil deterioration" (BIODEG) was presented as the session talk "Paths and degradation of PAHs in the Environment" at the conference "ISPAC 2013 - International Symposium on Polycyclic Aromatic Compounds" (Andersen et al, 2013). A detailed description of the toxicology effects of biodiesel blending was published as a chapter in the book "Unintended Consequences of Renewable Energy. Problems to be Solved" (Andersen, 2013):

References

Andersen O, Manzetti, S, Turek-Szytow J (2013) Paths and degradation of PAHs in the Environment. International Symposium on Polycyclic Aromatic Compounds (ISPAC 2013). Oregon State University, Corvallis, Oregon USA.

Andersen O (2013) Biodiesel and its Blending into Fossil Diesel. In: Unintended Consequences of Renewable Energy. Problems to be Solved.
Springer London, ISSN 1865-3529; ISBN 978-1-4471-5531-7; pp 55–70. Available at:
http://www.springer.com/energy/renewable+and+green+energy/book/978-1-4471-5531-7.

Comparison of the Fuel Impact on Exhaust Emission Using Swedish Environmental Class 1 (MK1) and Class 3 (MK3) diesel.

AVL SWEDEN

On behalf of the the Swedish Transport Administration

Comparison of the Fuel Impact on Exhaust Emission Using Swedish Environmental Class 1 (MK1) and Class 3 (MK3) diesel.

Content

significant effects in most cases, especially in the presence of S9 indicating a significant contribution by PAH rather that nitro-PAH. Strain TA 100 gave a few significant responses. With these low effects it is difficult to draw any conclusions regarding fuel differences (hot start vs cold start or fuel differences MK1 vs MK3).

For the analyses of the EGR vehicle the concentrations were increased as much as possible with the amount of samples available. In this case much higher mutagenic effects were seen. Since the mutagenicity is given per meter driving distance it is clear from the table that not only the slightly more concentrated samples are responsible for the higher mutagenicity. The exhaust extracts from the EGR vehicle are significantly more mutagenic that the corresponding extracts from the SCR vehicle. The data are more robust and it is possible to compare the exhausts from the two fuels used. All samples are significantly mutagenic both in the presence and in the absence of a metabolizing system. With strain TA98 all samples were more mutagenic in the presence of S9 and therefore demonstrate the presence of both direct acting components and indirect mutagens (e.g. PAH). When the effects are lower in the presence of a metabolizing system, i.e. with TA100 it could still be the results of both types of mutagens, although it cannot be determined without further tests. Comparing the difference between the fuels especially with hot start the mutagenicity within each group is not significantly different indicating that the samples seem to be representative, but the MK3 fuel generates a more genotoxic exhaust in these cases. With cold starts the situation is not as clear.

Conclusions

The use of diesel fuelled vehicles for transportation shows no tendency to decline, rather the opposite. For heavy duty vehicles and non-road mobile machinery diesel is, and will be (at least in the near future), the dominating fuel. Since vehicles are expensive, the transport sector will always be a mix of different emission standards. This puts focus on the fuel, since improvement of the fuel is the easiest way to improve the emissions from all diesel vehicles.
In this study a comparison between two different diesel fuels have been made – the Swedish Environmental class 1 (MK1) and diesel fulfilling the European diesel standard EN590 (MK3). The main difference between these fuels is the content of aromatics and polycyclic aromatics (PAH), of which many are known or potential carcinogen. The MK3 fuel contains more than 10 times the amount of PAH in %m/m compared to the MK1 fuel.
Earlier studies have shown significant differences between these two fuels. There have however been improvements both regarding the fuels and engine technologies. The goal for this study was to see if the differences persist.
Two modern (emission standard Euro V) heavy duty vehicles, equipped with a SCR and an EGR aftertreatment systems, were tested with the two diesel fuels. The vehicles were driven according to the WHVC test cycle on a chassis dynamometer. Regulated exhausts, CO2 and fuel consumption were measured. The gaseous components were sampled in bags as well as measured second-by-second. Particles were sampled on filters and analyzed gravimetrically. The size distribution of the

emitted particles was measured with an ELPI instrument. Unregulated components such as olefins, PAH and aldehydes have been analyzed. Extract of the particulate and semivolatile phase has been used to carry out the Ames’ bioassay test to analyze the level of mutagenicity in the exhausts.
This investigation has shown that there are still significant differences on emission level between these two fuel qualities even when tested on a modern, Euro V vehicle. There are discrepancies between the fuels regarding fuel properties and the effects on the emissions can depend on several parameters. The health effect of each of these parameters has however not been investigated in this study. For regulated components, the exhaust emission measurements have shown higher levels of NOx, PM and CO for the MK3 fuel. Regarding the unregulated components there are also some differences between the fuels. The total amounts of aldehydes are emitted to a higher extent when MK3 is used. The olifines investigated in this study, together with emissions of benzene, were too close to the detection level and no significant differences could be observed. The difference regarding emitted aromatics and polyaromatics must however be highlighted, where the higher levels of these compounds in MK3 is reflected in the exhaust emissions. The extracts of PAH used for Ames’ bio assay show higher mutagenic activity for the MK3 fuel.
The continuous development of engines and reduction of emissions is enforced through legislation. Since the first regulation was introduced, Euro I (effective from 1992), the limit value for NOx and PM have been reduced by more than 95%. The emission limits in Euro VI for heavy duty vehicles, effective from 2013 (new registrations) and 2014 (all registrations), will lead to a more extensive use of aftertreatment systems (such as SCR and diesel particulate filters) in vehicles, in order to comply with the legislation limits. It is however of importance to point out that an SCR system has to work during suitable conditions, i.e. engine load and exhaust temperature, in order to reduce emissions in a satisfying way outside the test cell (one great challenge is emission reduction on buses in urban areas). The more extensive use of diesel particulate filter will probably have positive effects on emissions of particle matter, particle number as well as PAH emissions. For non-road mobile machinery there is however no enforcement for diesel particulate filters driven through legislated emission limits. The major benefit when improving the fuel quality is that this factor affects emissions from all existent vehicles and non-road mobile machinery, whereas the legislation can affect emissions in the future.

Madden et al. Particle and Fibre Toxicology 2014, 11:37
http://www.particleandfibretoxicology.com/content/11/1/37

PARTICLE AND FIBRE TOXICOLOGY

RESEARCH Open Access

Diesel exhaust modulates ozone-induced lung function decrements in healthy human volunteers

Michael C Madden[1,4*], Tina Stevens[1,3], Martin Case[1], Michael Schmitt[1], David Diaz-Sanchez[1], Maryann Bassett[1], Tracey S Montilla[1], Jon Berntsen[2] and Robert B Devlin[1]

Abstract

The potential effects of combinations of dilute whole diesel exhaust (DE) and ozone (O_3), each a common component of ambient airborne pollutant mixtures, on lung function were examined. Healthy young human volunteers were exposed for 2 hr to pollutants while exercising (~50 L/min) intermittently on two consecutive days. Day 1 exposures were either to filtered air, DE (300 μg/m^3), O_3 (0.300 ppm), or the combination of both pollutants. On Day 2 all exposures were to O_3 (0.300 ppm), and Day 3 served as a followup observation day. Lung function was assessed by spirometry just prior to, immediately after, and up to 4 hr post-exposure on each exposure day. Functional pulmonary responses to the pollutants were also characterized based on stratification by glutathione S-transferase mu 1 (GSTM1) genotype. On Day 1, exposure to air or DE did not change FEV1 or FVC in the subject population (n = 15). The co-exposure to O_3 and DE decreased FEV1 (17.6%) to a greater extent than O_3 alone (9.9%). To test for synergistic exposure effects, i.e., in a greater than additive fashion, FEV1 changes post individual O_3 and DE exposures were summed together and compared to the combined DE and O_3 exposure; the p value was 0.057. On Day 2, subjects who received DE exposure on Day 1 had a larger FEV1 decrement (14.7%) immediately after the O_3 exposure than the individuals' matched response following a Day 1 air exposure (10.9%). GSTM1 genotype did not affect the magnitude of lung function changes in a significant fashion. These data suggest that altered respiratory responses to the combination of O_3 and DE exposure can be observed showing a greater than additive manner. In addition, O_3-induced lung function decrements are greater with a prior exposure to DE compared to a prior exposure to filtered air. Based on the joint occurrence of these pollutants in the ambient environment, the potential exists for interactions in more than an additive fashion affecting lung physiological processes.

Keywords: Diesel exhaust, Ozone, Co-exposure, Lung function, Greater than additive effects

Introduction

Numerous epidemiological studies have demonstrated an association between short-term exposure to ambient airborne particulate matter (PM) and adverse cardiopulmonary effects including premature mortality, increased hospitalizations for lung problems including infections, exacerbation of asthma symptoms, chronic bronchitis, and hospitalization for clinical cardiac events including arrhythmias, myocardial infarctions, and congestive heart failure [1,2]. The health effects are more strongly associated with PM that is smaller than 2.5 μm, i.e. $PM_{2.5}$, which typically is derived from human based activities such as vehicular emissions. $PM_{2.5}$ is a complex mixture of organic and inorganic compounds absorbed onto carbonaceous material with the composition varying across space and time. In this complex mixture of ambient air substances, the ubiquitous pollutants ozone (O_3) and diesel exhaust (DE) can be can be major and important components. DE can have "hotspots" such as bus terminals and major streets [3]. Levels of DE $PM_{2.5}$ reached transient concentrations of several hundred μg/m3 during drive-by studies [4]. O_3 levels have generally been decreasing in the US, but can reach over 0.1 ppm on a regular basis.

* Correspondence: madden.michael@epa.gov
[1]EPHD, NHEERL, US EPA, Research Triangle Park, Chapel Hill, NC 27711, USA
[4]U.S EPA Human Studies Facility, 104 Mason Farm Road, Chapel Hill, NC 27599-7315, USA
Full list of author information is available at the end of the article

ARTICLE IN PRESS

Chemosphere xxx (2013) xxx–xxx

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Diesel and biodiesel exhaust particle effects on rat alveolar macrophages with *in vitro* exposure

Laya Bhavaraju [a], Jonathan Shannahan [b], Aaron William [c], Robert McCormick [c], John McGee [d], Urmila Kodavanti [d], Michael Madden [d,*]

[a] *Currciculum in Toxicology, University of North Carolina, Chapel Hill, NC, United States*
[b] *School of Pharmacy, University of Colorado, Denver, CO, United States*
[c] *National Renewable Energy Laboratory, Golden, CO, United States*
[d] *EPHD, NHEERL, US EPA, Research Triangle Park, NC, United States*

HIGHLIGHTS

- Petroleum diesel and biodiesel exhaust particle composition varies by species.
- Macrophage exposure to exhaust particles results in prostaglandin production/release changes.
- Biodiesel exposure induced increased prostaglandin release compared to same dose of petroleum.
- Detection of prostaglandin release not inhibited by particle sequestering.
- Macrophage inflammation initiating pathways correlate in response to dose not particle type.

ARTICLE INFO

Article history:
Received 31 May 2013
Received in revised form 23 October 2013
Accepted 30 October 2013
Available online xxxx

Keywords:
Prostaglandin E_2
Alveolar macrophages
Biodiesel exhaust
Diesel exhaust

ABSTRACT

Combustion emissions from diesel engines emit particulate matter which deposits within the lungs. Alveolar macrophages (AMs) encounter the particles and attempt to engulf the particles. Emissions particles from diesel combustion engines have been found to contain diverse biologically active components including metals and polyaromatic hydrocarbons which cause adverse health effects. However little is known about AM response to particles from the incorporation of biodiesel. The objective of this study was to examine the toxicity in Wistar Kyoto rat AM of biodiesel blend (B20) and low sulfur petroleum diesel (PDEP) exhaust particles. Particles were independently suspended in media at a range of 1–500 μg mL^{-1}. Results indicated B20 and PDEP initiated a dose dependent increase of inflammatory signals from AM after exposure. After 24 h exposure to B20 and PDEP gene expression of cyclooxygenase-2 (COX-2) and macrophage inflammatory protein 2 (MIP-2) increased. B20 exposure resulted in elevated prostaglandin E_2 (PGE_2) release at lower particle concentrations compared to PDEP. B20 and PDEP demonstrated similar affinity for sequestration of PGE_2 at high concentrations, suggesting detection is not impaired. Our data suggests PGE_2 release from AM is dependent on the chemical composition of the particles. Particle analysis including measurements of metals and ions indicate B20 contains more of select metals than PDEP. Other particle components generally reduced by 20% with 20% incorporation of biodiesel into original diesel. This study shows AM exposure to B20 results in increased production of PGE_2 *in vitro* relative to diesel.

Published by Elsevier Ltd.

1. Introduction

Inhaled diesel exhaust particles deposit in the lungs where individual alveolar macrophages (AMs) engulf particles via phagocytosis. Phagocytosis initiates a response from AM to trigger an inflammatory response which includes release of cytokines, lipid mediators and other signals to recruit neutrophils to deposit site. *In vivo* exposures to petroleum diesel exhaust particles (PDEP) with guinea pigs and rats revealed phagocytosis by AM and increased inflammation response (Chen et al., 1980; Yang et al., 1997). Previous studies indicate human macrophages release cytokines IL-6 and TNFα after exposure to coarse and ultrafine particles of diesel exhaust indicating a heightened inflammatory response (Becker et al., 2003). Exposure to filtered diesel exhaust and unfiltered resulted in both types causing similar inflammation responses from human AM from bronchoalveolar lavage fluid (BALF), suggesting the particle and its composition plays a leading role in AM re-

* Coresponding author. Address: US EPA, Human Studies Facility, 104 Mason Farm Rd, Chapel Hill, NC 27599, United States. Tel.: +1 919 966 6257; fax: +1 919 966 6367.
E-mail address: madden.michael@epa.gov (M. Madden).

0045-6535/$ - see front matter Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.chemosphere.2013.10.080

http://informahealthcare.com/bmk
ISSN: 1354-750X (print), 1366-5804 (electronic)

Biomarkers

Biomarkers, Early Online: 1–8
 DOI: 10.3109/1354750X.2014.910553

informa
healthcare

RESEARCH ARTICLE

Are urinary PAHs biomarkers of controlled exposure to diesel exhaust?

Sixin S. Lu[1]#, Jon R. Sobus[2], Gerd Sallsten[3], Maria Albin[4], Joachim D. Pleil[2], Anders Gudmundsson[5], Michael C. Madden[6], Bo Strandberg[3], Aneta Wierzbicka[5], and Stephen M. Rappaport[7]#

[1]College of Natural Resources, University of California, Berkeley, CA, USA, [2]Human Exposure and Atmospheric Science Division, U.S. Environmental Protection Agency, National Exposure Research Laboratory, Research Triangle Park, NC, USA, [3]Occupational and Environmental Medicine, University of Gothenburg, Sweden, [4]Occupational and Environmental Medicine, Lund University, Sweden, [5]Ergonomics and Aerosol Technology, Lund University, Sweden, [6]Environmental Public Health Division, US Environmental Protection Agency, National Health and Environmental Effects Research Laboratory, Research Triangle Park, NC, USA, and [7]Center for Exposure Biology, School of Public Health, University of California, Berkeley, CA, USA

Abstract

Urinary polycyclic aromatic hydrocarbons (PAHs) were evaluated as possible biomarkers of exposure to diesel exhaust (DE) in two controlled-chamber studies. We report levels of 14 PAHs from 28 subjects in urine that were collected before, immediately after and the morning after exposure. Using linear mixed-effects models, we tested for effects of DE exposure and several covariates (time, age, gender and urinary creatinine) on urinary PAH levels. DE exposures did not significantly alter urinary PAH levels. We conclude that urinary PAHs are not promising biomarkers of short-term exposures to DE in the range of 106–276 μg/m^3.

