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

3rd Information Report for IEA Implementing Agreement AMF, Annex XLII, international activities 2013

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Report :

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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, [2] with some complements and modifications.

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. Now that the results of a number of programs are available, the need has arisen to evaluate the health hazard screening used so far. It seemed that no standard approach has been used and outcomes varied, which stresses the need to come to harmonised (standardised) test methods in order to compare the experimental results.

Comparability of the results is one of the reasons, why the Dutch Ministry of Environment (I&M) has requested the National Institute for Public Health and the Environment (RIVM) already in 2008 to:

a) develop an international network with both engineers and toxicologists/biologists in the area of testing new fuels and engine technologies,
b) coordinate and develop an international harmonized test procedure for toxicity testing of engine emissions.

The international harmonization of health screening has been started by the RIVM by organizing a number of workshops in 2008-2009. From that the network SETPOINT (Screening Emissions for Toxic Potential - Organizing INTernational harmonization) has been launched, which promotes knowledge transfer and harmonization of hazard screening of engine emissions and the critical evaluation of these developments to guide policymakers and regulators. During these workshops biomedical specialists, toxicologists and engineers from both the private and public sector were brought together to discuss e.g. important biological tests, sampling methods (dilution systems) and conditions relevant for toxicity screening of engine emissions.
For evaluation of the developed draft harmonised hazard screening and comparison of the test methods in different international laboratories (both engine and biomedical) the program EngToxNet (Engine Toxicity Network) is defined. The international harmonisation with a group of specialists within SETPOINT will be ongoing in parallel and the results of EngToxNet, along with the parallel projects, are needed for further harmonisation.

The outcome of the program EngToxNet, namely validation of the draft harmonised hazard screening and data-base with reference data for different engines and fuels, is especially meant to steer future government policies. Currently emission limits on regulated components are becoming more stringent every 3-5 years and billions of Euros are consequently spent to develop the engines that fulfil these requirements. However application of new technologies, new catalysts or fuels might change the chemical composition of the exhaust gas which may reflect a worse quality of exhaust gas with respect to health hazards. With an internationally harmonised health hazard screening it can be prevented that certain engine or emission control technologies or fuels are introduced which fulfil the requirements but actually form a greater health risks than the old situation.

During the IEA AMF 37th ExCoMeeting in Helsinki, May 2009, it was decided to reinforce the information activities and to help the international collaboration and coordination.

The Swiss and French delegates together with observers from Netherlands organized several meetings and prepared a proposal of an EU-project (per August 2010). As results of these coordinating activities and of the contacts with oversee partners the efforts of coordination and information of the worldwide research on toxicity of exhaust gases from engines with the unified methodology can be summarized with a flow-chart Annex 1 (the mentioned countries are members or observers of AMF).
In the proposal of the EU-project 9 countries participated. The topic did not fit in the objectives of EU research programs and the proposed project could not be performed. In the meantime there are several national activities and collaborations about the toxicity of engine exhaust gases.

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 (see activities SET POINT & EngToxNet, pt. 2.1.) the conditions of combined exposure were discussed and most of them were accorded in the meeting at ADEME, Sophia Antipolis (Nizza), Oct. 16th, 2009. 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.

The tests mentioned by project partners are given in annex A2. Most of the tests are normalized.

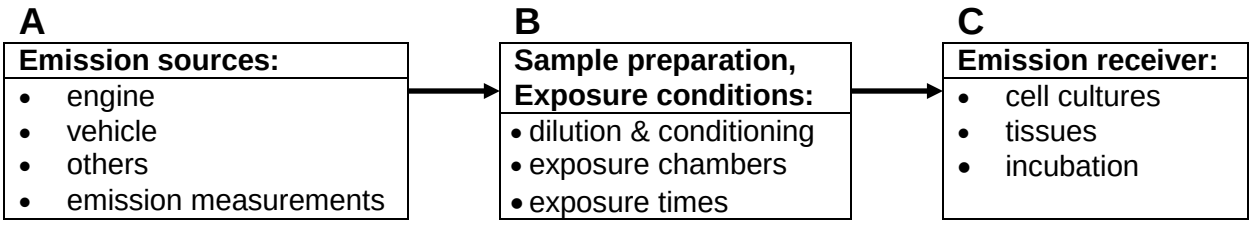
In free research the scientists may modify, or create other testing methods, according to different points of view and different objectives.
The efforts to establish a harmonized procedure nevertheless consider only standard tests.

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

Some further descriptions of biological processes and test methodologies are given in annex A3.

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.

