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

2nd Information Report for IEA Implementing Agreement AMF, Annex XLII, international activities 2012

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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 and 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 showed, 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 repeated from the last year report, [1] with only a few modifications.

We observe fast increasing 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 and in the near future also a limit for particulate number emission will be set (Euro 6, 2013). 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).
During the common works it became clear, that the activities have to be divided in several steps and subtasks. As already mentioned the activities on the political-administrative level and harmonization of hazard screening of engine emissions were called “SET POINT” and the research projects at technical-scientific level and validation of the draft hazard screening were called EngToxNet.
In the proposal of the EU-project 9 countries participated. The search of possibilities of financing this project is still in course. In the meantime there are several national activities and collaborations.

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.

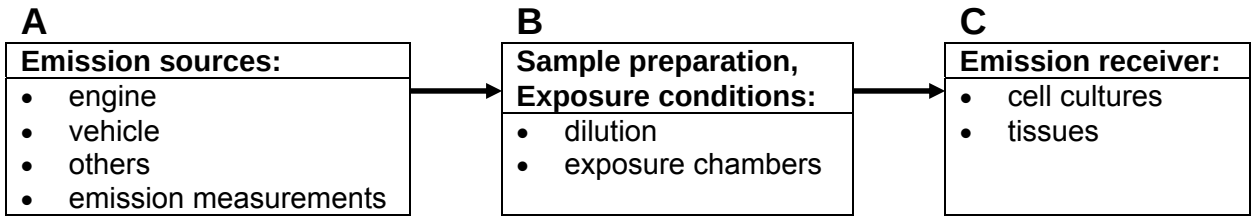
In free research the scientists may modify, or create other testing methods, according to different points of view and different objectives.
The efforts to establish a harmonized procedure nevertheless consider only standard tests.

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 unorganic analytics.

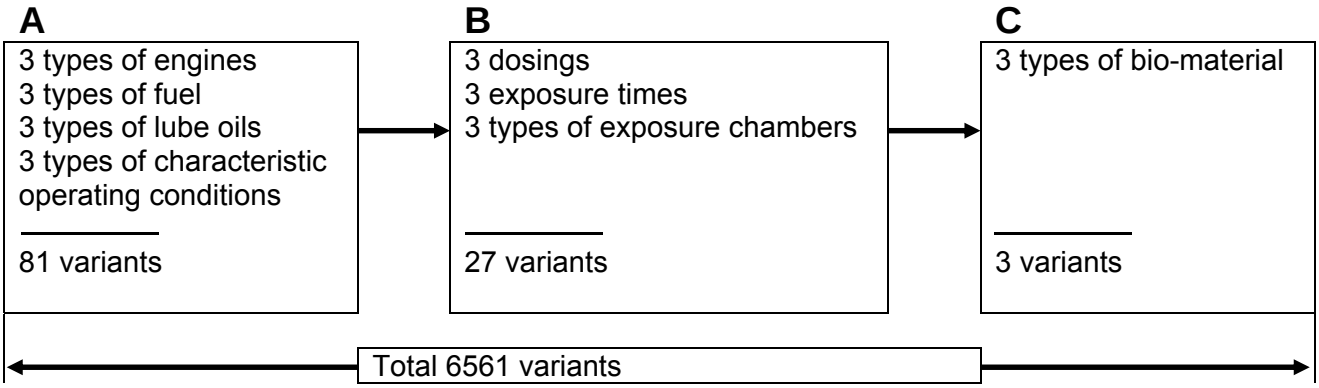
Part B – the conditioning of emission sampling – is prepared by specialists of measuring technics and control. It can be handled by the technicians from Part A.