Keywords

Biomarker, diesel exhaust, PAHs, polycyclic aromatic hydrocarbons, urine

History

Received 5 February 2014
Revised 27 March 2014
Accepted 28 March 2014
Published online 22 April 2014

Introduction

Diesel exhaust (DE) was classified in 2012 as a group 1 carcinogen by the International Agency for Research on Cancer (IARC) (Benbrahim-Tallaa et al., 2012) based on long-term occupational exposures. DE contributes to ambient particulate matter that has been linked to chronic cardiopulmonary and vascular effects (Sydbom et al., 2001; Wichmann, 2007). Yet, it has been difficult to quantify exposure–response relationships for DE due to the lack of quantitative data regarding exposures, which have primarily been classified by observational descriptors such as job title (Laumbach & Kipen, 2011; Pronk et al., 2009; Steenland et al., 1998). This reflects the complex nature of DE, which is comprised of both gaseous and fine particulate constituents.

Although gaseous DE contains predominantly small molecules such as nitrogen oxides and aldehydes, the particulate phase consists of elemental carbon coated with organic compounds (organic carbon), including particle-bound polycyclic aromatic hydrocarbons (PAHs) (Sobus et al., 2008a; Sydbom et al., 2001; Wichmann, 2007). The class of PAHs contains hundreds of chemicals with two or more fused-aromatic rings that are formed from incomplete combustion of hydrocarbons. As a class, PAHs have been associated with human lung and bladder cancers, and several of the five-ring PAHs, such as benzo(*a*)pyrene (BAP), are potent animal carcinogens (IARC, 2010). Volatile or semi-volatile PAH molecules (two or three rings) are found primarily in the gas phase, while the larger compounds (four to six rings) reside primarily in the particulate phase.

Concentrations of gas-phase PAHs are much greater in DE than those of particulate-phase PAHs. For example, Sobus et al. (2008a) reported air concentrations of naphthalene (NAP, two rings) and phenanthrene (PHE, three rings) during controlled human exposure to DE that were about three orders of magnitude greater than the BAP concentration. Because air levels of NAP and/or PHE were highly correlated with those of organic carbon as well as semivolatile and particulate PAHs, the authors speculated that NAP and PHE could be suitable surrogates for exposures to all DE-derived PAHs – and to DE more generally – in studies of health effects (Sobus et al., 2008a). In a separate analysis, Sobus et al. (2008b) showed that the levels of unmetabolized NAP and PHE in urine were also positively correlated in workers exposed to coke-oven emissions, asphalt fumes and DE.

Urinary PAHs are biomarkers of exposure, which offer attractive alternatives to air monitoring for determining exposure–response relationships (Lin, 2005). Many PAHs have been detected in urine from urban populations exposed to air pollutants (Campo et al., 2007, 2009, 2011; Serdar et al., 2003; Sobus et al., 2008b; Waidyanatha et al., 2003) as well as from workers exposed to emissions containing PAHs

#Sixin S. Lu and Stephen M. Rappaport are responsible for statistical design/analysis. E-mail: SL@berkeley.edu and srappaport@berkeley.edu

Address for correspondence: Dr. Stephen M. Rappaport, School of Public Health, University of California, Berkeley, CA 94720-7356, USA. Tel: + 1 510 642 4255. Fax: + 1 510 642 5815. E-mail: srappaport@berkeley.edu

Environmental Science & Technology

ACS Editors' Choice

Article

pubs.acs.org/est

Terms of Use

Comparisons of Ultrafine and Fine Particles in Their Associations with Biomarkers Reflecting Physiological Pathways

Jicheng Gong,[†] Tong Zhu,[‡] Howard Kipen,[§] Guangfa Wang,[∥] Min Hu,[‡] Qingfeng Guo,[‡] Pamela Ohman-Strickland,[⊥] Shou-En Lu,[⊥] Yuedan Wang,[#] Ping Zhu,[▽] David Q. Rich,[○] Wei Huang,[‡] and Junfeng Zhang*[,†]

[†]Duke University, Nicholas School of the Environment and Duke Global Health Institute, Durham, North Carolina, United States

[‡]Peking University, College of Environmental Sciences and Engineering and the Center for Environmental Health, Beijing, China

[§]Rutgers Robert Wood Johnson Medical School, Department of Environmental and Occupational Medicine, Piscataway, New Jersey, United States

[∥]Peking University First Hospital, Department of Pulmonary Medicine, Beijing, China

[⊥]Rutgers School of Public Health, Department of Biostatistics, Piscataway, New Jersey, United States

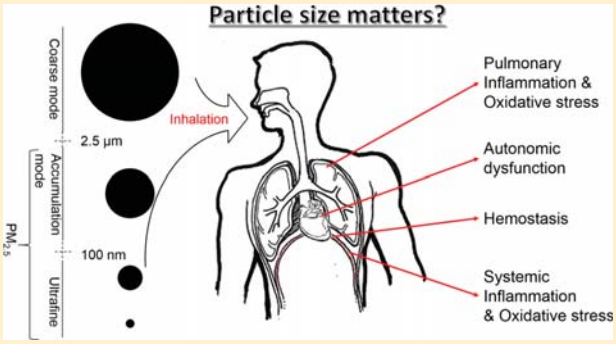
[#]Peking University Health Sciences Center, Department of Immunology, Beijing, China

[▽]Peking University First Hospital, Department of Hematology, Beijing, China

[○]University of Rochester, School of Medicine and Dentistry, Rochester, New York, United States

Supporting Information

ABSTRACT: Using a quasi-experimental opportunity offered by greatly restricted air pollution emissions during the Beijing Olympics compared to before and after the Olympics, we conducted the current study to compare ultrafine particles (UFPs) and fine particles ($PM_{2.5}$) in their associations with biomarkers reflecting multiple pathophysiological pathways linking exposure and cardiorespiratory events. Number concentrations of particles (13.0−764.7 nm) and mass concentrations of $PM_{2.5}$ were measured at two locations within 9 km from the residence and workplace of 125 participating Beijing residents. Each participant was measured 6 times for biomarkers of autonomic function (heart rate, systolic and diastolic blood pressures), hemostasis (von Willebrand factor, soluble CD40 ligand, and P-selectin), pulmonary inflammation and oxidative stress (exhaled nitric oxide and exhaled breath condensate pH, malondialdehyde, and nitrite), and systemic inflammation and oxidative stress (urinary malondialdehyde and 8-hydroxy-2′-deoxyguanosine, plasma fibrinogen, and white blood cells). Linear mixed models were used to estimate associations of biomarkers with UFPs and $PM_{2.5}$ measured 1−7 days prior to biomarker measurements (lags). We found that the correlation coefficient for UFPs at two locations (~9 km apart) was 0.45, and at the same location, the correlation coefficient for $PM_{2.5}$ vs UFPs was −0.18. Changes in biomarker levels associated with increases in UFPs and $PM_{2.5}$ were comparable in magnitude. However, associations of certain biomarkers with UFPs had different lag patterns compared to those with $PM_{2.5}$, suggesting that the ultrafine size fraction (≤100 nm) and the fine size fraction (~100 nm to 2.5 μm) of $PM_{2.5}$ are likely to affect PM-induced pathophysiological pathways independently.



INTRODUCTION

Over the past decades, a large body of literature has provided evidence for associations between exposures to ambient particulate matter (PM) and cardiorespiratory morbidity and mortality.[1−3] The vast majority of the epidemiological studies have assessed the relationships between health outcomes and $PM_{2.5}$ or PM_{10} mass concentrations.[4−6] Unlike a single gaseous pollutant, atmospheric PM is a mixture of heterogeneous components; and particles of different sizes may have different physicochemical and toxicological properties.[7] In a simplistic and practical fashion, $PM_{2.5}$ can be considered the sum of two distinct components, namely ultrafine particles (UFPs, ≤100 nm in aerodynamic diameter) and accumulation-mode particles (AMPs, ~100 nm to 1.0 μm).[8] UFPs make up a large number concentration but contribute little mass to $PM_{2.5}$.[9−12] Furthermore, results from animal studies have suggested that inhaled UFPs deposit more deeply into the lung and may even

Received: February 4, 2014
Revised: March 24, 2014
Accepted: March 25, 2014

   dx.doi.org/10.1021/es5006016 | Environ. Sci. Technol. XXXX, XXX, XXX−XXX

A Foundation of Science for Informed Policy on Ambient PM

Daniel L. Costa, Sc.D.
National Program Director for Air. Climate and Energy
Office of Research & Development, US EPA
Research Triangle Park, NC
919-541-2532
costa.dan@epa.gov

Health Effects of Fine Particles from Vehicle Emissions
April 1, 2014 Washington DC
(EFC, NIEHS)

Are We Missing Something… Ultrafine PM?

- Regulating by mass ignores constituent toxicities or unique physical attributes of UFPs
- UFPs contribute little to mass but some UFPs can possess high surface reactivity
- Lung deposition – mass vs number distribution
- Accumulation mode of PM comprises mostly UFP agglomerates that constitute much of $PM_{2.5}$
- Combustion and atmospheric chemistry constantly generate UFP
- High but uncertain exposure potential for UFPs

Some Things We Know

- PM (plus gases?) adversely impact health
- Acute / chronic …susceptible groups
- Respiratory / cardiac / cancer / other organs
- Mechanism(s) – composition / size / host factors
- Some PM sources may be worse than others
  - Combustion: fuels…oil, coal, nat. gas, biofuels, etc.
  - Stationary sources, moving vehicles, etc.
- Air pollution - is variable in composition and comprises a complex dynamic chemistry

Perhaps we know less than we think?!

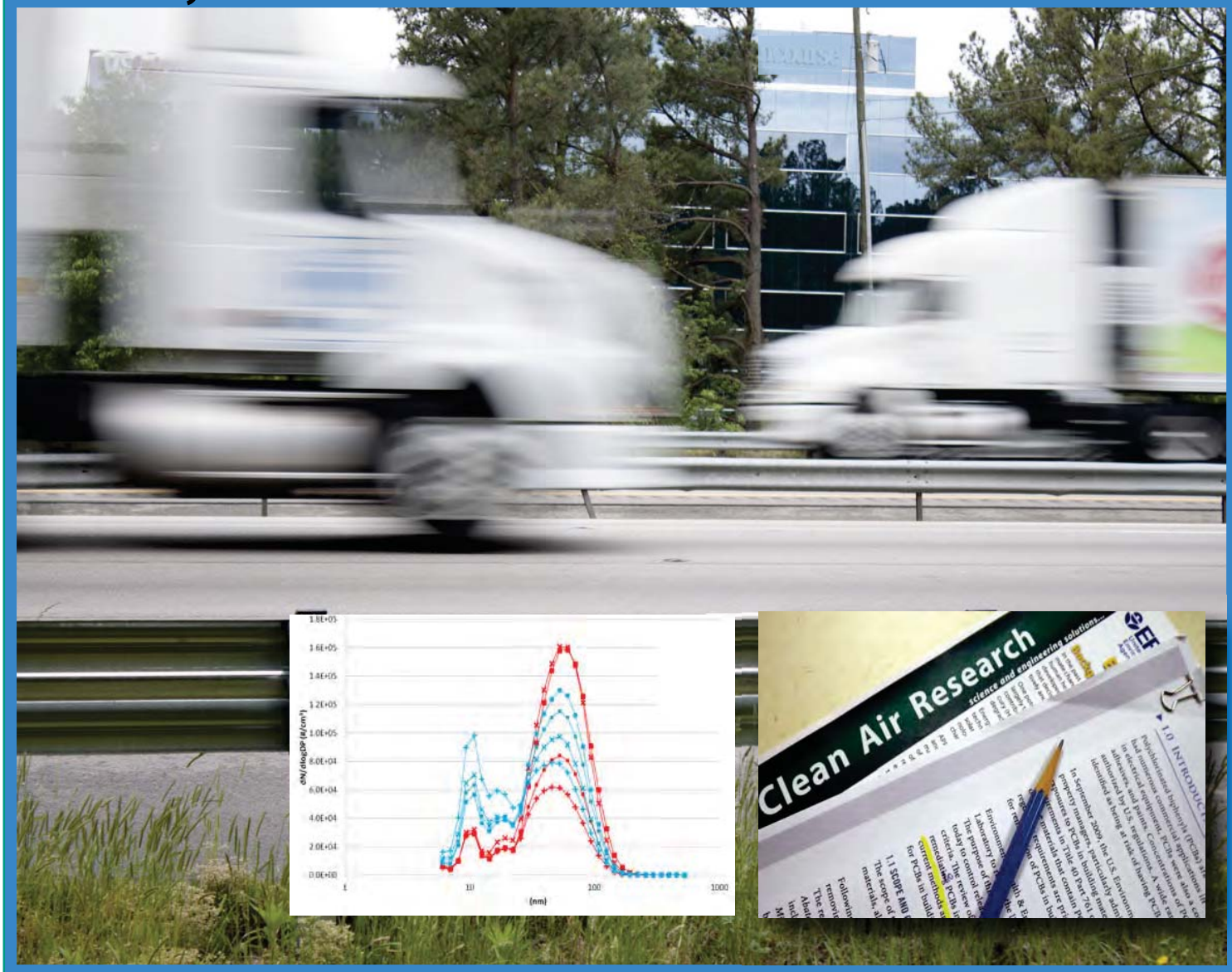
EPA Roadway-Related Research

- Emissions Characterization of Vehicles
  - Chassis dynamometers and on-board measurements
  - Analyses of direct and collected emissions
- Air Quality and Exposure Assessments
  - Fixed-site sampling strategies in near roadway campaigns
  - Mobile monitoring from specialized vehicles
  - Portable sensors for personal sampling
  - Computational Fluid Dynamics Modeling – emissions dispersion
  - Wind tunnel mock-ups for model testing
  - Inclusion into large area models
- Health Effects
  - Epidemiological and toxicological studies

EPA United States Environmental Protection Agency

Join us in Research Triangle Park: Save the Date!

Workshop on Ultrafine Particles (UFP workshop)
February 11-13, 2015



We will be bringing together international experts on emission, air quality, exposures, and health impacts of ultrafine particles to present and discuss the latest research and policy issues.

Please join us in the EPA Auditorium, 109 T. W. Alexander Drive, Research Triangle Park, North Carolina USA

For more information please contact Richard Baldauf at the U.S. EPA Baldauf.richard@epa.gov or visit the webpage at https://www.eventbrite.com/e/us-epa-workshop-on-ultrafine-particles-tickets-13583846651

Journal of Toxicology and Environmental Health, Part A, 76:907–921, 2013
ISSN: 1528-7394 print / 1087-2620 online
DOI: 10.1080/15287394.2013.825217

Taylor & Francis
Taylor & Francis Group

OXIDATIVE STRESS, INFLAMMATORY BIOMARKERS, AND TOXICITY IN MOUSE LUNG AND LIVER AFTER INHALATION EXPOSURE TO 100% BIODIESEL OR PETROLEUM DIESEL EMISSIONS

Anna A. Shvedova[1,2], Naveena Yanamala[1], Ashley R. Murray[1,2], Elena R. Kisin[1], Timur Khaliullin[1], Meghan K. Hatfield[1], Alexey V. Tkach[1], Q. T. Krantz[3], David Nash[4,5], Charly King[3], M. Ian Gilmour[3], Stephen H. Gavett[3]

[1]Health Effects Laboratory Division, Pathology and Physiology Research Branch, National Institute of Occupational and Safety Health, Morgantown, West Virginia, USA
[2]Department of Physiology and Pharmacology, School of Medicine, West Virginia University, Morgantown, West Virginia, USA
[3]Environmental Public Health Division, National Health and Environmental Effects Research Laboratory, U.S. Environmental Protection Agency, Durham, North Carolina, USA
[4]Oak Ridge Institute for Science and Education (ORISE), Oak Ridge, Tennessee, USA
[5]National Risk Management Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina, USA

Over the past decade, soy biodiesel (BD) has become a first alternative energy source that is economically viable and meets requirements of the Clean Air Act. Due to lower mass emissions and reduced hazardous compounds compared to diesel combustion emissions (CE), BD exposure is proposed to produce fewer adverse health effects. However, considering the broad use of BD and its blends in different industries, this assertion needs to be supported and validated by mechanistic and toxicological data. Here, adverse effects were compared in lungs and liver of BALB/cJ mice after inhalation exposure (0, 50, 150, or 500 μg/m^3; 4 h/d, 5 d/wk, for 4 wk) to CE from 100% biodiesel (B100) and diesel (D100). Compared to D100, B100 CE produced a significant accumulation of oxidatively modified proteins (carbonyls), an increase in 4-hydroxynonenal (4-HNE), a reduction of protein thiols, a depletion of antioxidant gluthatione (GSH), a dose-related rise in the levels of biomarkers of tissue damage (lactate dehydrogenase, LDH) in lungs, and inflammation (myeloperoxidase, MPO) in both lungs and liver. Significant differences in the levels of inflammatory cytokines interleukin (IL)-6, IL-10, IL-12p70, monocyte chemoattractant protein (MCP)-1, interferon (IFN) γ, and tumor necrosis factor (TNF)-α were detected in lungs and liver upon B100 and D100 CE exposures. Overall, the tissue damage, oxidative stress, inflammation, and cytokine response were more pronounced in mice exposed to BD CE. Further studies are required to understand what combustion products in BD CE accelerate oxidative and inflammatory responses.