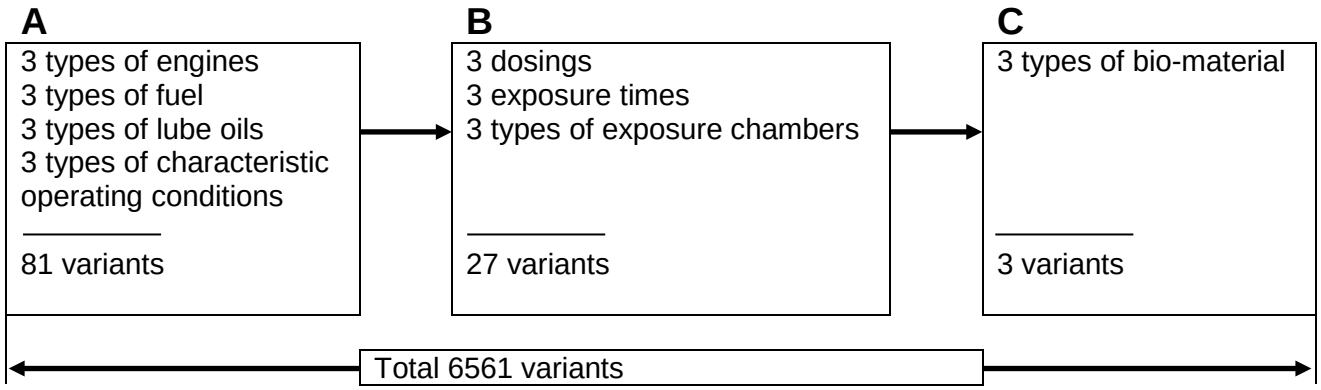
Part B – the conditioning of emission sampling – is prepared by specialists of measuring technics and control. It can be handled by the technicians from Part A.

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).

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. Finland

(contacts: Maija-Riita.Hirvonen@uef.fi; jorma.jokiniemi@vtt.fi)

At the University of Eastern Finland (UEF) there are activities with air-liquid exposure of cells to the whole emission aerosol. There is collaboration with the National Research Laboratories VTT. Information see in [1], (annex A16). Important to mention is the research with aged aerosols, so called secondary organic aerosols (SOA), which occur in the real world exposure. The ageing of aerosol for research is conducted in special ageing chambers using UV light radiation of controlled intensity.

Last message from UEF is:

In next spring we are going to have experiments using vehicle chassis dynamometer - diesel vehicle (fossil and biodiesel) - atmospheric chamber - Vitrocell air liquid exposure using cell line - parallel animal exposure using mice - analysis of toxicological parameters related to inflammation, oxidative stress, cell death, genotoxicity - and analysis of particle physico-chemical properties.

In a study (published in Sept. 2012) about the toxicity of exhaust gases from HD-engine powered with HVO, HVO blends and CNG it was found that, even in the best case, with lowest emissions some toxicological parameters were increased. More details see [3], annex A4.
The study applied an exposure method of a mouse macrophage cell line to the collected PM in suspension.

3.2. Netherlands

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

Information from TNO:

Publications:

- Bioassay-Directed Fractionation and Sub-fractionation for Mutagenicity and Chemical Analysis of Diesel Exhaust Particles.
  Esra Mutlu, Sarah H. Warren, Peggy P. Matthews, Charly King, William P. Linak, Ingeborg M. Kooter, Judith E. Schmid, Jeffrey A. Ross, M. Ian Gilmour, and David M. DeMarini (in press Environmental and Molecular Mutagenesis)

- Analytical in vitro approach for studying toxic effects of exhaust emissions using a heavy duty truck.
  Ingeborg M. Kooter, Marcel Alblas, Aleksandra D. Jedynska, Maaike Steenhof, Marc M.G. Houtzager, Martijn van Ras (submitted for publication)

Presentations:

- An air-liquid interface in vitro approach for studying toxic effects of exhaust emissions using a heavy duty truck.
  Ingeborg M. Kooter, Marcel Alblas, Jos van Triel, Aleksandra D. Jedynska, Maaike Steenhof, Marc M.G. Houtzager, Martijn van Ras, SOT conference 2013

- Development of an in vitro diesel exposure model for human bronchial epithelial cell.
  Maria C. Zarcone, Evert Duistermaat, Gimano D. Amatngalim, Pieter S. Hiemstra and Ingeborg Kooter, ERS congress Barcelona 2013

Further collaborations with different institutes on biodiesel studies; e.g. measurements of nitro-PAHs of biodiesel combustion products, based on the work of Kooter et.al., Atm. Env., 2011, 1574-1580

Within the national project NanoNextNL the release of nanomaterial from tires, as a non-combustion emission from road transport, is studied.

- 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.

Activities National Institute for Public Health and the Environment (RIVM),
Contacts: Miriam E. Gerlofs-Nijland, Centre for Environmental Health (MGO)
miriam.gerlofs@rivm.nl; flemming.cassee@rivm.nl

- Continuation of the network SETPOINT (Screening Emissions for Toxic Potential - Organising INTernational harmonisation) which promotes knowledge transfer and harmonization of hazard screening of engine emissions and the critical evaluation of these developments to guide policymakers and regulators.
- Continuation of activities: "Engine emission and Health" and “Toxicity testing of engine emissions”.
- Publication of a book: “Traffic-related air pollution – the health effects scrutinized”, see [4], annex A5.

