Toxicity of Exhaust Gases and Particles from IC-Engines – International Activities Survey (EngToxIn)*)

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*) Abbreviations see at the end of report

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1. ABSTRACT

Exhaust gases from engines, as well as from other technical combustion processes contain gaseous, semi volatile and solid compounds which are toxic. Some of these compounds are not yet limited by the respective legislations, but may need to be, based on ongoing health research findings. Some new substances did appear recently, due to the progressing technical developments providing new systems of exhaust gas aftertreatment.

A new approach described here is that the toxic effects of exhaust gases as an aerosol containing gaseous components as well as particulate matter and nanoparticles can be investigated in a global way, by exposing the living cells, or cell cultures to the aerosol, which means a simultaneous superposition of all toxic effects from all active components.
At several research sites it has been shown that this method offers more objective results of validation of toxicity, than other methods used until now. It also enables a relatively quick insight in the toxic effects with consideration of all superimposed influences of the aerosol.

This new methodology can be applied for all kinds of emission sources. It also bears the potential of giving new contributions to the present state of knowledge in this domain and can in some cases lead to a change of paradigma.

The present report gives information about activities concerning the research on toxicity of exhaust gases from IC-engines in different countries. It also gives some ideas about the available information sources.

The general situation and the basic information have not changed much so the chapters 1 & 2 are by a big part repeated from the last year report, [3] with some complements and modifications.

The information given in this report is complementary to the information from previous reports [1, 2, 3].

We observe intense research activities concerning health effects worldwide. They have different objectives, different approaches and methodologies and sometimes the results can be directly compared to each other. There are mostly common lines and with appropriate efforts there might be possible ways to establish even a harmonised biological test procedure.

2. INTRODUCTION

2.1. Actual situation

Emissions legislation for vehicles is in place in order to control air pollutant emissions of combustion engines and to protect human health. The legislation limits the emissions of the so called regulated components: NO_x, CO, HC and particulate matter. The particle number emissions have been limited for Diesel passenger cars and for gasoline cars with direct injection since Euro 6 2013 and in the near future also a limit for particulate number emission of trucks will be set (2014). However, there are still several components for which no limits are set and these unregulated components could also be harmful.

In the past the toxicity of engine emissions was mainly determined by measuring specific chemical compounds such as PAH (Polycyclic Aromatic Hydrocarbons), BTXE (Benzene, Toluene and Xylen and Ethyl-benzene), aldehydes and 1.3 butadiene.
This has considerable limitations since:

a) the possible combined effect of components may lead to a different toxicity and it is the mixture people are exposed to,
b) there may be chemical species which are toxic but which are not measured.

In order to fulfil the need for a more thorough health hazard screening, in recent years biological tests were performed with the exhaust gases (particles and volatiles). These consisted of, for example, the AMES test as an indicator for the mutagenicity of the compounds; cytotoxicity, as an indicator for cell viability; and oxidative potential, as an indicator for the potential to induce oxidative stress.

In the present report a special focus on the activities with exposure of human cells cultures or animal tissues to the entire aerosol (combined exposure, whole aerosol exposure) will be given.

The main objective is to make the things comprehensible for non-specialists as far as possible, with no obligation to enter too much into the technical and scientific details.

2.2. Technical and scientific remarks

Kinds of exposure

There are different ways of testing the toxic influences:

a) Epidemiological studies – research on groups of peoples, which were exposed to some notious influences over a longer time. This very work consuming method gives only retrospective information and the results can be cross-influenced by other factors in the research period.
b) Testing on living humans, or animals – "in vivo". Beside the ethical problems, there are tendencies to apply to low dosing for humans and to high dosing for animals. In both cases the observed effects are not realistic and they have to be extrapolated.
c) Testing of biological material in laboratory – "in vitro".
   - Most popular is to collect the toxic material from the emission source, to put it in suspension or in solvent and to expose the cells, cell cultures or tissues (bio-material) to the toxic substances, in liquid phase, independently of the emission source.
     An example is: collecting of exhaust particles, resuspension and testing in submersed cell cultures in vitro. Disadvantages are: no consideration of gas phase and gaseous toxic components, change of particle characteristics and composition during collection and resuspension, no realistic conditions (no air-liquid interface) for the cells from respiratory tract which is the principal way of air pollutants to penetrate into the human body.
   - New method, as mentioned in abstracts, is the combined exposure: exposure to the entire aerosol, (whole aerosol exposure) with all toxic substances acting simultaneously and with realistic repetitive conditions of temperature, humidity, dilution and air-liquid interface.

     In this method the exposed bio-material has to be near to the emission source during the all exposure time.

     In the case of IC-engines, or vehicles the cells are brought to the engine-, or chassis-dynamometer in a specialized vehicles laboratory. Special transportable exposure chambers have been developed for this purpose.

     A highly interdisciplinary collaboration between engine specialists and toxicologists is necessary.

     From both sides: engine as emission source and cell exposure as receiver of pollutants there is a large number of variables, which have to be fixed if a unified methodology should result. These variables are:
     for vehicle: type of engine, operating conditions, type of fuel, lube oil, exhaust gas aftertreatment, diverse technical modifications;
     for exposure: bio-material (cells, tissues), exposure conditions (temperature, humidity, dilution), exposure time, incubation, repetitions.

     The exposed biological material can be:
     - cell monocultures – focusing on one cell type, no cellular interplay

- multicellcultures (e.g. human airway triple cells model) – more advantageous,
- animal lung tissue – extrapolation from animal tissue to humans.

In several conferences the conditions of combined exposure were discussed and most of them were accorded in the meeting at ADEME, Sophia Antipolis (Nizza), Oct. 16th, 2009 (see [3], pt. 2.1.). It was accorded to continue the works on the common methodology with multicellcultures and with animal lung tissues.

Toxicological tests – endpoints

The toxicological tests can be divided in following groups:
- cell viability and genotoxicity – regarding cells modifications and mortality (number of dead cells),
- oxidative stress,
- inflammatory reactions.

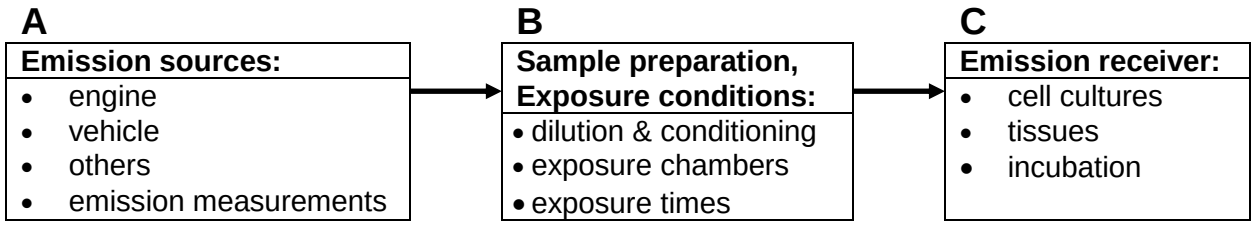
Descriptions of biological notions, processes and test methodologies are given in annex A1 and also in [1-3] (annexes 2&3). Most of the tests are normalized.