Epidemiologic and occupational studies demonstrated that ambient particular matter (PM) and diesel exhaust particles exert deleterious effects on human health, including

Received 29 May 2013; accepted 11 July 2013.
The authors are grateful to Bill Linak (U.S. EPA) for assistance in inhalation engineering and to Mary Daniels and Liz Boykin (U.S. EPA) for laboratory work. They also thank Dr. Vince Castranova and Dr. Teh-hsun B. Chen (CDC/NIOSH/HELD) and Dr. Mark Higuchi (U.S. EPA) for their discussion, comments, and feedback. This work was supported by NIOSH, 2927ZKCY.
This article has been reviewed by the U.S. Environmental Protection Agency and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Agency. 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 or U.S. EPA policy. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.
Address correspondence to Dr. Anna A. Shvedova, Pathology and Physiology Research Branch (MS-2015), 1095 Willowdale Road, Morgantown, WV 26505, USA. E-mail: ats1@cdc.gov

Toxicology and Applied Pharmacology 272 (2013) 373–383

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

journal homepage: www.elsevier.com/locate/ytaap

Biodiesel versus diesel exposure: Enhanced pulmonary inflammation, oxidative stress, and differential morphological changes in the mouse lung

Naveena Yanamala [a], Meghan K. Hatfield [a], Mariana T. Farcas [a], Diane Schwegler-Berry [a], Jon A. Hummer [c], Michael R. Shurin [e], M. Eileen Birch [d], Dmitriy W. Gutkin [e], Elena Kisin [a], Valerian E. Kagan [f], Aleksandar D. Bugarski [c], Anna A. Shvedova [a,b,*]

[a] Pathology & Physiology Research Branch/NIOSH/CDC, Morgantown, WV 26505, USA
[b] Department Physiology and Pharmacology, WVU, Morgantown, WV 26505, USA
[c] Office of Mine Safety and Health Research/NIOSH/CDC, Pittsburgh, PA 15236, USA
[d] NIOSH/CDC, 4676 Columbia Parkway, Cincinnati, OH 45226, USA
[e] Department of Pathology, University of Pittsburgh Medical Center, Pittsburgh, PA, USA
[f] Department of Environmental and Occupational Health, University of Pittsburgh, PA, USA

ARTICLE INFO

Article history:
Received 22 April 2013
Revised 12 July 2013
Accepted 13 July 2013
Available online 22 July 2013

Keywords:
Aspiration exposure
Cytokine panel
Pulmonary toxicity
Biodiesel particle retention
Inflammation
Lipid droplets

ABSTRACT

The use of biodiesel (BD) or its blends with petroleum diesel (D) is considered to be a viable approach to reduce occupational and environmental exposures to particulate matter (PM). Due to its lower particulate mass emissions compared to D, use of BD is thought to alleviate adverse health effects. Considering BD fuel is mainly composed of unsaturated fatty acids, we hypothesize that BD exhaust particles could induce pronounced adverse outcomes, due to their ability to readily oxidize. The main objective of this study was to compare the effects of particles generated by engine fueled with neat BD and neat petroleum-based D. Biomarkers of tissue damage and inflammation were significantly elevated in lungs of mice exposed to BD particulates. Additionally, BD particulates caused a significant accumulation of oxidatively modified proteins and an increase in 4-hydroxynonenal. The up-regulation of inflammatory cytokines/chemokines/growth factors was higher in lungs upon BD particulate exposure. Histological evaluation of lung sections indicated presence of lymphocytic infiltrate and impaired clearance with prolonged retention of BD particulate in pigment laden macrophages. Taken together, these results clearly indicate that BD exhaust particles could exert more toxic effects compared to D.

Published by Elsevier Inc.

Introduction

Despite the widespread use of petroleum-based diesel (D) fuels, interest in vegetable oils as an alternative fuel source was reported in several countries as early as the 1920s and 1930s. The potential interest in alternative fuels was not evidenced until the fuel-energy crisis in the late 1970s and early 1980s, after which vegetable oil derived fuels gained their prominence as a potential alternative energy source (Hill et al., 2006; Ragauskas et al., 2006). One of the key issues of biodiesel (BD) use is to reduce the emissions of particulate matter (PM) and greenhouse gasses (GHG). The combustion of vegetable oil-derived biodiesel fuels was proven effective in producing similar or less emissions compared to petroleum-based D (Koonin, 2006). Regardless of its broad use in different operational areas, including transportation (on- and off-road vehicles), and other manufacturing/production (mining, oil and gas industry) sectors, inadequate attention has been paid to the possible health hazards of BD (Bunger et al., 2007; Krahl et al., 2001; Swanson et al., 2007).

Exposure to diesel exhaust in humans has been shown to cause a number of adverse health outcomes. For instance, acute exposure to diesel particulate matter (DPM) was shown to facilitate pulmonary inflammation with influx of phagocytic cells (Holgate et al., 2003a, 2003b), while long-term exposure was strongly associated with a greater incidence of cough, phlegm, and chronic bronchitis (Pronk et al., 2009). Additionally, exposure to DPM has been associated with

* Corresponding author at: Pathology and Physiology Research Branch (MS-2015), 1095 Willowdale Road, Morgantown, WV 26505, USA. Fax: +1 304 285 5938.
E-mail addresses: wqu1@cdc.gov (N. Yanamala), wla4@cdc.gov (M.K. Hatfield), woe7@cdc.gov (M.T. Farcas), qzh3@cdc.gov (J.A. Hummer), shurinmr@upmc.edu (M.R. Shurin), mib2@cdc.gov (M.E. Birch), dwgutkin@hotmail.com (D.W. Gutkin), edk8@cdc.gov (E. Kisin), kagan@pitt.edu (V.E. Kagan), zjl1@cdc.gov (A.D. Bugarski), ats1@cdc.gov (A.A. Shvedova).

0041-008X/$ – see front matter. Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.taap.2013.07.006

Gardner et al. Particle and Fibre Toxicology 2014, 11:1
http://www.particleandfibretoxicology.com/content/11/1/1

PARTICLE AND FIBRE TOXICOLOGY

RESEARCH Open Access

Ambient fine particulate air pollution triggers ST-elevation myocardial infarction, but not non-ST elevation myocardial infarction: a case-crossover study

Blake Gardner[1], Frederick Ling[1], Philip Hopke [2], Mark Frampton [3], Mark J Utell[3], ojciech areba [1], Scott J Cameron[1], David Chalupa[3], Cathleen ane [4], Suresh ulandhaisamy [1], Michael C Topf[1] and David Rich [4*]

Abstract

Background: e and others have shown that increases in particulate air pollutant (PM) concentrations in the previous hours and days have been associated with increased risks of myocardial infarction, but little is known about the relationships between air pollution and specific subsets of myocardial infarction, such as ST-elevation myocardial infarction (STEMI) and non ST-elevation myocardial infarction (NSTEMI).

Methods: Using data from acute coronary syndrome patients with STEMI (n = 338) and NSTEMI (n = 339) and case-crossover methods, we estimated the risk of STEMI and NSTEMI associated with increased ambient fine particle (2.5 um) concentrations, ultrafine particle (10-100 nm) number concentrations, and accumulation mode particle (100-500 nm) number concentrations in the previous few hours and days.

Results: e found a significant 18% increase in the risk of STEMI associated with each 7.1 $\mu g/m^3$ increase in $PM_{2.5}$ concentration in the previous hour prior to acute coronary syndrome onset, with smaller, non-significantly increased risks associated with increased fine particle concentrations in the previous 3, 12, and 24 hours. e found no pattern with NSTEMI. Estimates of the risk of STEMI associated with interquartile range increases in ultrafine particle and accumulation mode particle number concentrations in the previous 1 to 96 hours were all greater than 1.0, but not statistically significant. Patients with pre-existing hypertension had a significantly greater risk of STEMI associated with increased fine particle concentration in the previous hour than patients without hypertension.

Conclusions: Increased fine particle concentrations in the hour prior to acute coronary syndrome onset were associated with an increased risk of STEMI, but not NSTEMI. Patients with pre-existing hypertension and other cardiovascular disease appeared particularly susceptible. Further investigation into mechanisms by which PM can preferentially trigger STEMI over NSTEMI within this rapid time scale is needed.

eywords: Myocardial infarction, Acute coronary syndrome, Epidemiology, Air pollution

Previous studies investigating triggering of myocardial infarction by particulate air pollution (PM) concentrations have, in most cases, reported an increased risk of myocardial infarction associated with increases in PM on the same and previous day 1-9. Similar acute effects of fine particulate air pollution have been reported for other cardiovascular outcomes 10,11. Some studies of myocardial infarction and PM have used symptom onset time, rather than the arrival time at the emergency room, to define myocardial infarction onset, thereby providing a better estimate of the myocardial infarction onset time and less exposure error 1,4,5,7 . Although Peters et al. 4reported a significantly increased risk of myocardial infarction associated with increased fine particle (particles 2.5 μm in diameterPM $_{2.5}$) concentrations in the preceding 2 hours,

* Correspondence: davidrichurmc.rochester.edu
[4]Department of Public Health Sciences, University of Rochester School of Medicine and Dentistry, 265 Crittenden Boulevard, CU 420644, Rochester, NY, USA
Full list of author information is available at the end of the article

144 Biophysical Journal Volume 99 July 2010 144–152

Simulation of Nanoparticle Permeation through a Lipid Membrane

Steven L. Fiedler and Angela Violi*
Department of Mechanical Engineering, University of Michigan, Ann Arbor, Michigan

ABSTRACT A metric of nanoparticle toxicity is the passive permeability rate through cellular membranes. To assess the influence of nanoparticle morphology on this process, the permeability of buckyball-sized molecules through a representative lipid bilayer was investigated by molecular-dynamics simulation. When C_{60} was compared with a prototypical opened C_{60} molecule and a representative combustion-generated particle, $C_{68}H_{29}$, the calculated free-energy profiles along the permeation coordinate revealed a sizable variation in form and depth. The orientation of the anisotropic molecules was determined by monitoring the principal axis corresponding to the largest moment of inertia, and free rotation was shown to be hindered in the bilayer interior. Diffusion constant values of the permeant molecules were calculated from a statistical average of seven to 10 trajectories at five locations along the permeation coordinate. A relatively minor variation of the values was observed in the bilayer interior; however, local resistance values spanned up to 24 orders of magnitude from the water layer to the bilayer center, due primarily to its exponential dependence on free energy. The permeability coefficient values calculated for the three similarly sized but structurally distinct nanoparticles showed a significant variance. The use of C_{60} to represent similarly sized carbonaceous nanoparticles for assessments of toxicity is questioned.

INTRODUCTION

It is known that particle toxicity can scale inversely with size (1). Although the relative detrimental health effects of micrometer-sized particles have received significant attention over the past century, less is known about the potential toxicity of ultrafine particles ($\leq$100 nm). Since nanometer-sized particles (NPs) that are capable of crossing cellular barriers can migrate into systemic circulation, attention is given to factors that influence the permeation process. Additionally, the presence of trapped, hydrophobic NPs can instigate changes in lipid packing and influence the phase behavior of the bilayer (2,3). Reciprocally, the permeability of molecular NPs into lipid bilayers is regulated by the fluidity and composition of the bilayers themselves, as well as the morphology and polarity of the permeant molecules (4,5). It is currently hypothesized that small molecules (molecular mass $<$ 100 amu) hop from dynamic stochastic voids within the bilayer (4). The permeation of NPs larger than the free volumes would be expected to proceed by a different mechanism (6). A clear difference in the permeation of small molecules vis-à-vis NPs has been shown by computational simulation and calculation of the so-called local diffusion constant as a function of permeant depth, z, within the bilayer. Variability of the diffusion constant values of the small molecular permeants with respect to z has been asserted to correlate to membrane heterogeneity, i.e., differences in free volume as a function of permeant position along the bilayer norm. NP diffusion constant values, however, have been observed to be relatively independent of molecular position in bilayer interior (6).

One defined property of both the small molecule and NP permeation process is the tendency of anisotropic molecules to preferentially orient with the major axis aligned parallel to the bilayer norm. This alignment has been observed by NMR (7), fluorescence depolarization (8,9), x-ray diffraction (10), and second harmonic spectroscopic measurements (11), and matched by analysis of molecular-dynamics (MD) simulations (6,12). According to the free-volume model (13), the cross-sectional area of the permeant could then be a key parameter in the diffusion process, since the permeant advancement would be based on encounters with voids exceeding the areal dimensions. Assessment of the alignment also provides a connection to a thermodynamic description of the permeation process (12,14). Conventionally, the translocation of hydrophobic particles into lipid bilayers has been attributed to an overall entropically driven process (15,16), considered to be a consequence of the hydrophobic effect. The size of the permeant molecule, however, has also been shown to influence the physics of aqueous solvation. Simulations have demonstrated that larger solutes are capable of disrupting water hydrogen bonds in the first solvation shell (17). This in turn, can alter the contribution of enthalpy versus entropy to the free energy of permeation or solvation. In a more general sense, evaluation of the total free energy can be used to determine the probability of the permeation process (18) (i.e., the lipophilicity of the permeant), quantitatively map entry barriers (15,19), indicate equilibrated locations of the permeants in the membrane (15), and obtain temporal estimates for membrane/permenat dynamics (14). Partition coefficient values can also be determined by assessing free-energy differences within the rubric of the inhomogeneous solubility-diffusion model (20). As such, the influence of the lipid bilayer microstructure is directly reflected. Similarly,

Submitted February 3, 2010, and accepted for publication March 10, 2010.

*Correspondence: avioli@umich.edu

Editor: Gregory A. Voth.