There are clear health effects from the particle mass from transport and efforts to reduce the emissions and to limit the exposure are recommended.

3.3. Switzerland

Different activities and collaborations in Switzerland are represented in [1], (annex A6).

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. 4.1.).

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.

A short version of final report of this project is given in annex A6. The project will be finished in 2013 with a Ph.D. Thesis of Mr. S. Steiner. Some published results are:

- influences of Cerium dioxide nanoparticles on the cellular response, [5]; annex A7
- reduction of (pro)inflammatory responses due to non-catalyzed diesel particle filter, [6]; annex A8.

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 perform a research project about SOA's from different vehicle groups. There are efforts to join the *in vitro* exposure of cell cultures with the ageing of aerosol in a flow-reactor, which was developed by PSI. Annex A9 gives some information about the SOA-project.

Exposure of workers
(contact: michael.riediker@hospvd.ch)

In an extensive study of the Swiss Institute for Work and Health in collaboration with US EPA the exposure of highway maintenance workers to fine particles and noise was deeply investigated.
18 persons were monitored and an increased probability of cardiovascular deceases was confirmed, [7], annex A10.

3.4. Germany

Diesel alternative fuels & bacterial mutagenicity
(contact: buenger@ipa-dguv.de)

Many activities concerning the detailed physico-chemical characterization and mutagenicity of engine exhaust gases, especially Diesel engines with bio-fuels, are known from the network FJRG (Fuel Joint Research Group).

There is following actual information on behalf of the FJRG (http://www.fuels-jrg.de):
In Germany a very successful project on neat HVO "Diesel regenerative" was completed; see press release of StMUG (in German). The next project (R33) started this month with over 280 vehicles combusting a fuel with 33% content from renewable sources; see press release of Hochschule Coburg (in German). Further information is provided at: http://www.tankzukunft.de/. Members of the Fuel Joint Research Group (FJRG) are involved in both projects as principal researchers.

Please, find also attached some recent publications of the Fuel Joint Research Group (FJRG):

Westphal GA, Krahl J, Munack A, Rosenkranz N, Schröder O, Schaak J, Pabst C, Brüning T, Bünger J. Combustion of Hydrotreated Vegetable Oil and Jatropha Methyl Ester in a Heavy Duty Engine: Emissions and Bacterial Mutagenicity. Environ Sci Technol. 2013;47:6038-6046, [8], annex A11 (supported by IEA AMF).

Schröder O, Bünger J, Munack A, Knothe G, Krahl J. Exhaust emissions and mutagenic effects of diesel fuel, biodiesel and biodiesel blends. Fuel 2013;103:414–420, [9], annex A12.

Bünger J, Krahl J, Schröder O, Schmidt L, Westphal GA. Potential hazards associated with combustion of bio-derived versus petroleum-derived diesel fuel. Crit Rev Toxicol. 2012;42:732-750, [10], annex A13 .

The studies of FJRG are strongly oriented on variation of fuel quality on different Diesel engines with a strong physico-chemical characterization of the emissions (limited & unlimited components, PM, PN, differentiated HC etc.). As biological test the standard Ames-test is used.

As main results of these studies, it can be mentioned: lower mutagenicity of HVO & GTL, maximum mutagenicity of PM-extract from B20 and a valuable literature study concerning health effects in [10]; (this last study was performed for EUGT and was already mentioned in [2], reference [9]).

Toxicity of nanoparticles
(contacts: Dang Sheng Su, dssu@imr.ac.cn, [11]; Federal Institute for Occupational Safety and Health BAuA, [13])

Research of toxic effects of Diesel soot were performed at the Fritz Haber Institute of the Max Planck Society, Berlin with collaboration of scientists from China and Italy, [11, 12], annexes 14 & 15. It was found, that the soot from vehicle with lower PM-emission causes stronger oxidative, cytotoxic and inflammatory potentials. Before the exposure of macrophage cells in suspension the soot was sterilized by heating to 180°C.

In a study of the Federal Institute for Safety and Health, [13], annex 16, the TiO2 particles were instillated to rats in suspension to simulate the working exposure. Dose-dependent effects were found. The particles agglomerated in suspension.

In an older study, [14], annex 17 it was shown, that the finest nanoparticles penetrate easily across the cellular membranes. In this case, the particles were inhaled and could not agglomerate quickly.

The search of realistic exposure conditions is important, since it has influence on the biological results.

3.5. Denmark

(contacts: j.bonlokke@dadlnet.dk; 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 Aarhus University there is a study with DTI in preparation. A plan is to use the experimental climate chamber for studies on human volunteers which is under restoration and modernization currently and will become an up-to-date gold standard facility in its field. Exposures to the exhaust emissions from modern diesel/gasoline direct injection or other modern technology engines - and on new types of fuels will be performed.