In free research the scientists may modify, or create other testing methods, according to different points of view and different objectives.
The efforts to establish a harmonized procedure nevertheless consider only standard tests.

A well known standard test of mutagenicity on salmonella bacteria is the AMES-test. This test can be attached to each research activities, it is simple and largely applied, but the toxicologists working with combined exposure do not consider it as representative for human cells.

2.3. Interdisciplinarity & complexity

The new exposure method of cells to the entire aerosol, which is described in pt. 2.2. (c) (aerosol exposure), can be graphically represented as in following chart:



Interdisciplinarity

Part A is performed by a laboratory, which can measure the emissions of engines, or vehicles according to the legal methods. This requires certain complexity of installations and measuring systems and a specialization of the participating personnel.
Emission measurements i.e. physico-chemical characterization concerns both: the legally limited and unlimited gaseous and particulate components.
Usually the limited components (CO, HC, NO_x, particle mass & counts) are analysed as standard by the legally measuring laboratories. The analytics of other unlimited components, like differential HC including PAH, nitric compounds or traces of substances is performed in collaboration with specialists for organic or inorganic analytics.

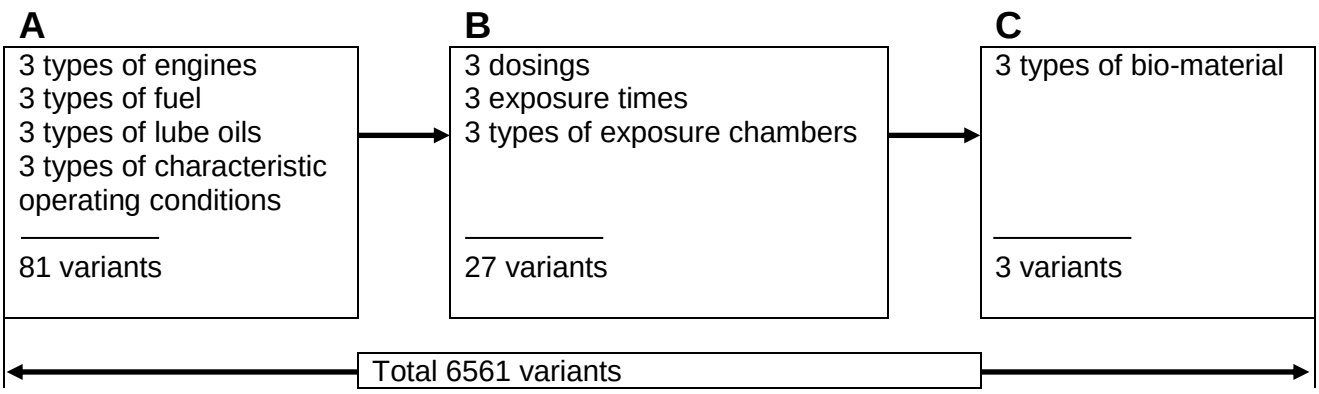
Part B – the conditioning of emission sampling – is prepared by specialists of measuring technics and control. It can be handled by the technicians from Part A.

Part C – the preparation, transport, exposure, incubation and toxicological tests are performed by toxicologists.

These remarks show that the research with combined aerosol exposure is a highly indisciplinary activity.

Complexity

The complexity of the investigated matter can be depicted by a simple example with a very modest supposition of 3 variants of some variables:



It is clearly to see that for a harmonized procedure certain variables have to be fixed.

Which combinations of emission sources and biological materials have to be investigated with preference?
The search for the "right way" is similar, like the "design of experiment DoE" for optimizing of systems with high number of variables.
In toxicological research the desired combinations of test variants are usually set by the experience of the working specialists (see: meetings and conferences of SETPOINT & EngToxNet, [1, 2, 3]).

In addition to the objective complexity of the investigated matter, the other complexities of analytical procedures and of organization can be mentioned.

2.4. How shall we call the research?

The research on emissions (part A) can be called "physico-chemical characterization" of exhaust gases.

In some research programs with profound analytics of nonlegislated components the toxicity research is mentioned. This is not right in strict meaning, since there was no research about biological responses of exposed bio-material.

There are officially used methods of describing the toxic potential of mixture of substances, like EPA toxicity equivalence TEQ.

These methods assume a linear dependence of toxic effects and amount of toxic substance and they neglect the possibilities of non linear influences and of other possible effects connected with the simultaneous interaction of different substances (multiple effects, superposition of effects, cross influences).

This supposition of proportionality between the concentration of toxic substance and the toxic effects (dose-response) is surely sufficient in most simple cases. But it is not satisfactory for special applications with many complex pollutants.

This opinion is supported by many biologists and toxicologists and it is the reason for proposing new universal exposure methods.
If we accept that the relationship: toxic substance – toxic effects is not always known, we should make difference in terminology between the research on the emission source and the research on the bio-material.

The authors propose to use following convention:

The research on emission source:
- physico-chemical characterization of emissions, or
- investigation of toxic potentials of the emission source.

The research on living cells: (epidemiological, in vivo, in vitro):
- research on toxicity.

This terminology will be considered in the present report.

3. ACTIVITIES

The information obtained from several partners is shortly commented and the received information notes and reports, as well as some positions from literature are given as annexes of this report.

3.1. France

(contact: jean-paul.morin@univ-rouen.fr)

Important developments of the biological exposure to the complex aerosol (aerosol exposure) were initiated and performed in the French network.

Since the methodology of whole aerosol exposure was developed in the French Network, let us remind the advantages of whole aerosol exposure system which are:

- no alteration of both gaseous phase and PM physicochemical properties,
- interactions of aerosol and biological sample simulating the real “in vivo” situation (sedimentation and diffusion),
- no alteration of pollutant bioavailability, global approach of exhaust impact,
- global approach of exhaust impact.

In this year the toxicological research concentrated mainly on nitric substances (Ammonia and Ammonium Nitrate), project PEAR (ADEME). It was found that there are no clear toxicological, or pathological responses on the cell lines, but there are clear irritating effects of respiratory tract. Regarding the fact of increasing emission potential of those substances by the road traffic and the non-adequate consideration of them in the present legislations a further research and screening of health effects is recommended.
A short summary of the PEAR program is given in annex A2 (French).