0006-3495/10/07/0144/9 $2.00

doi: 10.1016/j.bpj.2010.03.039

JOURNAL OF ENVIRONMENTAL SCIENCES 26 (2014) 2027–2033

Available online at www.sciencedirect.com

ScienceDirect

www.journals.elsevier.com/journal-of-environmental-sciences

Unregulated emissions from diesel engine with particulate filter using Fe-based fuel borne catalyst

Hong Zhao[1,*], *Yunshan Ge*[2], *Tiezhu Zhang*[1], *Jipeng Zhang*[1], *Jianwei Tan*[2], *Hongxin Zhang*[1]

1. *College of Mechanical & Electronic Engineering, Qingdao University, Qingdao 266071, China*
2. *National Lab of Auto Performance & Emission Test, Beijing Institute of Technology, Beijing 100081, China*

ARTICLE INFO

Article history:
Received 19 November 2013
Revised 8 April 2014
Accepted 11 April 2014
Available online 10 August 2014

Keywords:
Fuel-borne catalyst
DPF
VOCs
Carbonyl compounds
Particle-phase PAHs

ABSTRACT

The alteration and formation of toxic compounds and potential changes in the toxicity of emissions when using after-treatment technologies have gained wide attention. Volatile organic compound (VOC), carbonyl compound and particle-phase polycyclic aromatic hydrocarbon (PAH) emissions were tested at European Steady State Cycle (ESC) to study unregulated emissions from a diesel engine with a fuel-borne catalyst and diesel particulate filter (FBC–DPF). An Fe-based fuel-borne catalyst was used for this study. According to the results, brake specific emissions of total VOCs without and with DPF were 4.7 and 4.9 mg/kWh, respectively, showing a 4.3% increase. Benzene and n-undecane emissions increased and toluene emission decreased, while other individual VOC emissions basically had no change. When retrofitted with the FBC–DPF, total carbonyl compound emission decreased 15.7%, from 25.8 to 21.8 mg/kWh. The two highest carbonyls, formaldehyde and acetaldehyde, were reduced from 20.0 and 3.7 to 16.5 and 3.3 mg/kWh respectively. The specific reactivity (SR) with DPF was reduced from 6.68 to 6.64 mg/kWh. Total particle-phase PAH emissions decreased 66.4% with DPF compared to that without DPF. However, the Benzo[a]pyrene equivalent (BaP_{eq}) with DPF had increased from 0.016 to 0.030 mg/kWh. Fluoranthene and Pyrene had the greatest decrease, 91.1% and 88.4% respectively. The increase of two- and three-ring PAHs with DPF indicates that the fuel-borne catalyst caused some gas-phase PAHs to adsorb on particles. The results of this study expand the knowledge of the effects of using a particulate filter and a Fe-based fuel-borne catalyst on diesel engine unregulated emissions.

Introduction

Engine emissions constitute an environmental and health hazard. Good alternative fuels including alcohols, liquefied petroleum gas and biodiesel have solved some environmental problems as well as some energy problems (Agarwal, 2007; Gong et al., 2011; He et al., 2010). However, more stringent regulations set forth in the United States, Europe, and other locations motivated the development of after-treatment devices for diesel engines. After-treatment technologies have led to a significant reduction in the emission levels of particulate matter (PM) and nitrogen oxides (NOx). Among PM control technologies, the diesel particulate filter (DPF), which can reduce PM mass emissions more than 90%, has become the most effective strategy for reducing PM emissions (Biswas et al., 2009; Shah et al., 2007; Liu et al., 2012). However, the collected particles must be removed by oxidation to prevent excessive pressure drop in the exhaust system, which would otherwise adversely affect engine operation. Therefore, the ability to regenerate the DPF on which particulates are deposited is considered one of the major issues in diesel engine applications of DPF systems (Song et al., 2006).

* *Corresponding author*. E-mail: qdlizh@163.com (Hong Zhao).

http://dx.doi.org/10.1016/j.jes.2014.08.004
1001-0742/

KKU Res.j. 2014; 19(2) : 354-361
http://resjournal.kku.ac.th

การรับสัมผัสสารเบนซีนในพนักงานสถานีบริการน้ำมันเชื้อเพลิง: กรณีศึกษาเทศบาลนครขอนแก่น เมืองขอนแก่น

Exposure to benzene among workers in gasoline stations: a case study in Khon Kaen municipality, Muang Khon Kaen

ฉัตรสุดา พิมพาแสง[1] สุนิสา ชายเกลี้ยง[2]*
Chatsuda Pimpasaeng[1] Sunisa Chaiklieng[2]*

[1]หลักสูตรสาธารณสุขศาสตรมหาบัณฑิต สาขาอนามัยสิ่งแวดล้อม คณะสาธารณสุขศาสตร์ มหาวิทยาลัยขอนแก่น
[2]ภาควิชาวิทยาศาสตร์อนามัยสิ่งแวดล้อม คณะสาธารณสุขศาสตร์ มหาวิทยาลัยขอนแก่น
[1]MPH program in Environmental Health, Faculty of Public Health, Khon Kaen University, Khon Kaen
[2]Department of Environmental Health Science, Faculty of Public Health, Khon Kaen University, Khon Kaen, Thailand
**Correspondent author: csunis@kku.ac.th*

บทคัดย่อ

การศึกษาเชิงสำรวจนี้มีวัตถุประสงค์เพื่อศึกษาเส้นทางการรับสัมผัสสารเบนซีนในพนักงานสถานีบริการน้ำมันเชื้อเพลิง และประเมินความเข้มข้นของสารเบนซีนในบรรยากาศการทำงานของพนักงานสถานีบริการน้ำมันเชื้อเพลิง โดยใช้กรณีศึกษาในพนักงานบริการน้ำมันเชื้อเพลิง เขตเทศบาลนครขอนแก่น เมืองขอนแก่น (n=34) เก็บข้อมูลโดยใช้การสัมภาษณ์ สำรวจและตรวจวัดปริมาณสารเบนซีนในบรรยากาศ จำนวน 7 สถานี วิเคราะห์โดยใช้วิธีแก๊สโครมาโตกราฟี (GC-FID) พบว่า พนักงานทุกคนมีการสัมผัสไอระเหยน้ำมันที่มีสารเบนซีนผ่านทางเดินหายใจและรองลงมาเป็นทางผิวหนังและทางการกินที่ปนเปื้อนมากับอาหารเท่ากัน คือร้อยละ 97.06 พนักงานตำแหน่งเติมน้ำมันมีโอกาสสัมผัสด้วยความถี่สูงสุดผ่านเส้นทางการกินและการสูดดม ความเข้มข้นของเบนซีนในบรรยากาศการทำงานคือ 0.019 - 0.050 ppm (part per million) คิดเป็นร้อยละ 50.0 ของค่ามาตรฐานของ NIOSH (0.1 ppm) และพนักงานมีการใช้อุปกรณ์ป้องกันอันตรายส่วนบุคคลร้อยละ 26.47 จึงเสนอแนะให้มีอบรมพนักงานด้านความปลอดภัยในการทำงาน และส่งเสริมให้มีการเฝ้าระวังสุขภาพของพนักงาน

Abstract

The aims of this survey study were to investigate route of exposure to benzene and benzene concentrations in working environments of workers in gasoline stations by a case study in Khon Kaen municipality, Khon Kaen province (n=34). Data were collected by a structured questionnaire, a survey form and air monitoring for benzene concentrations from seven stations and analysis with Gas Chromatography (GC-FID). All workers potentially exposed to benzene through gasoline inhalation, followed by direct contact through a skin and food ingestion in an equal percentage (97.06%). Work position of fuelling had the highest frequency on benzene exposure through food ingestion and inhalation route. The concentrations of benzene in working environment were ranged between 0.019 - 0.050 ppm. Those levels did not exceed 50% of recommended exposure limit by NIOSH (0.1 ppm). Most workers did not use personal protective equipment (used mask = 26.47%). The suggestions were that there should be safety at work training provided to workers and employers should perform the health surveillance program among workers annually.

คำสำคัญ:เส้นทางการสัมผัส เบนซีน สถานีบริการน้ำมันเชื้อเพลิง สภาพแวดล้อมการทำงาน
Keywords: route of exposure, benzene, gasoline station, working environment

Effects of long-term exposure to air pollution on natural-cause mortality: an analysis of 22 European cohorts within the multicentre ESCAPE project

Rob Beelen, Ole Raaschou-Nielsen, Massimo Stafoggia, Zorana Jovanovic Andersen, Gudrun Weinmayr, Barbara Hoffmann, Kathrin Wolf, Evangelia Samoli, Paul Fischer, Mark Nieuwenhuijsen, Paolo Vineis, Wei W Xun, Klea Katsouyanni, Konstantina Dimakopoulou, Anna Oudin, Bertil Forsberg, Lars Modig, Aki S Havulinna, Timo Lanki, Anu Turunen, Bente Oftedal, Wenche Nystad, Per Nafstad, Ulf De Faire, Nancy L Pedersen, Claes-Göran Östenson, Laura Fratiglioni, Johanna Penell, Michal Korek, Göran Pershagen, Kirsten Thorup Eriksen, Kim Overvad, Thomas Ellermann, Marloes Eeftens, Petra H Peeters, Kees Meliefste, Meng Wang, Bas Bueno-de-Mesquita, Dorothea Sugiri, Ursula Krämer, Joachim Heinrich, Kees de Hoogh, Timothy Key, Annette Peters, Regina Hampel, Hans Concin, Gabriele Nagel, Alex Ineichen, Emmanuel Schaffner, Nicole Probst-Hensch, Nino Künzli, Christian Schindler, Tamara Schikowski, Martin Adam, Harish Phuleria, Alice Vilier, Françoise Clavel-Chapelon, Christophe Declercq, Sara Grioni, Vittorio Krogh, Ming-Yi Tsai, Fulvio Ricceri, Carlotta Sacerdote, Claudia Galassi, Enrica Migliore, Andrea Ranzi, Giulia Cesaroni, Chiara Badaloni, Francesco Forastiere, Ibon Tamayo, Pilar Amiano, Miren Dorronsoro, Michail Katsoulis, Antonia Trichopoulou, Bert Brunekreef, Gerard Hoek

Summary

Background Few studies on long-term exposure to air pollution and mortality have been reported from Europe. Within the multicentre European Study of Cohorts for Air Pollution Effects (ESCAPE), we aimed to investigate the association between natural-cause mortality and long-term exposure to several air pollutants.

Methods We used data from 22 European cohort studies, which created a total study population of 367 251 participants. All cohorts were general population samples, although some were restricted to one sex only. With a strictly standardised protocol, we assessed residential exposure to air pollutants as annual average concentrations of particulate matter (PM) with diameters of less than 2·5 μm ($PM_{2·5}$), less than 10 μm (PM_{10}), and between 10 μm and 2·5 μm (PM_{coarse}), $PM_{2·5}$ absorbance, and annual average concentrations of nitrogen oxides (NO_2 and NO_x), with land use regression models. We also investigated two traffic intensity variables—traffic intensity on the nearest road (vehicles per day) and total traffic load on all major roads within a 100 m buffer. We did cohort-specific statistical analyses using confounder models with increasing adjustment for confounder variables, and Cox proportional hazards models with a common protocol. We obtained pooled effect estimates through a random-effects meta-analysis.

Findings The total study population consisted of 367 251 participants who contributed 5 118 039 person-years at risk (average follow-up 13·9 years), of whom 29 076 died from a natural cause during follow-up. A significantly increased hazard ratio (HR) for $PM_{2·5}$ of 1·07 (95% CI 1·02–1·13) per 5 μg/m³ was recorded. No heterogeneity was noted between individual cohort effect estimates (I^2 p value=0·95). HRs for $PM_{2·5}$ remained significantly raised even when we included only participants exposed to pollutant concentrations lower than the European annual mean limit value of 25 μg/m³ (HR 1·06, 95% CI 1·00–1·12) or below 20 μg/m³ (1·07, 1·01–1·13).

Interpretation Long-term exposure to fine particulate air pollution was associated with natural-cause mortality, even within concentration ranges well below the present European annual mean limit value.

Funding European Community's Seventh Framework Program (FP7/2007–2011).

Introduction

Studies have shown the effects of long-term exposure to air pollution on mortality,[1,2] with most, especially those in the USA, reporting on the mass concentration of particulate matter (PM) smaller than 10 μm (PM_{10}) or 2·5 μm ($PM_{2·5}$) in diameter. Few European studies have investigated $PM_{2·5}$, partly because of the low availability of routine monitoring data. However, some European studies have shown associations between mortality and nitrogen dioxide (NO_2) or nitrogen oxides (NO_x).[3–8]

In urban areas, NO_2, NO_x, and $PM_{2·5}$ absorbance (a marker for black carbon or soot) have larger spatial concentration contrasts than PM because they are more closely related to motorised traffic. Interest in the health effects of coarse particles (2·5–10 μm in diameter) has also increased.[9] However, the comparability of previous studies is limited by the different exposure methods used.[10]

In the framework of the multicentre European Study of Cohorts for Air Pollution Effects (ESCAPE), we added standardised exposure assessment for PM, NO_2, and NO_x to health data from 22 ongoing cohort studies across Europe. The objective of ESCAPE was to investigate the association between long-term exposure to air pollution and mortality. In this Article, we report associations for natural-cause mortality. Cause-specific results will be published separately.

Published **Online** December 9, 2013 http://dx.doi.org/10.1016/S0140-6736(13)62158-3

See **Online/Comment** http://dx.doi.org/10.1016/S0140-6736(13)62570-2

Institute for Risk Assessment Sciences, Utrecht University, Utrecht, Netherlands (R Beelen PhD, M Eeftens MSc, K Meliefste BSc, M Wang MSc, Prof B Brunekreef PhD, G Hoek PhD); **Danish Cancer Society Research Center, Copenhagen, Denmark** (O Raaschou-Nielsen PhD, Z J Andersen PhD, K T Eriksen PhD); **Department of Epidemiology, Lazio Regional Health Service, Rome, Italy** (M Stafoggia MSc, G Cesaroni MSc, C Badaloni MSc, F Forastiere MD); **Center for Epidemiology and Screening, Department of Public Health, University of Copenhagen, Copenhagen, Denmark** (Z J Andersen); **Institute of Epidemiology and Medical Biometry, Ulm University, Ulm, Germany** (G Weinmayr PhD, Prof G Nagel PhD); **IUF – Leibniz Research Institute for Environmental Medicine, Germany and Medical Faculty, University of Düsseldorf, Düsseldorf, Germany** (G Weinmayr, Prof B Hoffman MD, D Sugiri MSc, Prof U Krämer PhD); **Institute of Epidemiology II, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany** (K Wolf PhD, Prof A Peters PhD, R Hampel PhD); **Department of Hygiene, Epidemiology and Medical**

Environment International 66 (2014) 1–10

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

Review

Ultrafine particles in cities

Prashant Kumar [a,b,*], Lidia Morawska [c], Wolfram Birmili [d], Pauli Paasonen [e,f], Min Hu [g], Markku Kulmala [e], Roy M. Harrison [h,i], Leslie Norford [j], Rex Britter [k]

[a] Department of Civil and Environmental Engineering, Faculty of Engineering and Physical Sciences (FEPS), University of Surrey, Guildford GU2 7XH, United Kingdom
[b] Environmental Flow (EnFlo) Research Centre, FEPS, University of Surrey, Guildford GU2 7XH, United Kingdom
[c] International Laboratory for Air Quality and Health, Queensland University of Technology, 2 George Street, Brisbane, Qld 4001, Australia
[d] Leibniz Institute for Tropospheric Research, Permoserstraße 15, 04318 Leipzig, Germany
[e] Department of Physics, University of Helsinki, 00014 Helsinki, Finland
[f] International Institute for Applied Systems Analysis (IIASA), Laxenburg, Austria
[g] State Key Joint Laboratory of Environmental Simulation and Pollution Control, College of Environmental Sciences and Engineering, Peking University, Beijing 100871, China
[h] Division of Environmental Health & Risk Management, School of Geography, Earth & Environmental Sciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, United Kingdom
[i] Department of Environmental Sciences / Center of Excellence in Environmental Studies, King Abdulaziz University, Jeddah, 21589, Saudi Arabia
[j] Department of Architecture, Massachusetts Institute of Technology, Boston, MA 02139, USA
[k] Urban Studies and Planning, Massachusetts Institute of Technology, Cambridge, MA 02139, USA

ARTICLE INFO

Article history:
Received 21 November 2013
Accepted 16 January 2014
Available online xxxx

Keywords:
City environment
Particle exposure
Health impacts
Particle number concentration
Ultrafine particles

ABSTRACT

Ultrafine particles (UFPs; diameter less than 100 nm) are ubiquitous in urban air, and an acknowledged risk to human health. Globally, the major source for urban outdoor UFP concentrations is motor traffic. Ongoing trends towards urbanisation and expansion of road traffic are anticipated to further increase population exposure to UFPs. Numerous experimental studies have characterised UFPs in individual cities, but an integrated evaluation of emissions and population exposure is still lacking. Our analysis suggests that the average exposure to outdoor UFPs in Asian cities is about four-times larger than that in European cities but impacts on human health are largely unknown. This article reviews some fundamental drivers of UFP emissions and dispersion, and highlights unresolved challenges, as well as recommendations to ensure sustainable urban development whilst minimising any possible adverse health impacts.