Another project is a work of a PhD-student, Yuduo Zheng, working with different toxicity assays on particles sampled from diesel engines, samples, which were received from RIVM (Flemming Cassee). The samples are diesel and biodiesel exhaust particles from different cycles, with and without filters. A short description of this work is given in annex A18. The exposures of cells to the particles matter will be done in suspension.

3.6. Norway

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

Health-related activities and research are performed at the Norwegian Institute of Public Health. 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.

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

Our research goals have been to elucidate the importance of the organic fraction of diesel exhaust particles (DEP) for inflammatory responses (no 2 and 5 in particular). In addition another publication (Kubatova A et al., 2013 in press) elaborates further the possible importance of groups of PAHs extracted from DEP. We also investigated if the addition of biodiesel and the use of particle filters (DPF) would modify the toxicity of the particles. Particles collected from a EURO 4 engine with B50 fuel (50% biodiesel) and DPF had around 90% less mass, but were on an equal mass basis much more potent than conventional DEP. Calculated per km driven, however, the B50/DPF particles were still less potent than conventional DEP (no 4). Publication 6 investigated the combined effect of a biological agent and PAHs. The epidemiological study (no7) did not specifically investigate engine exhaust, but those particles constitute a substantial part of the PM mass associated with increased incidence of lung cancer found in this ESCAPE study. In further studies already funded (INFLAME), the importance of mixtures will be studied, in particular engine exhaust fractions. In the other study (FUELHEALTH) we will investigate more the toxicity of biodiesel and DPF diesel particles.

Publications on engine toxicity:

1. Particulate matter and nanoparticles toxicology.
Alfaro-Moreno E, Nawrot TS, Nemmar A, Rosas I, **Schwarze P**.
Biomed Res Int. 2013;2013:642974. doi: 10.1155/2013/642974. Epub 2013 Jul 10. No abstract available.

2. Differential proinflammatory responses induced by diesel exhaust particles with contrasting PAH and metal content.
Totlandsdal AI, Låg M, Lilleaas E, Cassee F, **Schwarze P**.
Environ Toxicol. 2013 Jul 31. doi: 10.1002/tox.21884. [Epub ahead of print]

3. Recent advances in particulate matter and nanoparticle toxicology: a review of the in vivo and in vitro studies.
Nemmar A, Holme JA, Rosas I, Schwarze PE, Alfaro-Moreno E.
Biomed Res Int. 2013;2013:279371. doi: 10.1155/2013/279371. Epub 2013 Jun 20

4. Cell toxicity and oxidative potential of engine exhaust particles: impact of using particulate filter or biodiesel fuel blend.
Gerlofs-Nijland ME, Totlandsdal AI, Tzamkiozis T, Leseman DL, Samaras Z, Låg M, **Schwarze P**, Ntziachristos L, Cassee FR.
Environ Sci Technol. 2013 Jun 4;47(11):5931-8. doi: 10.1021/es305330y. Epub 2013 May 20.

5. Inflammation-related effects of diesel engine exhaust particles: studies on lung cells in vitro.
Schwarze PE, Totlandsdal AI, Låg M, Refsnes M, Holme JA, Øvrevik J.
Biomed Res Int. 2013;2013:685142. doi: 10.1155/2013/685142. Epub 2013 Feb 14.

Publications related to engine toxicity:

6. Mechanisms of chemokine responses by polycyclic aromatic hydrocarbons in bronchial epithelial cells: sensitization through toll-like receptor-3 priming.
Øvrevik J, Refsnes M, Holme JA, Schwarze PE, Låg M.
Toxicol Lett. 2013 May 23;219(2):125-32. doi: 10.1016/j.toxlet.2013.02.014. Epub 2013 Feb 28.

7. Air pollution and lung cancer incidence in 17 European cohorts: prospective analyses from the European Study of Cohorts for Air Pollution Effects (ESCAPE).
Raaschou-Nielsen O, Andersen ZJ, Beelen R, Samoli E, Stafoggia M, Weinmayr G, Hoffmann B, Fischer P, Nieuwenhuijsen MJ, Brunekreef B, Xun WW, Katsouyanni K, Dimakopoulou K, Sommar J, Forsberg B, Modig L, Oudin A, Oftedal B, Schwarze PE, Nafstad P, De Faire U, Pedersen NL, Ostenson CG, Fratiglioni L, Penell J, Korek M, Pershagen G, Eriksen KT, Sørensen M, Tjønneland A, Ellermann T, Eeftens M, Peeters PH, Meliefste K, Wang M, Bueno-de-Mesquita B, Key TJ, de Hoogh K, Concin H, Nagel G, Vilier A, Grioni S, Krogh V, Tsai MY, Ricceri F, Sacerdote C, Galassi C, Migliore E, Ranzi A, Cesaroni G, Badaloni C, Forastiere F, Tamayo I, Amiano P, Dorronsoro M, Trichopoulou A, Bamia C, Vineis P, Hoek G.