Further interesting information about health research in France can be found on the homepage of INSERM (www.inserm.fr) - Institut National de la Santé et de la Recherche Médicale.

3.2. Netherlands

(contacts: ruud.verbeck@tno.nl; ingeborg.kooter@tno.nl)

Information from TNO:

Publications:

- Jedynska, A., Hoek, G., Eeftens, M., (...), Brunekreef, B., Kooter, I.M. Spatial variations of PAH, hopanes/steranes and EC/OC concentrations within and between European study areas. Atmospheric Environment 2014; 87, pp. 239-248
- Yang, A., Jedynska, A., Hellack, B., Kooter, I.M. (...), Cassee, F.R., Janssen, N.A.H. Measurement of the oxidative potential of PM2.5 and its constituents: The effect of extraction solvent and filter type. Atmospheric Environment 2014; 83, pp. 35-42
- Mutlu E, Warren SH, Matthews PP, King C, Linak WP, Kooter IM, Schmid JE, Ross JA, Gilmour MI, Demarini DM. Bioassay-directed fractionation and sub-fractionation for mutagenicity and chemical analysis of diesel exhaust particles. Environ Mol Mutagen. 2013;54(9):719-36
- Kooter IM, Alblas MJ, Jedynska AD, Steenhof M, Houtzager MM, van Ras M. Alveolar epithelial cells (A549) exposed at the air-liquid interface to diesel exhaust: First study in TNO's powertrain test center. Toxicol In Vitro. 2013 Dec;27(8):2342-9

Presentations:

- Development of an in vitro diesel exposure model for human bronchial epithelial cells, Maria C. Zarcone, Gimano Amatngalim, Evert Duistermaat, Pieter S. Hiemstra, Ingeborg Kooter, Nanotoxicology conference 2014 Antalya
- Preliminary validation study of a 3D in vitro inhalation model, using cytokine and gene expression responses of copper oxide nanoparticles, Ingeborg M. Kooter, Mariska Grollers-Mulderij, Evert Duistermaat, Frieke Kuper, Eric Schoen, Eugene van Someren, Nanotoxicology conference 2014 Antalya
- Development of an Innovative in Vitro Inhalation Model for Studying the Effects of Diesel Exhaust, M.C. Zarcone, G. Amatngalim, E. Duistermaat, P.S. Hiemstra, I.M. Kooter, 18th ETH-Conference on Combustion Generated Nanoparticles, June 2014 Zurich

Whole aerosol exposure:

Exposure to diesel exhaust (DE), a major traffic-related component of air pollution in urban areas, is associated with a wide spectrum of negative health effects, especially in vulnerable populations such as patients with respiratory or cardiovascular disease. The underlying molecular mechanisms that explain these health effects are not fully understood, and mechanistic studies require a realistic in vitro inhalation model to whole DE. Therefore the aim of the present study was to develop a model in which well-differentiated primary bronchial epithelial cells (PBECs) cultured at the air liquid interface (ALI) are exposed to whole diesel exhaust (gas and particles). A diesel generator was used to generate exhaust (4 mg/m3 PM) which was collected in a tank where humidification level, PM size distribution (SMPS) and O2 /CO2 concentration were measured. The DE was directed (undiluted and further diluted to 1.2 and 0.4 mg/m3) to 3 dedicated exposure modules (Vitrocell®) in which 3 cell inserts per unit are exposed to the mixture, followed by a filter for PM concentration measurement. A fourth module was exposed to humidified air as control exposure. We first assessed the variability of the exposure in 4 independent exposure sessions of 1hr using cells from the same donor, and measured the activation of the oxidative stress response (HMOX1) at 6hr after the exposure. Next, we developed a time curve of exposure to DE for 01:00, 02:30 and 06:15hr; samples were collected at 6 and 24hr after exposure, and assessed for cytotoxicity (LDH release), epithelial barrier function (TEER measurement), oxidative stress (HMOX1 mRNA) and endoplasmic reticulum (ER) stress response (CHOP, GADD34 and spliced XBP1 mRNA). No cytotoxic effects or effects on barrier function were noted after 1 hour exposure, but prolonged exposures resulted in cytotoxicity at the highest DE dose (4 mg/m3; up to 30% LDH release after 06:15hr exposure and 24hr incubation). Dose-dependent increases in HMOX1 (till 15~f.i.), CHOP (till 2~f.i.) and GADD34 (till 2.5~f.i.) expression were observed at 6hr after exposure, and were no longer observed after 24hr. No increase in XBP1 spl was observed. These results demonstrate the feasibility of an in vitro model of

fully differentiated airway epithelial cells to whole DE. Use of this model will enable comparisons of the susceptibility of PBECs from different patient populations, investigations into the effect of exposure dose and exposure duration on the cellular response, and mechanistic studies. This study was supported by the Lung Foundation Netherlands (grant# 3.2.11.009).

- Continuation of a 4 year project (started 2012) named "Effect of exposure of diesel exhaust emissions on bronchial epithelial cells from COPD and asthma patients" co-sponsored by the Dutch Astma Fonds. The main objectives of this project are:
  - To study and understand the putative difference in susceptibility between airway epithelial cells of COPD and asthma patients and healthy subjects
  - To evaluate the relative importance of the concentration levels of DE in comparison with the exposure duration by varying these parameters (C*t = constant)
  - To evaluate the effect of a repetitive exposure compared to a constant exposure.

TNO (Netherlands National Laboratories www.tno.nl) have excellent possibilities of interdisciplinary collaboration of engine specialists with toxicologist. TNO also collaborates with RIVM (Netherlands Institute of Environment and Public Health www.rivm.nl).
TNO stresses the necessity of international harmonization and validation of bio-toxicological test methods.

3.3. Switzerland

In both domains: physico-chemical characterization of the pollutants and bio-toxicological responses there are several deeply specialized institutes.

The question of nanoparticles (NP) and health effects was early recognized and investigated on several places. In the domain of NP-measurements Swiss NP-Network contributed a lot to the PMP-Program of the ECE GRPE. In the domain of health effects the yearly organized Nanoparticle Conference at the Federal Institute of Technology (ETH Zürich) contributed very much to the interdisciplinary knowledge exchange between toxicologists and engineers
(www.nanoparticles.ethz.ch, see chap. 4.2.).

In several studies it was shown, that there is an increased penetration of ultrafine particles into the]cells and there are dose-dependent effects on the cells function. The biological responses depend also on the type of cells used for the investigations.

Whole aerosol exposure
(contacts: jan.czerwinski@bfh.ch, barbara.rothen@unifr.ch)

The activities with whole aerosol exposure started 2007 with the research on 2-stroke scooters, which was ended with the Ph.D. Thesis 2010 (available on the AFHB homepage at: www.afhb.bfh.ch → reports → toxicity → Thesis L. Müller).