Contents

1. Introduction

Whilst cities are facing challenges in addressing the problem of conventional air pollutants that are part of current regulatory frameworks, the emergence of unregulated pollutants, such as airborne ultrafine particles (UFPs; diameter less than 100 nm), has added an additional

* Corresponding author at: Department of Civil and Environmental Engineering, Faculty of Engineering and Physical Sciences (FEPS), University of Surrey, Guildford GU2 7XH, United Kingdom. Tel.: +44 1483 682762; fax: +44 1483 682135.
E-mail addresses: P.Kumar@surrey.ac.uk, Prashant.Kumar@cantab.net (P. Kumar).

0160-4120/$ – see front matter © 2014 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.envint.2014.01.013

(Choi et al., 2009; Kumar et al., 2010c). Their unique, highly reactive physicochemical characteristics are of particular concern in terms of human health (Xia et al., 2009), but very little is currently known about their concentration levels in the European environment, and even lesser for Asian environments (Kumar et al., 2012). A proactive research approach is required to fully reveal their injurious effects and at the same time, targeted efforts are needed to quantify their ambient concentrations by developing instruments that are able to distinguish them from the UFPs produced by other sources, such as traffic.

Acknowledgements

PK thanks the EPSRC and the University of Surrey for the DTA and instrument grants, respectively. RB and LN acknowledge the support of the Singapore National Research Foundation through the Singapore-MIT Alliance for Research and Technology (SMART), Center for Environmental Sensing and Modeling (CENSAM).

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.envint.2014.01.013.

References

AEA. UK emissions of air pollutants 1970 to 2008. Department for Environment, Food and Rural Affairs; 2010 [http://uk-air.defra.gov.uk/reports/cat07/1009030925_2008_Report_final270805.pdf].
Alam A, Shi JP, Harrison RM. Observations of new particle formation in urban air. J Geophys Res 2003;108:4093–107.
Andersen ZJ, Wahlin P, Raaschou-Nielsen O, Ketzel M, Scheike T, Loft S. Size distribution and total number concentration of ultrafine and accumulation mode particles and hospital admissions in children and the elderly in Copenhagen Denmark. Occup Environ Med 2008;65:458–66.
Atkinson RW, Fuller GW, Anderson HR, Harrison RM, Armstrong B. Urban ambient particle metrics and health: a time-series analysis. Epidemiology 2010;21:501–11.
Beddows DCS, Harrison RM. Comparison of average particle number emission factors for heavy and light duty vehicles derived from rolling chassis dynamometer and field studies. Atmos Environ 2008;42:7954–66.
Beddows DCS, Dall'Osto M, Harrison RM, Kulmala M, Asmi A, Wiedensohler A, et al. Variations in tropospheric submicron particle size distributions across the European continent 2008–2009. Atmos Chem Phys Discuss 2013;13:31197–249.
Birmili W, Tomsche L, Sonntag A, Opelt C, Weinhold K, Nordmann S, et al. Variability of aerosol particles in the urban atmosphere of Dresden (Germany): effects of spatial scale and particle size. Meteorol Z 2013;22:195–211.
Buccolieri R, Sandberg M, Di Sabatino S. City breathability and its link to pollutant concentration distribution within urban-like geometries. Atmos Environ 2010;44:1894–903.
Buonanno G, Fuoco FC, Stabile L. Influential parameters on particle exposure of pedestrians in urban microenvironments. Atmos Environ 2011a;45:1434–43.
Buonanno G, Giovinco G, Morawska L, Stabile L. Tracheobronchial and alveolar dose of submicrometer particles for different population age groups in Italy. Atmos Environ 2011b;45:6216–24.
Buonanno G, Marini S, Morawska L, Fuoco FC. Individual dose and exposure of Italian children to ultrafine particles. Sci Total Environ 2012a;438:271–7.
Buonanno G, Morawska L, Stabile L, Wang L, Giovinco G. A comparison of submicrometer particle dose between Australian and Italian people. Environ Pollut 2012b;169:183–9.
Buonanno G, Fuoco FC, Morawska L, Stabile L. Airborne particle concentrations at schools measured at different spatial scales. Atmos Environ 2013;67:38–45.
Carpentieri M, Kumar P. Ground-fixed and on-board measurements of nanoparticles in the wake of a moving vehicle. Atmos Environ 2011;45:5837–52.
Choi J-Y, Ramachandran G, Kandlikar M. The impact of toxicity testing costs on nanomaterial regulation. Environ Sci Technol 2009;43:3030–4.
Choi W, Hu S, He M, Kozawa K, Mara S, Winer AM, et al. Neighborhood-scale air quality impacts of emissions from motor vehicles and aircraft. Atmos Environ 2013;80:310–21.
Costabile F, Birmili W, Klose S, Tuch T, Wehner B, Wiedensohler A, et al. Spatio-temporal variability and principal components of the particle number size distribution in an urban atmosphere. Atmos Chem Phys. 2009;9:3163–95.
Dall'Osto M, Thorpe A, Beddows DCS, Harrison RM, Barlow JF, Dunbar T, et al. Remarkable dynamics of nanoparticles in the urban atmosphere. Atmos Chem Phys 2011;11:6623–37.
Defra. Adapting to climate change in England: a framework for action PB13137. London: Department for Environmental and Rural Affairs HM Government; 2008 [http://www.comeap.org.uk/images/stories/Documents/Reports/comeap%20the%20mortality%20effects%20of%20long-term%20exposure%20to%20particulate%20air%20pollution%20in%20the%20uk%202010.pdf;].
Eastwood P. Particulate emissions from vehicles. Chichester: Wiley; 2008.
EC. Directive on ambient air quality and cleaner air for Europe (Directive 2008/50/EC). http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2008:152:0001:0044:EN:PDF, 2008.
Ehn M, Junninen H, Petaja T, Kurten T, Kerminen V-M, Schobesberger S, et al. Composition and temporal behavior of ambient ions in the boreal forest. Atmos Chem Phys 2010;25:14897–946.
Fontaras G, Karavalakis G, Kousoulidou M, Tzamkiozis T, Ntziachristos L, Bakeas E, et al. Effects of biodiesel on passenger car fuel consumption, regulated and non-regulated pollutant emissions over legislated and real-world driving cycles. Fuel 2009;88:1608–17.
Fruin S, Westerdahl D, Sax T, Sioutas C, Fine PM. Measurements and predictors of on-road ultrafine particle concentrations and associated pollutants in Los Angeles. Atmos Environ 2008;42:207–19.
Fujitani Y, Kumar P, Tamura K, Fushimi A, Hasegawa S, Takahashi K, et al. Seasonal differences of the atmospheric particle size distribution in a metropolitan area in Japan. Sci Total Environ 2012;437:339–47.
Gurjar BR, Jain A, Sharma A, Agarwal A, Gupta P, Nagpure AS, et al. Human health risks in megacities due to air pollution. Atmos Environ 2010;44:4606–13.
Hamed A, Joutsensaari J, Mikkonen S, Sogacheva L, Dal Maso M, Kulmala M, et al. Nucleation and growth of new particles in Po Valley, Italy. Atmos Chem Phys 2007;7:355–76.
Harris SJ, Maricq MM. Signature size distributions for diesel and gasoline engine exhaust particulate matter. J Aerosol Sci. 2001;32:749–64.
Harrison RM, Shi JP, Xi S, Khan A, Mark D, Kinnersley R, et al. Measurement of number, mass and size distribution of particles in the atmosphere. Philos Trans R Soc Lond A 2000;358:2567–80.
Harrison RM, Giorio C, Beddows DC, Dall'Osto M. Size distribution of air-borne particles controls outcomes of epidemiological studies. Sci Total Environ 2010;409:289–93.
Harrison RM, Beddows DCS, Dall'Osto M. PMF analysis of wide-range particle size spectra collected on a major highway. Environ Sci Technol 2011;45:5522–8.
Heal MR, Kumar P, Harrison RM. Particles, air quality, policy and health. Chem Soc Rev 2012;41:6606–30.
HEI. HEI review panel on ultrafine particles. Understanding the health effects of ambient ultrafine particles. HEI Perspectives 3. Boston, MA: Health Effects Institute; 2013122 [http://pubs.healtheffects.org/getfile.php?u=893 (accessed 127 August 2013);].
Holmes N. A review of particle formation events and growth in the atmosphere in the various environments and discussion of mechanistic implications. Atmos Environ 2007;41:2183–201.
ICRP. ICRP publication 66: human respiratory tract model for radiological protection. A report of a task group of the International Commission on Radiological Protection; 1994. p. 1–482.
Int Panis L, de Geus B, Vandenbulcke G, Willems H, Degraeuwe B, Bleux N, et al. Exposure to particulate matter in traffic: a comparison of cyclists and car passengers. Atmos Environ 2010;44:2263–70.
Jones AM, Harrison RM, Barratt B, Fuller G. A large reduction in airborne particle number concentrations at the time of the introduction of "sulphur free" diesel and the London Low Emission Zone. Atmos Environ 2012;50:129–38.
Kaur S, Nieuwenhuijsen M, Colvile R. Personal exposure of street canyon intersection users to PM2.5, ultrafine particle counts and carbon monoxide in Central London, UK. Atmos Environ 2005;39:3629–41.
Kaur S, Clark RDR, Walsh PT, Arnold SJ, Colvile RN, Nieuwenhuijsen MJ. Exposure visualisation of ultrafine particle counts in a transport microenvironment. Atmos Environ 2006;40:386–98.
Keogh DU, Ferreira L, Morawska L. Development of a particle number and particle mass vehicle emissions inventory for an urban fleet. Environ Model Software 2009;24:1323–31.
Ketzel M, Wahlin P, Kristensson A, Swietlicki E, Berkowicz R, Nielsen OJ, et al. Particle size distribution and particle mass measurements at urban, near-city and rural level in the Copenhagen area and Southern Sweden. Atmos Chem Phys 2004;4:281–92.
Knibbs LD, Cole-Hunter T, Morawska L. A review of commuter exposure to ultrafine particles and its health effects. Atmos Environ 2011;45:2611–22.
Kuang C, McMurry PH, McCormick AV, Eisele FL. Dependence of nucleation rates on sulfuric acid vapor concentration in diverse atmospheric locations. J Geophys Res 2008;113:D10209.
Kulmala M, Pijrola L, Makela JM. Stable sulphate clusters as a source of new atmospheric particles. Nature 2000;404:66–9.
Kulmala M, Vehkamaki H, Petaja T, Dal Maso M, Lauri A, Kerminen V-M, et al. Formation and growth rates of ultrafine atmospheric particles: a review of observations. J Aerosol Sci. 2004;35:143–76.
Kulmala M, Kontkanen J, Junninen H, Lehtipalo K, Manninen HE, Nieminen T, et al. Direct observations of atmospheric aerosol nucleation. Science 2013;339:943–6.
Kumala M, Asmi A, Lappalainen HK, Baltensperger U, Brenguier J-L, Facchini MC, et al. General overview: general overview European Integrated project on Aerosol Cloud Climate and Air Quality interactions (EUCAARI) — integrating aerosol research from nano to global scales. Atmos Chem Phys 2011;11:13061–143.
Kumar P, Fennell P, Britter R. Effect of wind direction and speed on the dispersion of nucleation and accumulation mode particles in an urban street canyon. Sci Total Environ 2008a;402:82–94.
Kumar P, Fennell P, Langley D, Britter R. Pseudo-simultaneous measurements for the vertical variation of coarse, fine and ultra fine particles in an urban street canyon. Atmos Environ 2008b;42:4304–19.
Kumar P, Fennell P, Hayhurst A, Britter RE. Street versus rooftop level concentrations of fine particles in a Cambridge street canyon. Bound-Layer Meteorol 2009a;131:3–18.
Kumar P, Robins A, Britter R. Fast response measurements of the dispersion of nanoparticles in a vehicle wake and a street canyon. Atmos Environ 2009b;43:6110–8.