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. France

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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 progressing and will be terminated by the end of this year (final report on this program due February 2013).
> 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. Main preliminary results in terms of genotoxicity is the lack of PAH related DNA adduct occurrence which is correlated with the only trace levels of organosoluble PAH evidenced on the collected particles downstream the oxidation catalyst. On the other hand, 8OHdG has been detected suggesting DNA oxidative damage downstream the oxidation catalyst and to a slightly lesser extent downstream Oxycat + DPF.
>
> In the frame of the ITMO Cancer research program call, we will participate to 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.
>
> In the recent context of IRCC classification of diesel emissions as cancerogenic entity mainly based on US miners exposed to old heavy duty engine emissions prior year 1980, we do suggest, in agreement with McLellan paper, that research results have to be produced with newer engine emissions (most recent technologies) in order to revisit their potential toxic insults that due to major physicochemical changes will with no doubt significantly differ from mid or late 20th century diesel engine emission impacts.

The medical and scientific literature frequently misses the fact that profound evolution of diesel engine emissions occurred within the last twenty years due to injection pressure improvement, combustion efficiency improvement, fuel quality improvement and exhaust aftertreatment strategies.

I also attach the paper by Gamble et al. which is giving a very critical review of epidemiology literature on diesel emission induced cancer hypothesis, (fragments of the paper see annex 4). *Results from all occupational cohort studies with quantitative estimates of exposure have limitations, including weak and inconsistent exposure-response associations that could be explained by bias, confounding or chance, exposure misclassification, and often inadequate latency. In sum, the weight of evidence is considered inadequate to confirm the diesel-lung cancer hypothesis.*

Our project clearly aim at revisiting diesel emission toxicity using continuous flow exposures to continuously produced years 2010 representative diesel engine emissions with both in vitro and in vivo approaches.

Actually we are now routinely using Air Liquid interface exposures for lung slices, lung epithelial cell lines on porous supports and direct aerosol exposure for Ames test for in vitro approaches. Direct in vivo inhalation exposure of rodents. This strategy offers the great advantage that all the biological targets are exposed to the same aerosol with diffusion interactions and identical dose deliveries to the biological targets."

In annexes some fragments of publications treating about the causality between diesel emissions exposition and lung cancer are given. French scientists participated on those international studies.

Annex 4, [1], 2012: in sum the weight of evidence is considered inadequate to confirm the diesel – lung cancer hypothesis. Interesting are: the list of references and the declaration of interest.

Annex 5, [2], 2005: the group of researchers concludes some limited support for the hypothesis of an excess lung cancer risk due to diesel exhaust. See also a large list of references.

Annex 6, [3], 2010: the results show a consistent association between occupational exposure to diesel exhaust and increased risk of lung cancer.

Annex 7, [4], 2012: an increased risk of lung cancer by exposure to diesel engine emissions was confirmed.

It can be remarked (from the author), that the causality of exposure-response was officially recognized and this conducted to the new statement of WHO (chap. 3.12.), which declares diesel emissions as carcinogenic (with no doubt).

On the other hand it is known, that the solid nanoparticles can penetrate through the air-blood barrier at slow translocation rates and may be transported to all organs, where they may exert variable toxic potentials according to their physicochemical properties. In this situation the regard on lung cancer only may be a little simplified objective and the response of cell cultures or living tissues in the aerosol exposure appears as a more objective evaluation method.

Advantages of whole aerosol exposure system 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.

3.2. Netherlands

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

Information from TNO:

Collaborations with different institutes on biodiesel studies; e.g. measurements of nitro-PAHs of biodiesel combustion products, based on the earlier work at TNO published 2011:

- Toxicological characterization (cytotoxicity and mutagenicity via Ames test) of diesel engine emissions (DAF XE355 Euro III truck engine) is using biodiesel and a closed soot filter [6] (some fragments see [1] annex A5).