The toxicological research of exhaust aerosols from Diesel passenger car was continued in the Swiss Network with the project “BioToxDi” (Biofuels, Toxicity, Diesel). In this project the principal influences of the emission source, like different fuels, lube oils, aftertreatment, etc. on the biological responses of a triple cell cultures were investigated.

The project was finished in 2013 with a Ph.D. Thesis of Mr. S. Steiner.

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.

The Ph. D. Thesis of Dr. Steiner, as well as the "Final Report" summarizing this Thesis can be found on the AFHB Homepage < www.afhb.bfh.ch > under "Reports" & "Toxicity". The abstract of the "Final Report" is given in annex A3. Some publications of this thesis are [4, 5, 6], annexes A4, A5, A6, (also [3] ref. 5 & 6).

Further activities about toxicity of gasoline cars, mainly with direct injection, were started 2014 in the project EngToxGas. One of the first research topics was a non-coated GPF (gasoline particle filter) with very high (near 100%) nanoparticle filtration efficiency. It resulted that:

- In contrast to unfiltered gasoline engine exhaust, GPF-filtered exhaust did not induce activation of the xenobiotic-responsive aryl hydrocarbon receptor and acted not or only marginally genotoxic. However, filtration was found to increase the pro-inflammatory potential of the exhaust.
- It could be concluded that tested GPF changes exhaust toxicity, but is not sufficient for exhaust detoxification.

A similar picture was obtained in previous studies on the effects of diesel particle filters. Besides reducing the pro-inflammatory potential of diesel exhaust, an uncatalyzed diesel particle filter increased exhaust genotoxicity, which was abolishable by adding catalytic activity.
The arising recommendation is therefore to perform further tests with coated filters.

Secondary organic aerosols (SOA)
(contact: andre.prevot@psi.ch; urs.baltensperger@psi.ch)

The Paul Scherrer Institute (PSI) together with the Institute for Energy and Transport of EC-JRC Ispra and some other partners performed a research project about SOA's from different vehicle groups.
The research with the ageing of aerosol in a flow-reactor, which was developed by PSI is in course in the Swiss Network project GasOMeP (Gasoline Organic & Metal Particles). These tests with a deep physicochemical characterization of aerosol are by a big part joint with the *in vitro* exposure of cell cultures.

Further information sources

A large number of studies concerning air pollution & health, ultrafine particles, ozon and traffic pollutants can be found on the homepage of the Swiss Tropical- and Public Health Institute (Swiss TPH) at: http://ludok.swisstph.ch/.
A study from INFRAS, CH, [7], annex 7, resumes the traditional knowledge and methods of valuation of the toxicity of diesel exhaust components.

3.4. Germany

Interesting information is given from the University of Rostock
(contact:benjamin.stengel@uni-rostock.de)

It refers to a European network project at the University called **HICE**: *Helmholtz Virtual Institute of Complex Molecular Systems in Environmental Health* (www.hice-vi.eu). In this study, the aim is to investigate comprehensively and integratively the gas and PM emissions from different sources in the framework of health effects study by combining online and off-line techniques which is in conjunction with a cell exposure system.
Annex A8 shows some information slides about the objectives, hypotheses and results of this network project. The methodology of toxicity research responds to the whole aerosol exposure: "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".

Further contact persons are:

Prof. Dr. Ralf Zimmermann
Spokesperson of the Virtual Institute HICE
Helmholtz Zentrum München – German Research Centre for Environmental Health
Cooperation Group "Comprehensive Molecular Analytics"
Joint Mass Spectrometry Centre @ Uni Rostock and Helmholtz Zentrum München
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Helmholtz Zentrum München – German Research Centre for Environmental Health
Ingolstädter Landstraße 1
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3.5. Denmark
(contacts: jb@mil.au.dk; Steffen Loft: stl@sund.ku.dk)

There is collaboration between the universities of Aarhus (au) and Copenhagen (ku) and Danish Technological Institute (DTI).

At the University of Copenhagen 4 lines of research address engine toxicity:
1) Epidemiology based on cohorts with traffic-related exposure modeled at the address in a long- or short-term context and outcomes in terms of mortality, disease incidence and biomarkers and functions related to inflammation and aging;
2) Panel studies with personal exposure to ultrafine particles in different environments including engine exhaust and health effects assessed by biomarkers related to oxidative stress and inflammation as well as physiological especially cardiovascular functions;
3) Controlled exposure human studies involving diesel exhaust (20 subjects studied in collaboration with Lund University, Sweden) and air from a busy street (60 elderly obese subjects studied) and health effects assessed by biomarkers related to oxidative stress and inflammation as well as physiological especially cardiovascular and pulmonary functions;
4) experimental studies with diesel exhaust particles from different engines and fuels in complex cell (co-)cultures and animal exposures.

Several publications related to engine toxicity are given annex A9.

Contact person is Profl Steffen Loft:

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Section of Environmental Health
University of Copenhagen
Øster Farimagsgade 5
1014 Copenhagen

stl@sund.ku.dk
publichealth.ku.dk/about/departments/environment/

3.6. Norway

(contacts: per.schwarze@fhi.no; otto.andersen@vestforsk.no)

Information of the Norwegian Institute of Public Health about engine toxicity research is:

In the health-related activities and research there are among others extensive experiences with exposure of different cells types to the "extracted and fractionated" organic material. There also are collaborations in different international projects.

Other relevant activities: Development of updated emission factors (also for traffic emissions). Particularly relevant in Norway, since the average annual temperature in Oslo is 5 degrees. Thus most of the driving occurs at low temperatures. Studies at the Institute of Transport Economics have shown a 10x increase of particle emissions from a EURO 5 vehicle if driven according to the Helsinki cycle (-7 degrees).
This activity is followed by new modelling procedures that will result in air pollution concentrations for most (all) of the country. These models will include traffic derived roadside dust.

Last publications on engine toxicity:

Bach NS, Låg M, Øvrevik J. Toll like receptor-3 priming alters diesel exhaust particle-induced cytokine responses in human bronchial epithelial cells. Toxicol Lett. 2014 Jul 3;228(1):42-7. doi: 10.1016/j.toxlet.2014.03.021. Epub 2014 Apr 4. PubMed PMID: 24709138

Totlandsdal AI, Øvrevik J, Cochran RE, Herseth JI, Bølling AK, Låg M, Schwarze P, Lilleaas E, Holme JA, Kubátová A. The occurrence of polycyclic aromatic hydrocarbons and their derivatives and the proinflammatory potential of fractionated extracts of diesel exhaust and wood smoke particles. J Environ Sci Health A Tox Hazard Subst Environ Eng. 2014;49(4):383-96. doi: 10.1080/10934529.2014.854586. PubMed PMID: 24345236.