Kumar P, Robins A, Vardoulakis S, Britter R. A review of the characteristics of nanoparticles in the urban atmosphere and the prospects for developing regulatory controls. Atmos Environ 2010a;44:5035–52.
Kumar P, Robins A, ApSimon H. Nanoparticle emissions from biofuelled vehicles — their characteristics and impact on the number-based regulation of atmospheric particles. Atmos Sci Lett 2010b;11:327–31.
Kumar P, Fennell P, Robins A. Comparison of the behaviour of manufactured and other airborne nanoparticles and the consequences for prioritising research and regulation activities. J Nanopart Res 2010c;12:1523–30.
Kumar P, Gurjar BR, Nagpure A, Harrison RM. Preliminary estimates of nanoparticle number emissions from road vehicles in megacity Delhi and associated health impacts. Environ Sci Technol 2011a;45:5514–21.
Kumar P, Ketzel M, Vardoulakis S, Pirjola L, Britter R. Dynamics and dispersion modelling of nanoparticles from road traffic in the urban atmospheric environment — a review. J Aerosol Sci. 2011b;42:580–603.
Kumar P, Robins A, Vardoulakis S, Quincey P. Technical challenges in tackling regulatory concerns for urban atmospheric nanoparticles. Particuology 2011c;9:566–71.
Kumar P, Kumar A, Lead JR. Nanoparticles in the Indian environment: known, unknowns and awareness. Environ Sci Technol 2012;46:7071–2.
Kumar P, Jain S, Gurjar BR, Sharma P, Khare M, Morawska L, et al. New directions: can a "blue sky" return to Indian megacities? Atmos Environ 2013a;71:198–201.
Kumar P, Pirjola L, Ketzel M, Harrison RM. Nanoparticle emissions from 11 non-vehicle exhaust sources — a review. Atmos Environ 2013b;67:252–77.
Kumar P, Morawska L, Harrison RM. Nanoparticles in European cities and associated health impacts. In: Viana M, editor. Urban air quality in Europe. The Handbook of Environmental ChemistrySpringer Berlin Heidelberg; 2013c. p. 339–65. [http://dx.doi.org/10.1007/698_2012_161].
Liu H, Rönkkö T, Keskinen J. Impact of vehicle development and fuel quality on exhaust nanoparticle emissions of traffic. Environ Sci Technol 2013;47:8091–2.
Lowry GV, Gregory KB, Apte SC, Lead JR. Transformations of nanomaterials in the environment. Environ Sci Technol 2012;46:6893–9.
Mazaheri M, Clifford S, Jayaratne R, Megat Mokhtar MA, Fuoco F, Buonanno G, et al. School children's personal exposure to ultrafine particles in the urban environment. Environ Sci Technol 2014;48:113–20.
Mejia JF, Morawsk L, Mengersen K. Spatial variation in particle number size distributions in a large metropolitan area. Atmos Chem Phys 2008;8:1127–38.
Meng X, Ma Y, Chen R, Zhou Z, Chen B, Kan H. Size-fractionated particle number concentrations and daily mortality in a Chinese city. Environ Health Perspect 2013;121:1174–8.
Mirme A, Tamm E, Mordas G, Vana M, Uin J, Mirme S, et al. A wide-range multi-channel air ion spectrometer. Boreal Environ Res 2007;12:247–64.
Mishra VK, Kumar P, Van Poppel M, Bleux N, Frijns E, Reggente M, et al. Wintertime spatio-temporal variation of ultrafine particles in a Belgian city. Sci Total Environ 2012;431:307–13.
Monkkonen P, Koponen IK, Lehtinen KEJ, Hameri K, Uma R, Kulmala M. Measurements in a highly polluted Asian mega city: observations of aerosol number size distribution, modal parameters and nucleation events. Atmos Chem Phys 2005a;5:57–66.
Monkkonen P, Pai P, Maynard A, Lehtinen KEJ, Hameri K, Rechkemmer P, et al. Fine particle number and mass concentration measurements in urban Indian households. Sci Total Environ 2005b;347:131–47.
Morawska L, Wang H, Ristovski Z, Jayaratne ER, Johnson G, Cheung HC, et al. JEM spotlight: environmental monitoring of airborne nanoparticles. J Environ Monit 2009;11:1758–73.
Nel A, Xia T, Mädler L, Li N. Toxic potential of materials at the nanolevel. Science 2006;311:622–7.
O'Dowd CD, Aalto P, Hameri K, Kulmala M, Hoffmann T. Aerosol formation: atmospheric particles from organic vapours. Nature 2002;416:497–8.
Paasonen P, Visshedjik A, Kupiainen K, Klimont Z, van der Gon HD, Kulmala M, et al. Aerosol particle number emissions and size distributions: implementation in the GAINS model and initial results. IIASA interim report; 2013.
Pathak AK, Yadav S, Kumar P, Kumar R. Source apportionment and spatial-temporal variations in the metal content of surface dust collected from an industrial area adjoining Delhi, India. Sci Total Environ 2012;443:662–72.
Peters A, Wichmann HE, Tuch T, Heinrich J, Heyder J. Respiratory effects are associated with the number of ultrafine particles. Am J Respir Crit Care Med 1997;155:1376–83.
Pirjola L, Paasonen P, Pfeiffer D, Hussein T, Hämeri K, Koskentalo T, et al. Dispersion of particles and trace gases nearby a city highway: mobile laboratory measurements in Finland. Atmos Environ 2006;40:867–79.
Qian S, Sakurai H, McMurry PH. Characteristics of regional nucleation events in urban East St. Louis. Atmos Environ 2007;41:4119–27.
Quiros DC, Zhang Q, Choi W, He M, Paulson SE, Winer AM, et al. Air quality impacts of a scheduled 36-h closure of a major highway. Atmos Environ 2013;67:404–14.
Reche C, Querol X, Alastuey A, Viana M, Pey J, Moreno T, et al. New considerations for PM, black carbon and particle number concentration for air quality monitoring across different European cities. Atmos Chem Phys 2011;11:6207–27.
Reddington CL, Carslaw KS, Spracklen DV, Frontoso MG, Collins L, Merikanto J, et al. Primary versus secondary contributions to particle number concentrations in the European boundary layer. Atmos Chem Phys 2011;11:12007–120036.
Rimnácová D, Zdímal V, Schwarz J, Smolík J, Rimnác M. Atmospheric aerosols in suburb of Prague: the dynamics of particle size distributions. Atmos Res 2011;101:539–52.
Rückerl R, Schneider A, Breitner S, Cyrys J, Peters A. Health effects of particulate air pollution: a review of epidemiological evidence. Inhal Toxicol 2011;23:555–92.
Sabaliauskas K, Jeong, C-H, Yao X, Jun Y-S, Jadidian P, Evans GJ. Five-year roadside measurements of ultrafine particles in a major Canadian city. Atmos Environ 2012;49:245–56.
Shah AP, Pietropaoli AP, Frasier LM, Speers DM, Chalupa DC, Delehanty JM, et al. Effect of inhaled carbon ultrafine particles on reactive hyperemia in healthy human subjects. Environ Health Perspect 2008;116:375–80.
Sharma P, Sharma P, Jain S, Kumar P. An integrated statistical approach for evaluating the exceedence of criteria pollutants in the ambient air of megacity Delhi. Atmos Environ 2013;70:7–17.
Shi JP, Evans DE, Khan AA, Harrison RM. Sources and concentration of nanoparticles (<10 nm diameter) in the urban atmosphere. Atmos Environ 2001;35:1193–202.
Stanier C, Khlystov A, Pandis S. Nucleation events during the Pittsburgh air quality study: description and relation to key meteorological, gas phase, and aerosol parameters. Aerosol Sci Technol 2004;38:253–64.
Stölzel M, Breitner S, Cyrys J, Pitz M, Wölke G, Kreyling W, et al. Daily mortality and particulate matter in different size classes in Erfurt, Germany. J Exposure Sci Environ Epidemiol 2007;17:458–67.
Vanhanen J, Mikkila J, Lehtipalo K, Sipila M, Manninen HE, Siivola E, et al. Particle size magnifier for nano-CN detection. Aerosol Sci Technol 2011;45:533–42.
Wåhlin P. Measured reduction of kerbside ultrafine particle number concentrations in Copenhagen. Atmos Environ 2009;43:3645–7.
Wallace L, Ott W. Personal exposure to ultrafine particles. J Exposure Sci Environ Epidemiol 2011;21:20–30.
Wang ZB, Hu M, Sun JY, Wu ZJ, Yue DL, Shen XJ, et al. Characteristics of regional new particle formation in urban and regional background environments in the North China Plain. Atmos Chem Phys Discuss 2013;13:20531–60.
WHO. Review of evidence on health aspects of air pollution — REVIHAAP. World Health Organisation, Regional Office for Europe; 201333 [http://www.euro.who.int/__data/assets/pdf_file/0020/182432/e96762-final.pdf (accessed 09 September 2013)].
Wu ZJ, Hu M, Liu S, Wehner B, Bauer S, Maßling A, et al. New particle formation in Beijing, China: statistical analysis of a 1-year data set. J Geophys Res 2007;112:D09209.
Wu Z, Hu M, Lin P, Liu S, Wehner B, Wiedensohler A. Particle number size distribution in the urban atmosphere of Beijing, China. Atmos Environ 2008;42:7967–80.
Xia T, Li N, Nel AE. Potential health impact of nanoparticles. Annu Rev Public Health 2009;30:137–50.
Zhu Y, Hinds WC, Kim S, Shen S, Sioutas C. Study of ultrafine particles near a major highway with heavy-duty diesel traffic. Atmos Environ 2002;36:4323–35.

References about health effects due to exposures to atmospheric pollution, traffic-, or vehicle emissions.

- **Cell cycle alterations induced by urban PM2.5 in bronchial epithelial cells: characterization of the process and possible mechanisms involved**, Eleonora Longhin, Jørn A Holme, Kristine B Gutzkow, Volker M Arlt, Jill E Kucab, Marina Camatini, Maurizio Gualtieri; *Particle and Fibre Toxicology* (December 2013), Vol. 10 (63), doi: 10.1186/1743-8977-10-63.
- **Exposure to vehicle emissions results in altered blood brain barrier permeability and expression of matrix metalloproteinases and tight junction proteins in mice**, Hannah A Oppenheim, JoAnn Lucero, Anne-Cécile Guyot, Lindsay M Herbert, Jacob D McDonald, Aloïse Mabondzo, Amie K Lund; *Particle and Fibre Toxicology* (December 2013), Vol. 10 (62), doi: 10.1186/1743-8977-10-62.
- **Increasing emergency room visits for stroke by elevated levels of fine particulate constituents**, Szu-Ying Chen, Yu-Lun Lin, Wei-Tien Chang, Chung-Te Lee, Chang-Chuan Chan; *Science of The Total Environment* (1 March 2014), Vol. 473-474, pp. 446-450, doi: 10.1016/j.scitotenv.2013.12.035.
- **Patterns of traffic polycyclic aromatic hydrocarbon pollution in mountain areas can be revealed by lichen biomonitoring: A case study in the Dolomites (Eastern Italian Alps)**, Juri Nascimbene, Mauro Tretiach, Federica Corana, Fiorella Lo Schiavo, et al.; *Science of The Total Environment* (15 March 2014), Vol. 475, pp. 90-96, doi: 10.1016/j.scitotenv.2013.12.090.
- **Health risk assessment for residents exposed to atmospheric diesel exhaust particles in southern region of Taiwan**, Chia-Pin Chio, Chung-Min Liao, Ying-I Tsai, Man-Ting Cheng, Wei-Chun Chou; *Atmospheric Environment* (March 2014), Vol. 85, pp. 64-72, doi: 10.1016/j.atmosenv.2013.11.072.
- **Detailed diesel exhaust characteristics including particle surface area and lung deposited dose for better understanding of health effects in human chamber exposure studies**, Aneta Wierzbicka, Patrik T. Nilsson, Jenny Rissler, Gerd Sallsten, et al.; *Atmospheric Environment* (April 2014), Vol. 86, pp. 212-219, doi: 10.1016/j.atmosenv.2013.11.025

18th ETH Conference on Combustion Generated Nanoparticles Zürich, Switzerland, June 2014

	Session 6 A: Health Effects 16.40 – 18.30 Chair: Gehr P.
1	Von Garnier Chr. / Inselspital Bern, Switzerland *Health Effects of Nanoparticles in Susceptible Persons*
2	Gerlofs-Nijland M. / RIVM, The Netherlands *Health Effects of Combustion Sources in Perspective*
3	Amini H. / Kurdistan University of Medical Science, Iran *Estimating Spatial Variability of Ambient Particulate Matter Using Land-use Regression in Tehran*
4	Weise F. / NMI, Reutlingen, Germany *Toxic Effects of Nanoparticles from Biomass Combustion*
5	Violi A. / University of Michigan USA *How Chemical Composition of Nanoparticles Affects Interactions with Biological Systems*
6	Mayer A. / TTM Switzerland *PN versus PM: which Metric for Emission Limits and Air Quality Limits*

	Session 6 B: Health Effects 08.00 – 09.50 Chair: Rothen-Rutishauser B.
7	Geiser M. / University of Bern, Switzerland *Responses of Healthy and Diseased Airway Epithelia to Aged Aerosols from Wood Combustion*
8	Heeb N. / EMPA, Switzerland *Catalysis – a Key Property of Diesel Particle Filters to Lower Emissions of Genotoxic Compounds*
9	Steiner S. / Adolphe Merkle Institute, Fribourg, Switzerland *In-vitro Genotoxicity of Diesel Exhausts: Impact of Filtration and Catalysis*
10	Zarcone M. / Leiden University Medical Center and TNO, The Netherlands *Development of an Innovative in Vitro Inhalation Model for Studying the Effects of Diesel Exhaust*
11	Peters A. / Helmholtz Zentrum München, Germany *Health Effects of Ambient Ultrafine Particles – Do we know enough?*

FOCUS-Event

Field Inspection of Vehicle Emissions with Particle Number-based Instrumentation

	Focus Event Part 1: PN-PEMS for Vehicle Type Approval Chair: Leuenberger Chr.	**13.30 – 14.30**
1	Kasper M. *Introduction*	
2	Riccobono F. / JRC *How to Extend the Real Drive Emission Test Procedure to Particle Number*	
3	Cachón L. / Matter Aerosol AG, Switzerland *The Golden PEMS: Technical Aspects and Outlook*	

COFFEE BREAK **14.30 – 15.00**

	Focus Event Part 2 : Portable PN Instrumentation for Field Inspection Chair: Leuenberger Chr.	**15.00 - 16.40**
4	Krähenbühl S. / Federal Office for the Environment, Switzerland *New Instruments for PN-based Periodic Inspection: Results of a First Measurement Campaign*	
5	Andres H. / METAS, Switzerland *Field Measurement Instruments Ordinance: Calibration, Certification, Measurement Cycle*	
6	Horn H.-G. / TSI, USA. *Field Measurement, Technical Aspects of the First Generation PN Field Instrument*	
7	Fierz M. / FHNW, Switzerland *Towards Hand-Held DPF Inspection*	
8	Leuenberger Chr. *Conclusions*	

Concluding Remarks: Burtscher H. **16.45**

End of the 18th ETH-NPC **17.00**

WMA Statement on the Prevention of Air pollution due to Vehicle Emissions

Adopted by the 65th World Medical Assembly, Durban, South Africa, October 2014

Preamble

There are a number of ways in which the volume of harmful emissions can be reduced. These include encouraging fewer road traffic journeys, active transport for individuals undertaking relatively short journeys, the use of mass public transit in preference to individual vehicles, and alternative energy sources for vehicles, including electric and hybrid technologies. Where vehicle use is essential, means of reducing harmful emissions should be used.

Physicians around the world are aware of air pollution. It impacts the quality of life for hundreds of millions of people worldwide, causing both, a large burden of disease as well as economic losses and increased health care costs. According to WHO estimates, in 2012, urban outdoor air pollution was responsible for 3.7 million annual deaths, representing 6.7% of the total deaths (WHO, 2014).

Especially, diesel soot is acknowledged as a proven carcinogen (IARC, 07/2012). Furthermore, it has many other toxic effects, most prominently in the cardiovascular (Brook et al., 2010) and respiratory systems (ERS, 2010). Moreover, in the context of global warming, soot, along with methane, is identified as the second most important greenhouse driving force substance after CO2 (Kerr, 2013).

Despite the fact that new vehicles will have to comply with stricter emission standards which take into account most harmful ultra fine particles too, a high-polluting in-use fleet, including off-road vehicles such as construction engines and ships, will continue polluting for many more years.

Background

In many densely populated cities around the world, fine dust concentrations measurable as aerosols exceed up to 50 times the maximum WHO recommendation. High volumes of transport, power generated from coal, and pollution caused by construction machinery are among the contributing factors. People living and working near major (high density volume traffic) streets are most affected by pollutants.

For fighting the health risks mentioned above, there exist a variety of highly efficient and reliable filter systems on the market (Best Available Technology (BAT) filters[1]). They are applicable to all internal combustion engines and they reduce even most harmful ultra-fine particles by a factor of over one hundred.
As soon as 90% of heavy duty vehicles, both, new and upgraded ones, satisfy this standard, health problems attributable to emissions of heavy duty traffic will be greatly reduced, and no further tightening of emission standards will be possible or even needed at all because of an almost total elimination of the pollutant as such.

In a variety of countries on different continents and under varying conditions retrofit or upgrading programs have been successfully performed. The UN's Working Party on Pollution Prevention and Energy in Geneva has just proposed a technical standard for regulation in their member states, which will be applicable worldwide.

The WMA supports these efforts and calls on policy makers in all countries, especially in urban regions, to introduce regulatory restrictions of access for vehicles without filter, and/or to provide financial assistance to support the retrofitting of in-use vehicles.

Recommendations

The WMA therefore recommends that all NMAs should encourage their respective governments to:

1. Introduce BAT standards for all new diesel vehicles (on road and off-road)
2. Incentivise retrofitting with BAT filters for all in-use engines
3. Monitor and limit the concentration of nanosize soot particles in the urban breathing air
4. Conduct epidemiological studies detecting and differentiating the health effects of ultrafine particles
5. Build professional and public awareness of the importance of diesel soot and the existing methods of eliminating the particles
6. Contribute to developing strategies to protect people from soot particles in aircraft passenger cabins, trains, homes and in the general environment. These strategies should include plans to develop and increase use of public transportation systems.

Abbreviations:

EPA: Environmental Protection Agency (US)

ERS: European Respiratory Society

IARC: International Agency for Research of Cancer

BAT Standards: Emission standards for passenger cars, heavy-duty vehicles and off-road machinery, based on count of ultrafine particles rather than mass and aimed at the protection of human health from the most hazardous soot particles, the lung and even cell membrane penetrating ultra-fines.