Very interesting is the information about the strongly increased research activities worldwide about the topic of "Particulate Matter and Nanoparticles Toxicology ", annex A19. There were approximately 2000 publications in the first decade of this century.

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. Czech Republic

(contacts: michal.vojtisek@tul.cz; jtopinka@biomed.cas.cz)

There is an intense interdisciplinary collaboration between the Departments of Vehicles and Engines, Technical University of Liberec and Technical University of Prague and the Institute of Experimental Medicine of the Czech Academy of Science.

In the last years a lot of specific projects and progresses of infrastructure and measuring systems were performed. Also the interdisciplinarity between engine specialists and toxicologists was strengthen and developed. Detailed information about the recent activities, see annex A20.

3.8. Greece

(contact: A.G. Konstandopoulos: agk.@cperi.certh.gr)

The Aerosol & Particle Technology Laboratory (APTL) of the CERTH / CPERI has a long tradition in research on nanoaerosols from engines. There also is high competence of physico-chemical characterization, [1]. For toxicological research there is collaboration with the Department of Biology of the Aristotle University.

Prof. Konstandopoulos summarizes the activities in nanoparticle toxicity for 2013 as follows:

1. Asimakopoulou A., Daskalos E., Chasapidis L., Akritidis T., Vlachos N.D., Papaioannou E., Athanasios G. Konstandopoulos A.G. (2011) "Characterization of a Multiculture In-Vitro Cell Exposure Chamber for Assessing the Biological Impact of Diesel Engine Exhaust", Journal of Physics: Conference Series, 304 (1), Art No. 012005. Also in Proceedings of International Conference on Safe Production and Use of Nanomaterials, Nanosafe 2010, [CD-ROM], November 16-18, Grenoble, France ([15], annex A21).

2. Asimakopoulou A., Daskalos E., Lewinski N., Riediker M., Papaioannou E., Konstandopoulos A.G. (2013) "Development of a Dose-Controlled Multiculture Cell Exposure Chamber for Efficient Delivery of Airborne and Engineered Nanoparticles", Journal of Physics: Conference Series 429, Art. No. 012023; Also in International Conference on Safe production and use of Nanomaterials (Nanosafe 2012), November 13-15, Grenoble, France ([16], annex A22).

Furthermore, our proposal " System for preliminary in-vitro health impact assessment of size-selected nanoparticles" that describes our innovative system of exposing multiple biological samples (e.g. cell lines, tissues) to aerosol flows of size-selective selected size nanoparticle distributions, was among the 10 finalists in a national competition organized by EUROBANK and the Hellenic Federation of Enterprises (SEV).

3.9. Sweden

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

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

In a national commission in Sweden regarding evaluation of the health effects of Swedish environmental class 1 diesel and European diesel (higher aromatics) participates the Institute of Environmental Medicine (IMM). IMM is a department at Karolinska Institutet, an interdisciplinary research organization with internationally competitive research in the fields of epidemiology, toxicology, physiology and environmental medicine. The contact persons for the diesel fuel project were:
Professor Annika Hanberg (http://ki.se/ki/jsp/polopoly.jsp?d=28928&a=82347&l=en) and
Tomas Sandström, Professor in Public Health and Clinical Medicine at Umeå University.
http://www.umu.se/sok/english/staff-directory/view-person?languageId=1&uid=thsa0001

Following the information of the homepages the activities and publications can be found about subjects like:

- experimental determination of deposition of diesel exhaust particles in human respiratory tract, [17], annex A23.
- cardiovascular effects of particulate air pollution exposure, [18], annex A23.
- exposure to wood smok, [19], annex A24.

In this research principally the human exposure was applied.

3.10. 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.
Actually the French network works on a project MAETAC (Méthodes Alternatives pour l'Evaluation de la Toxicité des Aérosoles Complexes), which compares the results of exposures: on line (whole aerosol), off line (resuspended PM) and mutagenicity.

There is following information from Dr. J.-P. Morin:

"In Rouen, the MAETAC Program funded by ADEME is still in work.

Briefly Euro3 2 liter common rail injection turbocharged engine equipped with oxidation catalyst and Silicon carbide DPF on test bench runs with a low charge urban driving cycle.
There are nearly no toxic effects on the cell lines exposed to the gas after oxidation catalyst and DPF. At "engine-out" there are limited toxic impacts.

The project PEAR (ADEME) investigates the reduction potentials and the toxic effects of non-legislated components, like NH_3 and N_2O for gasoline cars and NO_2 for Diesel cars. Also the possibilities of secondary emissions from SCR systems, like hydrocyanic acid, isocyanic acid, ammonium nitrate and ammonia are considered and a toxicological research is planned.