Information of the Wesetern Norway Research Institute (WNRI):

The Western Norway Research Institute (WNRI) is active in international projects on bio-fuels and toxic potentials. WNRI is specialized in molecular dynamics simulations which allow investigating the interactions of nanoaerosols and chemicals with the cells.

3.7. Sweden

(contacts: magnus.lindgren@trafikverket.se; thomas.sandstrom@lung.umu.se; annika.hanberg@ki.se)

There is following message from Dr. Magnus Lindgren, Swedish Delegate for IEA AMF:

In Sweden we have performed some tests with different diesel fuels (European diesel fuel compared with Swedish environmental class 1 diesel fuel).

The tests were performed by the AVL Sweden. Information about the report is given in annex A11.

In addition to the regulated emission components the particle size distribution has been measured with an ELPI instrument. Unregulated components i.e. olefins, PAH and aldehydes have been analyzed. Extract of the particulate and semivolatile phase has been used to carry out the Ames' bio assay to analyze the level of mutagenicity in the exhausts.

A higher mutagenicity of one of the fuels could be found, there was nevertheless no further toxicological research.

3.8. USA

North Carolina
(contacts: madden.michael@epa.gov; www.epa.gov/rtp)

Information and references from EPA, Chapel Hill Laboratories, North Carolina are given in:

[8], annex A12: by means of exposures of human volunteers to the diluted whole diesel exhaust (DE) and ozone, it was found, that the presence of DE affects the lung physiological processes.
[9], annex A13: exposures of rat alveolar macrophages to diesel and biodiesel (B20) exhaust gas showed increased inflammatory potential with B20.
[10], annex A14: after exposures of human volunteers to diesel exhaust (DE) in controlled-chamber studies and research of urinary PAH's from different exposure conditions, it was concluded, that the urinary PAH's are no useful biomarkers of short-term exposures.

Duke University, Durham, Nord Carolina in collaboration with several universities in US and China gives following information in [11], annex A15 (contact: junfeng.zhang@duke.edu):

"Researchers Find Ultrafine Particles Have Health Impacts Independent from Larger Particles" – Using a quasi-experimental opportunity offered by greatly restricted air pollution emissions during the 2008 Beijing Olympics compared to before and after the Olympics, a team of U.S. and Chinese researchers conducted a study to compare ultrafine particles (UFPs) and fine particles (PM2.5) in their associations with biomarkers reflecting multiple pathophysiological pathways linking exposure and cardiorespiratory events.

Several participants were measured 6 times for biomarkers of autonomic function, hemostasis, pulmonary inflammation and oxidative stress and systemic inflammation and oxidative stress.

Changes in biomarker levels associated with increases in UFPs and PM2.5 were comparable in magnitude. However, associations of certain biomarkers with UFPs had different lag patterns compared to those with PM2.5, suggesting that the ultrafine size fraction (≤100 nm) and the fine size fraction (~100 nm to 2.5 µm) of PM2.5 are likely to affect PM-induced pathophysiological pathways independently.

The results of this study suggest that particle emission-controlling policies need to consider both the ultrafine and the fine size fraction of PM2.5 in order to protect human health.

Further information of US EPA, NC about health effects connected with emissions of ultrafine particles and necessities of epidemiological and toxicological studies is given in different conferences and workshops e.g. Washington, April 1st, 2014, annex A16, or UFP Workshop, NC, Feb. 11-13, 2015, annex A17.

West Virginia University & NIOSH, Morgantown

In two papers, dating from 2013, increased oxidative stress and pulmonary inflammation potentials with biodiesel are confirmed: [12], annex A18 & [13], annex A19.

University of Rochester, Department of Public Health, NY

[14], annex A20, investigated and found correlations of increased ambient fine particle concentrations with cardial functions.

University of Michigan, Ann Arbor, MI

[15], annex A21, investigated by means of simulation and modeling the nanoparticle permeation through a lipid membrane. There is a significant variance of permeability for particles with different structures (contact: avioli@umich.edu).

3.9. China

A study about the influences of atmospheric pollution, especially nanoparticles, on physiological responses of 125 Beijing residents before & after the Olympic Games, was performed mostly by Chinese scientists in collaboration with US scientists. This study is represented in the previous chapter 3.8, [11], annex A15.

Since 2013 there are intense efforts in China to retrofit the Diesel fleet with DPF-systems. A study about the non-legislated emissions of a diesel engine with non-coated DPF and Fe-based regeneration additive (fuel borne catalyst) is represented in [16], annex A22. This type of regeneration help and a non-catalyzed trap are the best retrofit solution for high-sulfur-fuels. It was found that the DPF reduced the PAH, but increased the B(a)P-equivalent. This work represented only the physico-chemical characterization of the total exhaust gas, no biological tests were performed. (In the Swiss Network it was found, that the additional catalytic activity (coated DPF) further lowers the toxic potentials).

3.10 Australia

A study highlights importance of fuel and particle chemistry on toxicity of biodiesel PM, [17]. A group of Australian researchers has recently published their work in characterizing the oxidative stress of biodiesel PM and relating it to the molecular structure of the fuel, such as saturation and chain length, and operating condition of the engine. The work was published in the online version of the American Chemical Society publication *Environmental Science and Technology.* In general, the research showed that more saturated biofuels with shorter carbon chain lengths resulted in lower PM mass but higher volatile fraction and higher levels of Reactive Oxygen Species (ROS) characteristic of more toxic exhaust. The authors suggest that policy makers need to develop new metrics beyond PM mass that take into account the potentially toxic parts of diesel PM.

3.11 Chile

Information from the IEA AMF ExCo 46 Meeting, Santigo de Chile, Nov. 18-21.2013
(contacts: poyola@cmmolina.cl; glopez@cmmolina.cl):

Centro Mario Molina, Chile, together with Harvard School of Public Health, US realizes a project "Health and Environmental Impacts of Exhaust from Biofuels". In focus of the research are flex-fuel-vehicles with Ethanol blend fuels and the project is working on four specific hypotheses:

1) blending gasoline and ethanol will alter biological outcomes to exposure to particulate matter in vehicle exhaust;
2) atmospheric aging, which alters the chemical composition of particle matter in vehicle exhaust, will affect the biological responses to exposure for all fuel types;
3) biological responses to exposure to particle matter in vehicle exhausts will exhibit seasonal variability due to changes in fuel formulations; and
4) blending gasoline and ethanol will change the amount of ozone and secondary organic aerosol that form during atmospheric photo-oxidation.