References:

- Brook, Robert D. et al. (2010): AHA Scientific Statement: Particulate Matter Air Pollution and Cardiovascular Disease. An Update to the Scientific Statement from the American Heart Association. Circulation 121: 2331-2378.
- ERS (2010): The ERS report on air pollution and public health. European Respiratory Society, Lausanne, Switzerland. ISBN: 978-1-84984-008-8
- IARC (2012): "IARC: Diesel Engine Exhaust Carcinogenic". Press Release No. 213. http://www.iarc.fr/en/media-centre/pr/2012/pdfs/pr213_E.pdf . (access: 14/02/14)
- Kerr, Richard R. (2013): "Soot is Warming the World Even More Than Thought". In: Science 339(6118), p. 382.
- WHO (2014): "Burden of disease from Ambient Air Pollution for 2012." http://www.who.int/phe/health_topics/outdoorair/databases/AAP_BoD_results_March2014.pdf?ua=1 (access: 26/08/14)

[1] Euro 6/VI, US/EPA/CARB, Chinese and equivalent standards.

Executive Bulletin

2200 Wilson Boulevard * Suite 310 * Arlington, VA 22201 * (202) 296-4797 * www.meca.org

For MECA Members Only

May 27, 2014

SPECIAL REPORT

Health Effects Institute's 2014 Annual Conference in Alexandria, VA

The Health Effects Institute (HEI) held its annual conference on May 4-6, 2014 in Alexandria, VA. MECA's Jamie Song attended the conference.

Topics discussed at the conference included:

Presentation slides from this conference are available at: http://www.healtheffects.org/annual.htm.

Highlights from the conference of interest to MECA members included:

- Rashid Shaikh, HEI, reviewed progress in HEI's activities. Shaikh noted that since 2010, HEI has initiated, conducted and/or completed 72 studies and 5 Special Reviews:
  - Multipollutant exposure, epidemiology and toxicology research
  - Measuring the health outcomes of air quality actions (accountability)
  - Assessing health effects of emerging fuels and technologies
  - An international perspective in the developed and developing world
  - Highlights of Progress (2010-2014):
    - NPACT: two major studies published on toxicity of PM components, by Lippman and Vedal (http://pubs.healtheffects.org/view.php?id=410)
    - ACES: Characterization of 2004 and 2010 heavy-duty diesel engines (http://pubs.healtheffects.org/view.php?id=377)
    - MOSES: rigorous testing of cardiovascular effects of ozone
    - SCET: report on emerging vehicle technologies and fuels
    - Ultrafines: review of sources, exposures and potential health effects published

    - Diesel epidemiology: assessment of data from new epidemiology studies for risk assessment underway
    - Health outcome/accountability: new research initiated
    - Near-road exposure to traffic related pollution: new research initiated

- Jacob McDonald, Lovelace Respiratory Research Institute, presented the final results from the Lifetime Animal Exposures to a 2007-Compliant Diesel Engine (Advanced Collaborative Emissions Study Phase 3B).
  - ACES is a cooperative effort to characterize emissions and assess the possible health impacts of the new, advanced heavy-duty diesel-engines and emission control systems that have been entering the U.S. market since 2007. Results from the emissions characterization of 2007- and 2010-compliant engines in Phases 1 and 2 of ACES were published in 2010 and 2012. A team conducted a lifetime (up to 3 months) inhalation bioassay in Phase 3B, in which rats were exposed to emissions from one of the four diesel engines tested in Phase 1 of the study. In addition to assessing the possible carcinogenicity of whole diesel exhaust, the bioassay has provided information on chronic toxicity, in vivo mutagenicity, and changes in non-cancer health endpoints that have been associated with exposure to diesel exhaust in other studies. In Phase 3B, animals were exposed to 4.1, 0.8 and 0.1 ppm NO_2 (as part of the diesel exhaust mixture) 16 hours/day, 5 days/week for up to 28 months (males) or 30 months (females). Rats were evaluated by respiratory function, hematology, serum chemistry, bronchoalveolar lavage, lung cell proliferation, and histopathological assays. Results of the measurements made at 12, 24, and up to 30 months were as follows:
    - Pulmonary lesions in the ACES study were dramatically different from the old technology diesel exhaust studies.
    - Many of the centriacini appeared normal in the lungs of high-exposure ACES rats with minimal thickening of alveoli walls in some centriacinar (gas-exchanging) regions.
    - Changes in the lungs of the ACES study were more similar to the Lovelace NO_2 study in which centriacinar thickening and occasional preterminal bronchiole hyperplasia was found.
    - The most dramatic differences were between the new 2007 technology diesel study in which few and mostly minimal lesions were found and the old diesel studies in which the lungs were loaded with pigment.
    - Concentrations of PM are generally very low and rise only during diesel filter regeneration, once or twice per 16-hour exposure period. Mild biological responses were observed, and were confined primarily to the respiratory tract and primarily at the highest exposure concentration. The primary histologic findings were minimal airway thickening in the central acinus. The severity of the lesions did not increase between 1 year of exposure and lifetime exposure.

  The study concluded that the new technology diesel exhaust after a rodent lifetime study did not cause neoplastic lesions that were observed in the traditional diesel exhaust studies. The observed changes were consistent with exposure to gaseous

components such as NO_2, and differed markedly from effects observed from traditional diesel exhaust. The ACES Phase 3B report will be issued by late 2014.

- Homer Boushey, University of California, San Francisco, discussed the panel review of the ACES Phase 3B report. The review panel determined that the investigators successfully completed a complex, lifetime exposure study of rats to 2007-compliant emissions. The review panel members were in overall agreement with the authors of the ACES study:
  - No increase in tumor formation over background in target organ (the lung) or any other organ
  - Major difference compared to long-term exposures to "traditional" diesel exhaust containing PM that found:
    - Cancer effects associated with PM exposure (>1 mg/m^3)
    - Evidence of inflammation and particles in lung tissue
  - Exposure to 2007-compliant emissions is consistent with effects of NO_2 exposure
  - Some histopathologic changes in the centriacinar region of the lung, similar to changes after long-term exposures to oxidizing pollutant gases, such as NO_2 and ozone
  - Few changes in inflammatory endpoints in blood, bronchoalveolar lavage or lung tissue. Where an exposure-related effect was seen, almost exclusively at highest doses and in females
  - Few changes in respiratory endpoints: mild obstructive airway effects at highest doses and in females.
  - Genotoxic endpoints: no exposure-related changes
  - Vascular endpoints: most unchanged, a few exposure-related changes
  - Review panel found no coherence among changes; biological significance is unclear

- John Balmes, University of California, San Francisco and Berkeley (and a member of the California Air Resources Board) gave a presentation on air pollution effects on chronic respiratory outcomes.
  - Obstructive airways diseases: asthma (reversible obstruction after inhaled bronchodilator); COPD (fixed obstruction not responsive to bronchodilator)
  - According to a literature study Balmes conducted and published in Lancet in 2014, the idea that outdoor air pollution can cause exacerbations of pre-exiting asthma is supported by an evidence base that has been accumulating for several decades, with several studies suggesting a contribution to new-onset asthma as well. From a mechanistic perspective, air pollutants probably cause oxidative injury to the airways, leading to inflammation, remodeling, and increased risk of sensitization.
    - Air pollution and asthma (epidemiology): multiple studies support short-term worsening of asthma with exposure to PM, O_3, NO_2 or traffic emissions. Evidence is less clear-cut for new onset of asthma.
    - Air pollution and COPD (epidemiology):

      - biological plausibility: oxidative stress, inflammation, decreased ciliary function, increased risk of infection
      - analogy with other products of combustion known to cause COPD: tobacco smoke and biomass smoke
      - exposures to outdoor air pollution can lead to exacerbations of both asthma and COPD; evidence that such exposures contribute to causation of these diseases is suggestive, but not definitive

- Karen Wesson, EPA Office of Air and Radiation, gave a presentation on EPA multipollutant initiatives.
  - Pollutants interact with each other within the atmosphere and across environmental media; many pollutants have common sources of direct and precursor emissions (control technologies can affect multiple pollutants)
  - Exposure pathways and risks are affected by multiple pollutants
  - Some pollutants contribute more to human or ecological risks than others
    - Health/ecosystem effects and toxicities vary greatly across pollutants
    - Transported pollutants (ozone and secondarily formed PM) can have more widespread impacts than pollutants with primarily local dispersion (certain air toxics and carbonaceous PM)
  - Health and ecosystem impacts of mixtures are not necessarily the sum of the impacts of individual pollutants
  - A better understanding of pollutant relationships and interactions could inform standard-setting and implementation decisions
  - EPA has been working to understand how more fully characterizing pollutant interactions could inform:
    - NAAQS review process: combined NOx/SOx secondary standard review; EPA's Multipollutant Science Documents
    - Choices made when implementing emissions reductions to more effectively improve air quality and reduce risk among pollutants
      - Air Quality Management Plans (AQMPs)
      - Multipollutant, risk-based planning
  - Secondary NOx/SOx standard
    - First NAAQS review that combines criteria pollutants: EPA combined review of the ecological effects of NOx and SOx (completed in 2012)
      - Deposition of nitrogen and sulfur is the primary driver of effects
      - Single, multipollutant Integrated Science Assessment (ISA)
      - Review secondary, welfare-based standards together
      - Based on the Administrator's judgments regarding scientific uncertainty, EPA did not set a combined NOx/SOx standard
    - Plan for current review is to evaluate these pollutants together (kickoff workshop was on March 2014)
  - EPA's Multipollutant Science Document (MSDs)
    - EPA is in the planning and preparation stages for two multipollutant science documents addressing specific welfare and health endpoints

        - One MSD intends to evaluate the effects of the climate-relevant criteria air pollutants on climate forcing
        - The other intends to facilitate the evaluation of the body of health-related multipollutant research, allowing for a more comprehensive evaluation of the potential for mixtures to drive health effects
      - Information provided in the MSDs, as well as in individual pollutant ISAs, will be considered to determine if there are opportunities to more effectively assess the effects of multiple pollutants on human health and the environment
      - Tentative peer input workshop in late 2014 to get additional input on the MSD for the effects of the Criteria Air Pollutants on Climate Forcing
  - Air Quality Management Planning Initiatives:
      - Detroit Multipollutant Study: identified how multipollutant, risk-based planning could inform control strategies and address at-risk populations
      - Air Quality Management Plan (AQMP) Pilot Studies: partnered with states to facilitates exploring comprehensive air quality management planning (www.epa.gov/air/aqmp)
      - South Carolina Multipollutant Project: working to implement integrated ozone and PM Advance programs in 10 county areas in northwestern SC
      - Revised Implementation Guidance: encouraging multipollutant, risk-based planning
  - An improved understanding of pollutant interactions could:
      - Better inform the NAAQS review process
        - Currently, available evidence focuses reviews on the contributions to health effects of individual pollutants
        - In the near future, reviews could consider how that unique contribution changes in the presence of other pollutants
        - In the longer term EPA could consider multipollutant standard-setting
      - Provide information to state and local policy makers to allow for choices made when implementing emissions reductions to more effectively improve air quality and reduce risk among pollutants

- Bob O'Keefe and Dan Greenbaum, HEI, presented the draft HEI Strategic Plan 2015-2020. HEI develops a strategic plan every five years to review what they've done, anticipate the policy and science challenges ahead and map out the most effective way for HEI to contribute to better decisions on air quality and health.
  - HEI has begun to identify key policy and science challenges for the coming years. Major areas the HEI might address going forward and are seeking public input are:
      - Informing challenging air quality standards decisions: targeted research into effects at low concentrations for PM and ozone and further examination of effects of different components of the PM mixture and potential short-lived climate pollutants (e.g. elemental and organic carbon, sulfate)

      - Examining traffic and ports exposure and health: building on HEI's current traffic exposure studies to initiate new studies of outdoor/indoor exposures and health near traffic and port facilities, especially potential effects in environmental justice communities; examining effects of key components of traffic exposures (e.g. older diesel , ultrafine PM); and considering a potential major update to the HEI Special Report on Traffic
      - Improving science for decisions: transparency and accountability: completing and planning the next generation of HEI accountability/health outcomes studies on key regulatory actions; enhancing our ability to build data access from inception into all HEI work; and bringing diverse parties together to identify the opportunities, limitations, and best practices for engaging in data sharing going forward
      - Climate, air quality, and health: new emissions and health testing of vehicles/fuel combinations likely to be considered in meeting upcoming and future vehicle greenhouse gas requirements; informing developing world decisions on air quality and climate; and building the science base on the potential health effects of climate change (e.g. heat, allergens)
  - Policy challenges ahead:
      - How low could/should ambient standards go?
      - Continued science and regulatory pressure on "traffic" effects, especially new NO_2 and PM roadside monitors in U.S.
      - Increased scrutiny of the effectiveness of air quality rules
      - Actions in Europe, China, elsewhere
      - Climate, air quality, and health
  - Major HEI research opportunities:
      - Informing challenging NAAQS/limit values decisions including:
        - Estimating the effects of long-term exposure to low levels of air pollution
        - Exploring further analyses in the National Particle Component Toxicity (NPACT) and European Study of Cohorts for Air Pollution effects (ESCAPE) studies
        - Testing the cardiovascular effects of ozone at lower levels of exposure
        - Research into effects at low concentrations for PM and ozone
        - Detailed examination of effects of different components of the PM mixture
        - Health effects of short-lived climate pollutants (EC, OC, sulfate)
      - Examining traffic and ports exposure and health: requirements for cleaner fuels and technologies promise progress for the future as transportation fleets are replaced. The advent of increased monitoring and potential regulatory attention to continuing roadside exposures from older technology has increased the need for targeted, advanced, and innovative exposure and health research to inform future questions on reducing such exposures and effects. These trends pose several scientific challenges and opportunities:

- Enhanced and innovative exposure assessment: HEI has already launched 5 targeted studies to enhance exposure assessment. It will be especially important to ensure that these studies, and the next generation, can examine microenvironments and the relationship between outdoor and indoor exposure.
- New health studies of road and port exposures: this study will identify opportunities to apply emerging tools to new targeted health studies, especially in sensitive populations.
- Exposure components of special interest: within the mix of components in traffic exposures, some stand out as areas that may be especially important for further investigation:
    - Older diesel engine exhaust: for countries outside Europe and the US, older diesel vehicles will continue to be a significant component of exposure. HEI's *Diesel Epidemiology Project* is expected to provide important guidance on the suitability of the newest diesel epidemiology studies for quantitative risk assessment of effects at typical ambient levels.
    - Ultrafine Particles: HEI's 2013 Perspectives *Understanding the Health Effects of Ambient Ultrafine Particles* summarized current science on exposure to and health effects of ultrafine particles, and concluded "The current evidence does not support a conclusion that exposures to UFPs alone can account in substantial ways for the adverse effects that have been associated with other ambient pollutants such as PM2.5." The perspectives also identified a number of continuing research needs, and questions continue to be raised about the potential role in effects observed in traffic exposure studies, especially because some new technologies, such as gasoline direct injection, emit UFPs. This set of issues continues to be of importance as decision makers in the U.S. and globally consider what, if any, action to take on particle number standards for light-duty and heavy-duty vehicles in light of European actions.
    - Non-tailpipe emissions: with a significant reduction of tailpipe PM emissions from new technology diesel vehicles, interest in non-tailpipe emissions of vehicles is increasing, and there is interest in understanding how the non-tailpipe emissions could affect exposures of individuals living near major roads. HEI issued a request for applications in early 2014 for studies to characterize such particulate emissions. Two or three studies will start early in the Strategic Plan 2015-2020.
    - Coarse Particles: While PM2.5 has been the focus of a large number of studies, the role of smaller, $PM_{<2.5}$

(including ultrafine particles) and larger, coarse $PM_{2.5-10}$ particles is not well understood. Both EPA and CASAC have emphasized the need for better understanding of possible associations between coarse particle exposure and health effects

- An updated HEI Traffic Special Review? Since HEI published their traffic review in 2010, a number of additional studies have been published and more are being published with some frequency. HEI is considering, and seeking input on, the value of updating that report
- Improving science for decisions; transparency and accountability:
    - New methods: HEI will build on new statistical methods to enable direct evaluation of well-defined, long-term regulatory interventions using national databases such as Medicare or census data, explore progress measures of air quality (e.g. non-attainment status) and health outcomes at the state, regional, and national levels.
    - Specific study areas: HEI will seek to fund studies examining air quality and health outcomes of selected major rules at the national and state levels
    - Environmental justice: HEI will seek studies that focus on potential disproportional impacts among sensitive populations, focusing on communities which may be at greater risk due to ethnicity, socio-economic status, proximity to roadway and stationary sources, and the cumulative effects of multiple pollutants
    - Air quality and climate: HEI could also identify accountability opportunities among climate-directed actions at the city to state level intended to reduce GHGs
    - Improving data access: recently, interest in ownership and control of data underpinning scientific research has increased in Congress and the scientific and stakeholder communities and request for HEI involvement have returned. HEI's commitment extends to making data from studies funded by the Institute widely available for reanalysis, replication, and extended analysis by others.