In the frame of the ITMO Cancer research program call, we participate in a program dedicated to the study of the genotoxic potential of diesel emissions after inhalation exposure in vivo in the rat. The consortium is coordinated by Dr. Thierry Douki (CEA Grenoble France) and is composed of our group (EA4651-ABTE), an Inserm Unit of Rennes (Valerie Lecureur) and a laboratory of Dunkerque Cote D'Opale University Pro Shirali.
Two sorts of inhalation are applied to the rats: 24 hours, simulating an acute exposure and 3 weeks, simulating a semi-chonical situation. End of the project and results are planned in 2014.

A project about cardiotoxic impacts of NO_2 project CARDIOX (ADEME) is planned for 3 years since 2014.

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.

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.

3.11. USA

California
(contacts: sioutas@usc.edu; www.usc.edu/aerosol; aayala@arb.ca.gov; www.arb.ca.gov)

There are many activities concerning air pollution, traffic emissions and health effects in California, which is regarded as a birthplace of the exhaust emissions legislation in the 60-ties and 70-ties.
Except of the Californian Air Resources Board (CARB) there are other known institutions supporting the research of academia. Some of them are:

- South Coast Air Quality Management District (SCAQMD),
- Southern California Airborne Particulate Matter Center (SCAPMC),
- Asthma Allergic Disease Research Center (AADRC).

Extensive information about project activities at the University of Southern California and UC Irvine is given in [1], (annex A18).

Further activities to be mentioned are:

- Presentation of Dr. Kleinman, UC Irvine, at CARB Sacramento, Oct. 9th, 2013, annex A25: http://www.arb.ca.gov/research/seminars/kleinman2/kleinman2.htm

- This study has demonstrated that the semi-volatile PM fraction of ambient ultrafine particulate matter is an important contributor to the development of atherosclerosis and heart disease.
- Thermal denuding technology such as afterburner emission controls not only reduce pollution but reduce the toxicity of the residual particles.

- Research on formation and growth of secondary organic aerosol (SOA) at UC Irvine, [20], annex 26: the airborne particles, their chemical changes and growth under the solar radiation have impacts on air quality, health effects, visibility and climate.
- Source-oriented sampling of atmospheric nanoparticles and analysis by a Particle Mass Spectrometer – a study at UC Davis, [21], annex A27: different emission sources and atmospheric transformations of aerosol have influence on the composition and toxicity. The more differentiated look on the air pollution allows more selective and efficient measures to reduce it. The work shows only the chemical characterization of atmospheric nanoparticles.

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

Information and references from EPA, Chapel Hill Laboratories, North Carolina are given in [1], (annex A20). There is a close collaboration with the University of North Carolina School of Public Health (http://www.sph.unc.edu).

West Virginia University, Morgantown
(contact: tnurkiewicz@hsc.wvu.edu)

There is following information from Prof. Timothy R. Nurkiewicz, Center for Cardiovascular and Respiratory Sciences, WVU, Morgantown:

Attached are several publications from my research program, [22-26], annex A28. I especially encourage you to look at two of these. The first is from my current post-doc, Phoebe Stapleton (AJOG 2013); wherein we report for the first time the fetal micro vascular consequences of maternal nanomaterial inhalation. The second is from my inhalation engineer, Jinghai Yi (JOVE); wherein a link to a video of our inhalation exposure technique is provided.

In these studies, with exposures of rats and inhalation to the manufactured aerosols, several pulmonary and cardiovascular effects are found.
Remark from the author: there are intense activities about engine emission at WVU, Faculty for Mechanical and Aerospace Engineering and also collaborations with NIOSH Morgantown. These are excellent conditions for the research with whole aerosol exposure on engines, or vehicles.

Harvard School of Public Health, Boston, MA
(contact: jlepeule@hsph.harvard.edu)

In an epidemiological Harvard Six Cities Study from 1974 to 2009 an increased cardiovascular and lung-cancer mortality by increased exposure to fine particles was confirmed, [27], annex A29.

Generally there are no doubts about the penetration of nanoparticles into the human organism and about the negative acute or potential health impacts. The last ones depend on many factors, like: composition of nanomaterial, target organs (or cells), dosing (i.e. exposure time & concentration). There are many variables and each project, like usually in the research, can open new questions.

3.12. Canada
(contacts: subramanian.karthikeyan@hc-sc.gc.ca, paul.white@hc-sc.gc.ca)

Information from the highly specialized laboratories of the National Research Council, Canada is provided in [1], (annex A21).

There are works and experiences with different types of exposure with the objectives to attain the most realistic exposure route. Due to the unchanged objectives, employed technologies and collaborations the preceding information is still actual.

In 2013, a study about the results of exposure of rats to the Diesel exhaust gas without and with catalyzed DPF was published at Oxford Toxicological Sciences, [28], annex A30.

The toxic effects were increased with this DPF-system due to increased NO_2 and reactive NP.