3.12 Thailand

Information from the delegate of Thailand, IEA AMF: the contact persons in Thailand who work for emission toxicology are:

Associate Professor Dr. Anamai Thetkathuek
Department of Industrial Hygiene and Safety, Faculty of Public Health, Burapa University
Email: anamai@buu.ac.th

Associate Professor Dr. Kanokrat Siripanichgon
Department of Microbiology, Faculty of Public Health, Mahidol University
Email: phksr@mahidol.ac.th

Prof. Thetkathuek informs about a study on exposure of workers in gasoline stations to increased benzene concentrations, paper coordinates and abstract see annex A23.
Since most workers did not use the personal protective equipment the suggestions are to increase and to make repetitively the safety at work trainings.

4. Other Information Sources

4.1. Literature

There is a huge amount of literature.
As already pointed out in the chap. 2.4., there is not always a clear differentiation of notions between physico-chemical characterization and toxicological research. Several studies dealing with profound analytics of pollutants composition attach to the study some toxicological elements like Ames Tests, or toxicity equivalence TEQ. This type of studies is usually done by engine specialists together with chemical analysts.

Several examples of references with physico-chemical characterization of exhaust emissions and some considerable lists of literature are given in [1], chap. 4.1.

Remarkable references from last year period are:

[18], annex A24, an analysis of 22 European cohorts concerning effects associated to long-term air pollution exposures, project ESCAPE.

In conclusion, the findings show that long-term exposure to fine particulate air pollution is associated with natural-cause mortality; even at concentration ranges well below the present European annual mean limit value.

[19], annex A25, an international consortium of authors shows the NP-emissions in different countries and cities – sources of emissions, unresolved challenges and recommendations. It estimates, that the average exposure to the UFP's in Asian cities is about four-times higher than that in European cities.

Further references treating about health effects due to exposures to atmospheric pollution, traffic-, or vehicle emissions are given in annex A26.

4.2. 18th ETH Conference on Combustion Generated Nanoparticles

The interdisciplinary information exchange is promoted since many years at the Nanoparticle Conference organized yearly at the Federal Institute of Technology ETH Zürich (www.nanoparticles.ethz.ch).

The principal motivations of this Conference on Combustion Generated Particles are the health effects. Since 2004 there are specific sessions about health effects, which promote the technical and scientific exchange between the specialists. Annex A27 shows the presentations of the sessions "Health effects" from the last year 2014. Further information can be asked and a CD can be ordered at: ttm.a.mayer@bluewin.ch.

At the last Conference, June 2014, a special Focus Event was organized about the possibilities of field inspection of vehicle emissions with PN-based instrumentation, see annex A28.

4.3. World Medical Association (WMA)

Annex A29 represents an important statement of the World Medical Assembly accepted during their meeting at Durban in October 2014 with respect to the health effects of combustion generated particles and the need to use best available technology particle filters for first fit as well as retrofit.

4.4. Health Effects Institute (HEI)

(http://www.healtheffects.org, contact: mcostantini@healtheffects.org)

There are intense activities of HEI together with the US engine- and emission control industry (MECA). A special report from the 2014 HEI Annual Conference in Alexandria, VA is given in annex A30.

Information about a study "Modeling of Size-resolved Propagation of Urban Aerosols" is given in annex A31 and the recent list of published studies and on-going research topics is given in annex A32.

4.5. Internet

There are vast possibilities of information research on internet.
We want to mention the homepages of the concerned institutions and universities, which are already given in this report and the Wikipedia addresses:
http://wikipedia.org//wiki/toxicity
http://en.wikipedia.org/wiki/Exhaust_gas
http://wikipedia.org//wiki/Diesel_exhaust

Further interesting information can be found at:

http://www.lrri.org	… Lovelace Respiratory Research Institute (LRRI), US
http://www.efca.net	… European Federation of Clean Air and Environmental Protection Associations
http://www.ceees.org	… Confederation of European Environmental Engineering Societies
http://www.aphekom.org	… Institut de Veille Sanitaire, Département Santé Environnement, France

5. CONCLUSIONS

- Research activities about toxicology have large extent in several countries and focus on different pollution sources.
- The principal methods of research are:

- epidemiological or cohort studies,
- exposures in vivo (humans or animals)
- exposures in vitro (different kinds of supply of pollutant, different exposed bio-material).

Behavior of particles at biological barriers, cells & tissues

- Only the nanoparticles penetrate through the air-blood tissue barriers into the blood; they translocate with the blood to other organs, where they interact with the cells, even cell cores and tissues,
- Within cells and in the vascular system the nanoparticles cause different adverse effects depending on organ, nanoparticle material and the conditions of exposure (also the inherited inclinations and external influences of the exposed individuum stimulate strongly the biological reactions).

Harmonized international biological test method

- The proposed wholistic approach of the exposure of human lung cells to the aerosol as emitted (gaseous & particulate compounds) is still not very wide spread – as major limits exist e.g. the necessity of a highly interdisciplinary approach and high personal / material efforts.
- Nevertheless, this methodology offers the best balance between the objectivity of the biological response and the time-to-results.
- A lot of work was done to pave the way of this method to become an international standard – further efforts are necessary.
- An important point in the discussions is to combine the research on physico-chemical characterization of the pollution source of gas (tox-potentials) and the toxicity research (bio-responses, tox-effects).
- The details of methodology of research are often not clearly to see from the publications, but there are several countries already working with the newest method of whole aerosol exposure and the other countries have excellent potentials to do it.
- The establishment of an harmonized international biological test method is possible.

The harmonized bio-toxicological test method, which is described in this report and which is already recognized and applied by a growing part of the scientific community can enable further progresses and can help to better apply the newest knowledge for the progressing legislation.

New look on particle toxicity 2013/2014

- the harmful effects of atmospheric pollution, especially NP, became an important research topic and requirement of the authorities in China,
- there are requirements in USA (MECA) to introduce more severe limits and to enforce the introduction of DPF-technology on the market,
- the health risks of exposure to NP were confirmed in several projects.

Remarks

The reader is kindly remained that the information given in this report is complementary to the information from previous reports, [1, 2, 3] and does not replace them.

Old & new Diesel engine technology: there are some opinions that new engines with very low PM (mass) emission have also a very low PN (number) emission and do not need a DPF. This nevertheless from the point of view of physics is wrong, since the invisible high PN exist always in untreated exhaust, even at engine idling and with newest combustion technics. A smallest amount of fuel burned heterogeneously suffices for a full emission of NP/UFP.

"New Diesel engine technology" is a synonym of reduced or eliminated NP/UFP-emissions only if in this notion a DPF with adequate quality is included.