▪ Climate, Air Quality, and Health: HEI has been encouraged to consider expanding its role in climate change to understand important aspects of the complex interactions between climate, conventional air pollution, and health. Growing issues include:

- The need, in response to tightening GHG vehicle emissions standards in many countries, to have the best available information on the emissions and health effects of a range of vehicle technologies and fuels, as well as enhanced life cycle analyses for these technology/fuel combinations

- The potential effects on climate of different conventional pollutants such as ozone, carbon particles and sulfate particles; and the need to assess those actions for the potential benefits and potential dis-benefits
- The potential direct health effects of climate change and actions to address climate change

HEI sees several important ways that it could engage more directly in helping to inform decision on climate change actions during the next strategic plan:

- Choosing fuel efficient vehicles: In view of concerns about climate change and energy security, there is a need to find new solutions to enable mobility for the public while overcoming problems to climate, energy security, and costs, along with air pollution. This complex situation also provides the impetus for development and introduction of a broad range of new fuels, technologies, and sources of energy to meet the needs of the transportation sector. Over the next five years, concerns that may arise from the use of new fuels and technologies will remain a priority for HEI research including:
  - Toxicity of emissions from emerging vehicle technologies and fuels: 1) HEI will convene leading experts in biological testing, both in vitro and short-term in vivo assays for several end points. HEI will develop an agreed upon set of tests and appropriate protocols, and test these across several laboratories to assess the procedures and their reproducibility, as well as develop base parameters of the assays. 2) HEI will then work with experts in engines, vehicles, fuels, and operating conditions to select a modest but diverse group of engines or vehicles. The result will be a systematic, side-by-side comparison program that will provide useful data for policy decisions.
  - Ultrafine particles are encountered under a number of emissions scenarios. These are emitted from LD engines that use GDI. Though UFPs are not specifically regulated in the U.S. they are regulated in Europe under a particle number mandate. The understanding of the health effects of larger size particle is far from complete, but it is better than that for the effects of UFPs. This issue is also important in the context of human exposure to traffic related air pollution. HEI will explore the scientific investigations that may shed light on the role various UFP characteristics, such as mass, number, surface area, age, composition, solubility, may play in health effects.
  - Aromatics: there is evidence that the degree of hydrocarbon unsaturation in gasoline, including the contribution from aromatic compounds, is proportional to

PM emissions. The use of ethanol blends would be expected to dilute unsaturated hydrocarbons in the fuels, and reduce PM formation. However, recent data from EPA suggest that ethanol's effects on PM emissions is complex and that at least for certain vehicles, ethanol augments the effect of unsaturated HCs in terms of PM production. Given that the Energy Policy Act (2005) mandates the use of increasing amounts of renewable fuels in the coming years, the most recent data on unsaturation and PM is a potentially significant issue, PM emitted under these conditions has not been characterized, or have any health effects studies been performed with them. HEI stands ready to test the emissions from a small number of vehicles, fueled by various blend levels and characterize their emissions, and perform short-term health effects testing.
  - Analysis of fuel/technology life cycle health impact: HEI is prepared to convene an expert panel to re-visit analysis of fuel/technology life cycle health impact, update it in light of newer data, and subject it to a rigorous peer review. This update would address two HEI Special Committee on Emerging Technologies' (SCET) recommendations: a better understanding of the impact of "displaced" emissions from electricity generation, and life-cycle impact of metals such as lithium widely used in batteries.
- Developing world decisions on air quality and climate: high current, and projected future increases in emissions of "traditional" pollutants and greenhouse gases in many parts of the developing world, and the decisions taken by governments to mitigate them, directly impact both health and climate. The *Global Burden of Disease* analysis found that extremely high levels of air pollution contributed to 2.1 million premature deaths in developing Asia in 2010. Transported SO_2, NO_2, and other pollutants contribute to increased ozone and particulate levels in neighboring Japan and as far as the western U.S. In Latin America and Eastern Europe emissions are lower, but in some areas pose concerns. HEI has established itself as a globally relevant, trusted provider of science.

The first draft Plan is released for public comments (requested by June 15, 2014). HEI plans to issue a revised plan for review by the sponsors in the June to October 2014 timeframe, with adoption by the HEI Board in February 2015. The Final HEI Strategic Plan will become effective on April 1, 2015. MECA will be providing HEI with comments on their draft plan by the June 15 request date. A copy of the draft Strategic Plan is available for public comments at: http://www.healtheffects.org/Pubs/HEI-Draft-StrategicPlan2015-2020-May2014.pdf.

HEI

STATEMENT

Synopsis of Research Report 179

HEALTH EFFECTS INSTITUTE

Development and Application of an Aerosol Screening Model for Size-Resolved Urban Aerosols

BACKGROUND

Dr. Charles O. Stanier, a recipient of HEI's Walter A. Rosenblith New Investigator Award, and Dr. Sang-Rin Lee developed, tested, and evaluated an aerosol screening model for estimating the number concentrations and size distribution of ultrafine particles, defined as particles less than 100 nm in aerodynamic diameter, in near-road environments with high spatial resolution (~10 m). In the urban atmosphere, ultrafine particles are derived primarily from motor vehicles, and their concentrations vary greatly because of steep concentration gradients near traffic sources. Thus assessing exposure to ultrafine particles is challenging, and there is a need for improved models.

APPROACH

The main goal of the study was to develop, test, and evaluate an aerosol screening model of hourly size-resolved number concentrations and distributions for particles in the size range of 3 nm to 2.5 μm. The aerosol screening model is an integrated model based on the Lagrangian modeling framework, which assumes columns of air parcels that move downwind with larger steps when far from receptors and smaller steps when close to receptors. The assumptions used by the aerosol screening model include rapid mixing of tailpipe emissions, emissions evenly mixed horizontally across the road width and carried beyond the edge of the road by diffusion and advection with the wind (i.e., downwind transport), and rapid mixing into a predefined vertical distribution.

Model design and construction were guided by the desire for the model, first, to have the ability to model concentrations over short (1-hour) and longer (24-hour) periods at sites with various traffic volumes and patterns and at various distances from roads and, second, to use a large database of road segments and emission factors derived from different data sources. It was also important that the model estimates could be compared with field measurements made with a condensation particle counter (CPC) and a scanning mobility particle sizer (SMPS), which have different lower size cutoffs.

What This Study Adds

- Stanier and Lee developed and tested an aerosol screening model to simulate the dispersion of ultrafine particles near roadways using a Lagrangian dispersion framework. The model estimated particle numbers and size distributions at 11 sites in Los Angeles and Riverside counties in California.
- The performance of the model was mixed. The model predictions for the 24-hour average number concentrations were close to the preset performance targets; the predictions for the 1-hour average number concentrations were poor and did not capture the diurnal variations observed at several sites. Particle size distributions also were not well represented by the model.
- The study demonstrates the challenges involved in modeling ultrafine particles in urban areas. Although it remains unclear what the most useful applications of this model will be, it offers promise for further improvements.

This Statement, prepared by the Health Effects Institute, summarizes a research project funded by HEI and conducted by Dr. Charles O. Stanier and Dr. Sang-Rin Lee at the University of Iowa, Iowa City. The complete report, *Development and Application of an Aerosol Screening Model for Size-Resolved Urban Aerosols* (© 2014 Health Effects Institute), can be obtained from HEI or our Web site (see next page). **STANIER 179**

The model was run to predict hourly and 24-hour concentrations and size distributions of particle number and mass at 11 sites in California where real-time measurements were made in previous studies. These included seven sites around the port of Long Beach (one of the busiest commercial ports in the United States) that were part of the Harbor Community Monitoring Study (HCMS) and four sites near retirement communities in Los Angeles and Riverside counties that were part of the Cardiovascular Health and Air Pollution Study (CHAPS).

RESULTS AND INTERPRETATION

The investigators assessed the performance of the aerosol screening model by comparing the 1-hour and 24-hour-average simulations with the corresponding measured concentrations. Correlations between the modeled and measured 1-hour and 24-hour average number concentrations differed.

For the 24-hour measurement, the model's performance was not far from the preset targets. For the 1-hour average number concentrations, the model's performance was poor and did not capture the diurnal variations observed at several sites. In general, the performance was better at the CHAPS sites, which were further from freeways and had a lower volume of heavy-duty vehicles compared with the majority of the HCMS sites. The investigators found that when the modeled values failed to fall in the specified ranges, the model typically underestimated the particle concentrations. Sensitivity analysis showed that the model was sensitive to traffic volume and type, as well as to road class.

The investigators compared modeled and measured size distributions at two of the Long Beach sites, LB4 and LB5. The modeled size distributions differed from the measured distributions for many of the simulations. The investigators concluded that the model underpredicts particle number concentrations for all particles sizes $\geq$ 15 nm and overpredicts concentrations for particle sizes $<$ 15 nm.

CONCLUSIONS

Modeling the number and size distributions of ultrafine particles in epidemiologic studies is challenging, and only a few approaches have so far been tested. Thus the Committee thought that the study addressed an important research need. This ambitious study was carefully planned and performed, and the work was of high quality. The Committee felt that Stanier and Lee had chosen a high level of complexity for a screening model, that the model would require additional simplifications for actual screening applications, and that additional information would be needed for more detailed applications.

The strengths of the model are its flexibility to incorporate additional processes, the automated procedure to process road network and traffic data, and the synthesis of emission data for particle number by size from various research groups (a complex task). Model limitations are implicit in the Lagrangian approach, which assumes that all the air parcels move downwind at the same rate and communicate by diffusion, but which does not allow any movement through their boundaries associated with changes in wind speed and direction.

Evaluation of the model indicated that the predictions of the 24-hour average number concentrations were close to the preset performance targets; the predictions of the 1-hour average number concentrations were poor and did not capture the diurnal variations observed at several sites. Particle size distributions were not well represented by the model, at least in part because of uncertainties in the emission factors.

The Committee agreed with the investigators' overall assessment that the performance of the model in predicting particle number and size distribution was mixed. The results suggest that the model might be more suitable for studies that require long-term (i.e., 24-hour or longer) averages.

The study reflected the challenges involved in modeling dynamic concentrations of UFPs in urban areas, including the complex behavior of UFPs in the atmosphere as well as our limited knowledge not only of size-resolved emission factors as a function of vehicle types and operating modes, but also of emissions from non-mobile sources. Given the complexity of the model and the limitations of the Lagrangian framework in modeling the behavior of ultrafine particles, it remains unclear what the most useful application of this model will be. However, the model offers promise for further improvements and has the flexibility of incorporating additional inputs such as fleet information and emissions from off-road sources.

HEALTH EFFECTS INSTITUTE

101 Federal Street, Suite 500
Boston, MA 02110, USA
+1-617-488-2300 phone
+1-617-488-2335 fax

pubs@healtheffects.org
www.healtheffects.org

HEI ACTIVITIES RELATED TO RESEARCH ON ENGINE EXHAUST GASES

(Prepared by Maria Costantini, Health Effects Institute)

RECENT PUBLICATIONS OF COMPLETED STUDIES

Characterization of emissions of heavy-duty diesel engines meeting the US EPA 2007 and 2010 emission standards

Khalek IA, Bougher TL, Merritt PM. 2009. Phase I of the Advanced Collaborative Emissions Study. Coordinating Research Council (CRC) Report ACES-Phase 1. Alpharetta, GA:CRC.

Khalek IA, Blanks MG, Sr, Merritt PM. 2013. Phase 2 of the Advanced Collaborative Emissions Study. Coordinating Research Council (CRC) Report ACES-Phase 1. Alpharetta, GA:CRC.

HEI reports on the exhaust characterization in the exposure chamber and toxicity of the exhaust of a 2007 compliant heavy duty diesel engine

Mauderly JL, McDonald JD. 2012. Advanced Collaborative Emissions Study (ACES) Phase 3A: Characterization of U.S. 2007-Compliant Diesel Engine and Exposure System Operation. Communication 17. Health Effects Institute, MA.

Advanced Collaborative Emissions Study (ACES.) 2012. Subchronic Exposure Results: Biologic Responses in Rats and Mice and Assessment of Genotoxicity Report # 166. Health Effects Institute, Boston, MA.

Part 1. Biologic Responses in Rats and Mice to Subchronic Inhalation of Diesel Exhaust from U.S. 2007-Compliant Engines: Report on 1-, 3-, and 12-Month Exposures in the ACES Bioassay.
McDonald JD, Doyle-Eisele M, Gigliotti A, Miller RA, Seilkop S, Mauderly JL, Seagrave J, Chow J, Zielinska B.

Part 2. Assessment of Genotoxicity After Exposure to Diesel Exhaust from U.S. 2007-Compliant Diesel Engines: Report on 1- and 3-Month Exposures in the ACES Bioassay.
Bemis, JC, Torous DK, Dertinger SD.

Part 3. Assessment of Genotoxicity and Oxidative Stress After Exposure to Diesel Exhaust from U.S. 2007-Compliant Diesel Engines: Report on 1- and 3-Month Exposures in the ACES Bioassay.
Hallberg LM, Ward JB, Hernandez C, Ameredes BT, Wickliffe JK.

Part 4. Effects of Subchronic Diesel Engine Emissions Exposure on Plasma Markers in Rodents: Report on 1- and 3-Month Exposures in the ACES Bioassay.
Conklin DJ and Kong M.

Results of the chronic bioassay (same studies as those listed above with exposure period up to 30 months) will be published at the end of the year.

Other studies

Zhu Yifang, Zhang Q, 2014. Characterizing Ultrafine Particles and Other Air Pollutants In and Around School Buses Report # 180. Health Effects Institute, Boston, MA.

Johnston MV, Klems JP, Zordan CA, Pennington MR, Smith JN. 2013. Selective Detection and Characterization of Nanoparticles from Motor Vehicles Report # 173. Health Effects Institute, Boston, MA.

Riedl MA, Diaz-Sanchez D, Linn WS, Gong H,Jr., Clark KW, Effros RM, Miller JW, Cocker DR, Berhane KT. 2012. Allergic Inflammation in the Human Lower Respiratory Tract Affected by Exposure to Diesel Exhaust Report # 165. Health Effects Institute, Boston, MA.

HEI Perspectives

Health Effects Institute. 2013. Understanding the Health Effects of Ambient Ultrafine Particles. Perspectives 3. Health Effects Institute, Boston, MA.

ON-GOING RESEARCH

Christopher Frey, *North Carolina State University, Raleigh, NC*
Characterizing the determinants of vehicle traffic emissions exposure: Measurement and modeling of land-use, traffic, transformation and transport

Allison Fryer, *Oregon Health and Science University, Portland, OR*
Air pollution and systemic inflammation of autonomic nerves

Nga Lee (Sally) Ng, *Georgia Institute of Technology, Atlanta, GA*
Composition and oxidative properties of particulate matter mixtures: Effects of particle phase state, acidity, and transition metalsRichard Peltier, *University of Massachusetts, Amherst, MA*
Development of a new method for measurements of reactive oxygen species associated with $PM_{2.5}$ exposure

Nga Lee (Sally) Ng, *Georgia Institute of Technology, Atlanta, GA*
Composition and oxidative properties of particulate matter mixtures: Effects of particle phase state, acidity, and transition metals