Remarks of the author:
The catalyzed DPFs, especially with Platinum as catalyst, increase the NO_2-portion in certain operating conditions. There is also an aptitude of producing the spontaneous condensates of substances, which pass the filter as vapor. This is the case especially with sulphates in sub 30 nm size range; with a strongly catalyzed DPF (or CRT). This part of the nanoaerosol is reactive and contributes to the generation of SOA together with NO_2. The increased toxicity is well known.
In the Swiss quality testing of DPF systems (VERT & OAPC) these facts are considered and the NO_2-producing systems are not recommended for use in closed spaces.
(see VERT List, www.vert-dpf.eu, www.vert-certifaction.eu).
On the other hand, there are big progresses of the exhaust aftertreatment technologies offering catalyzed systems with no production of NO_2 or/and using the deNO_x systems in line with DPF.

3.13. Korea

(contact: Prof. Jiyeon Yang; jyyang67@yuhs.ac)

Prof. Jiyeon Yang from the Institute for Environmental Research, Yonsei University (IERY) in Korea gave following information:

My major research fields are human exposure and risk assessment of hazardous chemicals, and I research in a new field, which is in vitro test of nano-materials and ultrafine particles since 2004. Recently, we are studying inflammation and genotoxicity of black carbon in the ultrafine particles.

A study of exposure and toxicity of ultrafine particles from urban traffic was performed,
[29], annex A31. In this study a sampling and chemical characterization of ambient particles were performed. The cell cultures were exposed to the "extractable organic matter from PM_{10}" in solution in vitro. The biological essays confirmed the induction of inflammatory responses and risks of respiratory injury. Seasonal differences with tendencies of more SOF and more risks in winter time were stated.

3.14. China

(contact: Prof. Lap Ah Tse; shelly@cuhk.edu.hk)

Prof. Lap Ah Tse from JC School of Public Health and Primary Care from the Chinese University of Hong Kong performed a research about the lung cancer risks of professional drivers, [30],
annex A32. In this work, 19 published cohort and case studies were systematically evaluated. It was found, that for the professional drivers, who are frequently exposed to diesel exhaust, there is 18% increased risk of lung cancer. The study (2012) suggests further implications for policy to reduce the emissions.

Prof. Kan Haidong from the School of Public Health at Fudan University in Shanghai performed research confirming higher toxicity of smaller size air pollution aerosols. There is a necessity of monitoring and limiting of the fine nanoparticles. A recent information from China Daily see annex A33.

3.15. WHO

The World Health Organization (WHO, http://www.euro.who.int/en/home) and the International Agency for Research on Cancer (IARC), which is part of WHO, published a study about air pollution and health effects (REVIHAAP), [31], annex A34. This study, with participation of many experts, clarifies the evidence of health effects of different air pollutants.
Further scientific contributions are: the book "Air Pollution and Cancer", [32], annex A35 and the press information from Oct. 2013 about air pollution and cancer, annex A36.

3.16. MECA

The Manufacturers of Emission Controls Association (MECA, www.meca.org) released a report outlining the health impacts of ultrafine particulates (UFP) from cars, trucks, and off-road equipment and the benefits of reducing both the mass and number of particulate emissions through the use of particulate filters. The report--Ultrafine Particulate Matter and the Benefits of Reducing Particle Numbers in the United States--was prepared for MECA by Gladstein, Neandross & Associates.

The report summarizes the current understanding of the potential adverse health impacts of UFPs; outlines the various control technologies that can be used to meet current and upcoming US EPA and California ARB emission standards; and documents the success story of using diesel particulate filters (DPF) to meet US and EU emission standards. The report indicates that a particle number (PN) measurement may offer a more robust unit than PM mass for determining compliance at very low particle emission levels. The report also quantifies the health benefits of the additional emission reductions that are realized when DPFs or gasoline particulate filters (GPFs) are used compared to only engine-based strategies.

In the report, MECA makes several recommendations for EPA and ARB to help achieve the maximum environmental and health benefits from their current and upcoming on-road and off-road emission standards:

- EPA and ARB should add a PN limit to its regulatory structure for mobile sources;
- EPA and ARB should consider a new set of heavy-duty diesel engine PM standards that would be equivalent in stringency to ARB's future LEV III standards for light-duty vehicles;
- EPA should increase its in-use compliance monitoring of nonroad diesel engines that are certified without DPFs;
- EPA and ARB should coordinate activities to develop a methodology measuring UFP emissions and particle numbers;
- Environmental agencies around the world should follow the U.S. lead and tighten evaporative emission limits as a way to control secondary organic aerosols; and
- Federal and state governments should play a greater role in accelerating the retirement or retrofitting of older, dirtier diesel engines and the introduction of cleaner diesel replacements.

Full Report: http://www.meca.org/resources/MECA_UFP_White_Paper_0713_Final.pdf

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.

4.2. 17th 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 A37 shows the presentations of the sessions "Health effects" from the last year 2013. Further information can be asked and a CD can be ordered at: ttm.a.mayer@bluewin.ch.