6. ACKNOWLEDGEMENTS

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7. REFERENCES

[1] Czerwinski, J.; Morin, J.-P.; Winkelmann, R.: Toxicity of Exhaust Gases and Particles from IC-Engines – International Activities Survey (EngToxIn). 1st information report B315, Sept. 2011 for IEA AMF Annex XLII.

[2] Czerwinski, J.; Morin, J.-P.; Winkelmann, R.: Toxicity of Exhaust Gases and Particles from IC-Engines – International Activities Survey (EngToxIn). 2nd information report B344, Oct./Nov. 2012 for IEA AMF Annex XLII.

[3] Czerwinski, J.; Morin, J.-P.; Winkelmann, R.: Toxicity of Exhaust Gases and Particles from IC-Engines – International Activities Survey (EngToxIn). 3rd information report B370, Oct./Nov. 2013 for IEA AMF Annex XLII.

[4] Steiner, S.; Czerwinski, J.; Comte, P.; Popovicheva O.; Kireeva, E.; Müller, L.; Heeb, N.; Mayer, A.; Petri-Fink, A.; Rothen-Rutishauser, B.: Comparison of the toxicity of diesel exhaust produced by bio- and fossil diesel combustion in human lung cells *in vitro*. Atmospheric Environment 81 (2013) 380-388. Journal homepage: www.elsevier.com.

[5] Steiner, S.; Heeb, N.V.; Czerwinski, J.; Comte, P.; Mayer, A.; Petri-Fink, A.; Rothen-Rutishauser, B.: Test-Methods on the Test-Bench: A Comparison of Complete Exhaust and Exhaust Particle Extracts for Genotoxicity/Mutagenicity Assessment. Environmental Science & Technology, April 2014, 48, 5237-5244, dx.doi.org/10.1021/es4056033.

[6] Steiner, S.; Czerwinski, J.; Comte, P.; Heeb, N.V.; Mayer, A.; Petri-Fink, A.; Rothen-Rutishauser, B.: Effects of an Iron-Based Fuel-Borne Catalyst and a Diesel Particle Filter on Exhaust Toxicity in Lung Cells in Vitro. Aerosols and Health, June 2014, Springer, doi 10.1007/s00216-014-7878-5

[7] de Haan, P.; Keller, M.: Carcinogenic and non-carcinogenic effects of diesel exhaust components (incl. PAH and nitro-PAH) using different particulate trap technologies. Final report B7048b1-02, INFRAS, Bern, Oct. 2001

[8] Madden, MC.; Stevens, T.; Case, M.; Schmitt, M.; Diaz-Sanchez, D.; Bassett, M.; Montilla, TS.; Berntsen, J.; Devlin, RB.: Diesel Exhaust Modulates Ozone-induced Lung Function Decrements in Healthy Human Volunteers. Accepted, Particle and Fibre Toxicology. 11:37. 2014.

[9] Bhavaraju,L.; Shannahan,J.; William, A.; McCormick, R.; McGee, J.; Kodavanti, U.; *Madden, MC*.: Diesel and biodiesel exhaust particle effects on rat alveolar macrophages with in vitro exposure. Chemosphere.104:126-33. 2014. www.elsevier.com.

[10] Lu, SS.; Sobus, JR.; Sallsten, G.; Albin, M.; Pleil, JD.; Gudmundsson, A.; *Madden, MC.;* Strandberg, B.; Wierzbicka, A.; Rappaport, SM.: Are urinary PAHs biomarkers of controlled exposure to diesel exhaust? Biomarkers. 2014 Apr 22. http:/informahealthcare.com

[11] Gong, J.; Zhu, T.; Kipen, K. ; Wang, G. ; Hu, M.; Guo, Q.; Ohman-Strickland, P.; Lu, S-E.; Wang, Y.; Zhu, P.; Rich, D.Q.; Huang, W.; Zahng, J.: Comparisons of Ultrafine and Fine Particles in Their Associations with Biomarkers Reflecting Physiological Pathways. Environmental Science & Technology, March 2014.

[12] Shvedova, A. A.; Yanamal, N.; Murry, A. R.; Kisin, E. R.; Khaliullin, T.; Hatfield, M.K.; Tkach, A. V.; Krantz, Q. T.; Nash, D.; King, Ch.; Gilmour, M. I.; Gavett, St.: Oxidative Stress, Inflammatory Biomarkers, and Toxicity in Mouse Lung and Liver after Inhalation Exposure to 100% Biodiesel or Petroleum Diesel Emissions. Journal of Toxicology and Environmental Health, Part A, 76:907-921, 2013.

[13] Yanamala, N.; Hatfield, M.K.; Farcas, M. T.; Schwegler-Berry, D.; Hummer J.A.; Shurin, M.R.; Birch, M. E.; Gutkin, D.W.; Kisin, E.; Kagan, V.E.; Bugarski, A.D.; Shvedova, A.A : Biodiesel versus Diesel Exposure : Enhanced Pulmonary Inflammation, Oxidative Stress, and Differential Morphological Changes in the Mouse Lung. Toxicology and Applied Pharmacology 272 (2013) 373-383.

[14] Garnder, B.; Ling, F.; Hopke, Ph.; Frampton, M.; Utell, M.J.; Areba, O.; Cameron, S. J.; Chalupa, D.; Ane, C.; Ulandhaisamy, S.; Topf, M. C.; Rich, D.: Ambient Fine Particulate Air Pollution Triggers ST-Elevation Myocardial Infarction, but no non-ST Elevation Myocardial Infarction: a Case-Crossover Study. Paricle and Fibre Toxicology 2014, 11:1

[15] Fiedler, S. L.; Violi, A.: Simulation of Nanoparticle Permeation through a Lipid Membrane. Biophysical Journal, Vol. 99, July 2010, 144-152.

[16] Zhao, H.; Ge, Y.; Zhang, T.; Zhang, J.; Tan, J.; Zhang, H.: Unregulated Emissions from Diesel Engine with Particulate Filter Using Fe-Based Fuel Borne Catalyst. Elsevier Journal of Environmental Sciences 26 (2014) 2027-2033.

[17] Pourkhesalian, A. M.; Stevanovic, S.; Salimi, F.; Rahman, M.; Wang, H.; Pham, P.X.; Bottle, S.E.; Masri, A.; Brown, J.R.; Ristovski, Z.D.: The Influence of Fuel Molecular Structure on the Volatility and Oxidative Potential of Biodiesel Particulate Matter. Environmental Science & Technology, Oct. 2014 (accepted).

[18] 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: Effects of Long-Term Exposure to Air Pollution on Natural-Cause Mortality: an Analysis of 22 European Cohorts within the Multicentre ESCAPE Project. www.thelancet.com, Dec. 2013.

[19] Kumar, P.; Morawska, L.; Birmili, W.; Paasonen, P.; Hu, M.; Kulmala, M.; Harrison, R. M.; Norford, L.; Britter, R.: Ultrafine Particles in Cities. Elsevier, Environment International 66 (2014) 1-10.

8. ABBREVIATIONS

ADEME	Agence de l'Environnement et de la Maîtrise de l'Energie, France
AECC	Association for Emission Control by Catalyst (www.aecc.be)
AFHB	Abgasprüfstelle der Fachhochschule, Biel CH, (www.afhb.bfh.ch) (Lab.For Exhaust Gas Control, Univ. of Appl. Sciences, Biel-Bienne, Switzerland)
AMF	Advanced Motor Fuels
BfE	Bundesamt für Energie, CH (SFOE)
B(a)P	Benzo (a) Pyrene
BAT	best available technology
BAFU	Bundesamt für Umwelt, (Swiss EPA, FOEN)
CARB	Californian Air Resources Board
CERTAM	Centre d'Etudes et de Recherche Technologique en Aérothermique et Moteur
CERTH	Center of Research & Technology Hellas
COPD	chronic obstructive pulmonary disorder
CPC	condensation particle counter
CPERI	Chemical Process Engineering Research Institute
CRT	continuously regenerating trap
CVS	constant volume sampling
DEP	Diesel exhaust particles
DMA	differential mobility analyser
DOE	US Department of Energy
DPF	Diesel Particle Filter
DTI	Danish Technological Institute
ECN	Energy Research Center Netherlands
EMA	Engines Manufacturers Association (US)
EMPA	Eidgenössische Materialprüfungs- und Forschungsanstalt
EngToxNet	Engine Toxicity Network
EPA	Environmental Protection Agency
ETHZ	Eidgenössische Technische Hochschule Zürich
EUGT	Europeische Forschungsvereinigung für Umwelt und Gesundheit im Transportsektor, www.eugt.org
EV	Erdöl Vereinigung, CH (www.swissoil.ch)
FJRG	Fuel Joint Research Group, D
FNR	Fachagentur Nachwachsender Rohstoffe, D
FOEN	Federal Office of Environment (BAFU)
GRPE	Groupe Rapporteur Pollution et Energie
GtL	gas to liquid synthetic fuel
HEI	Health Effects Institute
HVO	hydrogenated vegetable oil
I & M	NL Ministry of Infrastructure & Environment (www.government.nl/ministries)
IA	Implementing Agreement
IARC	International Agency for Research on Cancer
IEA	International Energy Agency
INSERM	Institut National de la Santé et de la Recherche Médicale, F

INSOF	insoluble fraction
JRC	EU Joint Research Center, Ispra It.
MECA	Manufacturers Emission Control Association
NIOSH	National Institute for Occupational Safety and Health
NP	nanoparticulates
OAPC	Ordinance on Air Pollution Control, CH
PAH	polycyclic aromatic hydrocarbons
PM	particulate matter, particulate mass
PMP	Particle Measuring Program of the UNO ECE GRPE
PN	particles number
PSI	Paul Scherrer Institut, Switzerland
RIVM	NL National Institute of Public Health
SAE	Society of Automotive Engineering (www.sae.org)
SAG	Swiss Aerosol Group (medical)
SMPS	scanning mobility particles sizer
SOA	Secondary Organic Aerosol
SOF	soluble organic fractions
SWRI	South West Research Institute
TEF	Toxicity Equivalence Factor
TEQ	Toxicity Equivalence TEQ = sum (TEF_i x $concentration_i$)
TNO	NL National Research Laboratories
TPN	total particle number
TTM	Technik Thermische Maschinen, Niederrohrdorf, CH
UC	University of California
UFP	ultra-fine particles
VERT	Verification of Emission Reduction Technologies
VSS	Verband der Schweizerischen Schmierstoffindustrie (www.vss-lubes.ch)
VTT	Technical Research Center of Finland
WHO	World Health Organisation
WMA	World Medical Association
WNRI	Western Norway Research Institute
WVU	West Virginia University

9. ANNEXES

A 1 Biological background and term definitions

A 2 Programme PEAR: Résumé synthétique (French)

A 3 Abstract of the “Final Report” project EngToxDi, PhD. Thesis Dr. Steiner

A 4 Reference [4], Toxicity bio- and fossil diesel, CH

A 5 Reference [5], Whole aerosol-PM extracts genotoxicity & mutagenicity, CH

A 6 Reference [6], Toxicity with DPF & Fe-fuel-borne catalyst, CH

A 7 Reference [7], Carcinogenicity, mutagenicity, weighing factors of diesel exhaust, CH

A 8 Uni Rostock, Helmhotlz, HICE-Aerosols and Health, D

A 9 Publications, engine toxicity, Universities Copenhagen & Aarhus, DK
A 10 Activities & publications, Western Norway Research Institute, N
A 11 Mutagenicity of different Diesel fuels, AVL Sweden, S
A 12 Reference [8], Diesel exhaust – decrements of lung function, US
A 13 Reference [9], Biodiesel exhaust – effects on rat alveolar macrophages, US
A 14 Reference [10], Diesel exhaust exposures – urinary PAH's biomarkers, US
A 15 Reference [11], Pathophysiological influences of ultrafine & fine nanoparticles, US
A 16 Statements from presentation D. L. Costa, EPA NC, April 1st, 2014, US
A 17 Workshop on Ultrafine Particles, EPA NC, Feb. 11-13, 2015, US
A 18 Reference [12], Biodiesel (B100) toxicity in mouse lung & liver, US
A 19 Reference [13], Toxic effects in mouse lung with biodiesel, US
A 20 Reference [14], Ambient particulate pollution vs.cardial functions, US
A 21 Reference [15], Nanoparticle permeation through a lipid membrane, US
A 22 Reference [16], Unregulated emissions with DPF & Fe-additive, China
A 23 Exposure of workers in gasoline stations, paper Thailand
A 24 Reference [18], 22 European cohorts exposures, project ESCAPE, EU
A 25 Reference [19], Ultra-fine particles in cities, International Authors
A 26 References: health effects due to exposure to polluted air, International Authors
A 27 Session "Health Effects" of the 18th NP Conference, ETH Zürich, June 2014
A 28 NP Conference 2014 Focus Event
A 29 WMA statement on air pollution and vehicles emissions
A 30 MECA / HEI 2014 Annual Conference in Alexandria, US
A 31 HEI: Modeling of propagation of urban aerosols, US
A 32 HEI: List of published studies and research topics, US