At the last Conference, June 2013, a special Focus Event was organized about the possibilities and necessity of more appropriate valuation and regulation of atmospheric aerosol pollution, see annex A38. This Focus Event was the continuation of the very important topic from 2012.

From these Focus Events two very important statements about the behavior of nanoparticles at biological barriers, cells and tissues are:

- Only nanoparticles may penetrate in the lung into cells and tissues and, therefore, in the alveolar region through the air-blood tissue barrier into the blood; they can translocate into other organs by the blood circulation, where they may interact with organ specific cells and tissues.
  There are further biological barriers, like the blood-brain barrier, the blood-blood placenta barrier, the blood-thymus barrier, the blood-testis barrier a.o. that may be penetrated by nanoparticles.
- Within cells nanoparticles may cause adverse effects. Moreover, adverse effects have been shown to be caused by nanoparticles in the vascular system. Combustion-generated nanoparticles may cause inflammatory effects in the brain; in most organs, however, the effects which nanoparticles may cause are not fully known yet. Size matters; nanosized particles are the most critical ones for health.

These statements are formulated and agreed from many independent scientists.

4.3. EU research programs

In the Frame Program FP7 several projects about the exposure and health impacts of nanoparticles were performed. The actual list of projects, annex A39, can be found under the link:

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

The projects focus mostly on the engineered nanoparticles, but they also treat many subjects, which are interesting for research on toxicity of exhaust gases.

4.4. 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.healtheffects.org … Health Effects Institute (HEI), 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.

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

- the harmful effects of atmospheric pollution, especially NP, became an important research topic and requirement to 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.

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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)

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
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
WVU	West Virginia University

9. ANNEXES

A 1	Efforts of coordination and information of the worldwide research on toxicity of exhaust gases from engines with unified methodology of aerosol exposure
A 2	Toxicological tests - endpoints
A 3	Introduction in test methodologies and some biological processes
A 4	Reference [3], toxicity HD-engine with bio diesel, HVO & CNG, Fin
A 5	Reference [4], traffic – air pollution – health effects; NL
A 6	Final report of BioToxDi / EngTox Di, short version, CH
A 7	Reference [5], Cerium dioxide nanoparticles – cellular response, CH
A 8	Reference [6], Reduction inflammatory responses – non catalysed DPF, CH
A 9	Secondary aerosol (SOA) from road vehicles, information PSI, CH
A 10	Reference [7], Exposure of Highway Maintenance Workers to Fine Particles & Noise, CH
A 11	Reference [8], Emissions & Mutagenicity, HD Diesel, HVO, Jathropa FAME, D
A 12	Reference [9], Emissions & Mutagenicity, Diesel with biofuel, D
A 13	Reference [10], Potential hazards, Bio Diesel, D
A 14	Reference [11], Diesel Soot Toxification (2013), D
A 15	Reference [12], Diesel Soot Particles-cytotoxicity, inflammatory potentials (2007), D
A 16	Reference [13], Toxic effects of nanoparticle inhalation (2013), D
A 17	Reference [14], Ultrafine particles cross cellular membranes (2005), D / CH

A 18 Effects of ultrafine particles in whole blood assay and cell exposure in suspension, DK
A 19 Research about "Particulate Matter and Nanoparticles Toxicology", N
A 20 Activities EngTox in Czech Republic, CZ
A 21 Reference [15], Characterization of cell exposure chamber, Gr
A 22 Reference [16], Development of a dose-controlled cell exposure chamber, Gr
A 23 Abstracts of references [17] & [18]: deposition of NP & cardiovascular effects, S
A 24 Reference [19], Exposure to wood smoke, S
A 25 Presentation Dr. Kleinman, UC Irvine, CARB Sacramento, Oct. '13 – CARB homepage, US
A 26 Reference [20], Secondary Organic Aerosol – formation & growth in the atmosphere, US
A 27 Reference [21], Source-oriented characterisation of atmospheric NP, US
A 28 References [22-26], from the research team WVU, Prof. T.R. Nurkiewicz, US
A 29 Reference [27], Increased mortality with chronic exposure to NP, US
A 30 Reference [28], Biological effects of NO_2 & NP with catalysed DPF, Can
A 31 Reference [29], Toxicity of ultrafine particles from urban traffic, Korea
A 32 Reference [30], Lung cancer risks of professional drivers; China
A 33 Information China Daily Oct. 28th, 2013: PM1 Air Pollution Most Harmful
A 34 Reference [31], Evidence of health aspects & air pollution, WHO
A 35 Reference [32], Air Pollution and Cancer, IARC Sc. Publication N° 161
A 36 WHO/IARC press Release N° 221, Oct. 2013; Air pollution & cancer
A 37 Session "Health Effects" of the 17th NP Conference, ETH Zürich, June 2013
A 38 NP Conference 2013 Focus Event
A 39 List of EU-projects FP7: Exposure to NP & health effects