2012 R&D at TNO:

- 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).
- Together with RIVM and ECN in the Netherlands a project on the oxidative potential of PM in the Netherlands was performed. Objective was on the relation between sources and health effects of the oxidative stress.
- Beginning of 2012 a 4 year project started 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),
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- Continuation of the network SETPOINT (Screening Emissions for Toxic Potential - Organising INTernational harmonisation) which promotes knowledge transfer and harmonisation of hazard screening of engine emissions and the critical evaluation of these developments to guide policymakers and regulators. A preliminary proposal for a COST Action has been submitted in September 2012 for funding network activities. This Action called NOHARM (Novel hazard identification methodology to establish the true toxicity of transport-related air pollution) will facilitate strategies for implementing new technologies and fuels with minimal health impact.
- Project "Engine emission and Health" funded by the Dutch Ministry of Infrastructure and the Environment to keep up with professional literature and support policy with ad hoc questions regarding traffic-related health effects.
- Toxicity testing of engine emissions (in vivo animal/human) in collaboration with divers partners. Part of this work on the impact of particulate filter and biodiesel blend use on the oxidative and pro-inflammatory potential of engine exhaust particles has been submitted for publication.

In June 2011 an European Workshop about wear emissions from road transport and their health effects was organized in Amsterdam. TNO & RIVM summarized the information of this workshop in a report [7], annexe A8. 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

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

In the present annex 9, there is information about the internationally recognized SAPALDIA, Swiss Tropical and Public Health Institute and the Institute for Work and Health.

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.

The activities with whole aerosol exposure started 2007 with the research on 2-stroke scooters, which was ended with the Ph.D. Thesis [8]. This PhD. Thesis is 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 is continued in the Swiss Network with the project "BioToxDi" (Biofuels, Toxicity, Diesel), see annex A10. 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 are investigated.

As conclusions up to date it can be remarked, that:

- there is a clear influence of exhaust gas quality on the cytotoxicity, oxidative stress and inflammatory reactions of cells,
- the exposure of cells to the combined aerosol (with gaseous and particulate toxic components) is a very useful method of research of toxicity; it is proposed to apply this method for all kind of pollution sources.

The tests of soot were performed at EMPA Federal Laboratories combining the methods of X-ray absorption spectroscopy and toxicological end points. Cellular toxic and inflammatory reactions were confirmed. Together with other studies from Norway and USA these results gave the base for WHO to reclassify the Diesel soot from "probably carcinogenic" to "carcinogenic", see EMPA note annex A11.

3.4. Germany

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

The group includes:

- Prof. Dr. med. Jürgen Bünger, Institute for Prevention and Occupational Medicine of the German Social Accident Insurance, at the Ruhr-Universität Bochum, (contact: buenger@ipa-dguv.de),
- Prof. Dr.-Ing. Peter Eilts, Institute of Internal Combustion Engines at the Technische Universität Braunschweig, p.eilts@tu-braunschweig.de
- Prof. Dr.-Ing. Axel Munack, Institute of Agricultural Technology and Biosystems Engineering at Johann Heinrich von Thünen-Institute, Braunschweig, (contact: axel.munack@fal.de), and
- Prof. Dr. Jürgen Krahl, Technology Transfer Center Automotive (TAC) at the University of Applied Sciences and Arts, Coburg (contact: juergen.krahl@hs-coburg.de).

All four professors have extensive experience in fuel research projects.

This year Prof. P. Eilts joined the FJRG completing the interdisciplinarity of the group.

In [9], a study founded by the European Research Group of Environment and Health of the Transport Sector (EUGT), the authors give an extensive overview of in vitro studies about biological effects of Diesel emissions with B100. From the 18 mentioned studies 14 use the bacterial mutagenicity AMES as principal end point. There are 120 references in this work.

In [10] is stated, that the use of Diesel oxidation catalyst lowers the mutagenicity of the gas phase, but has only a little impact on the mutagenicity from the particulate phase.

An interesting study was performed by another German research group from the Helmholtz Center Munich, concerning the correlation between the nanoparticle air pollution and the cardiovascular mortality in Beijing, [11], annex A12.

The results and conclusions of this study are:

- Observed were associations between daily cardiovascular mortality and particle NC (number concentration) for a 2-days delay. Moreover, nearly all particle metrics showed 2-days delayed associations with ischemic heart disease mortality. The strongest association was found for particle NC in the size range 0.03–0.1 µm.

- Results show an elevated risk of cardiovascular mortality in Beijing from short-term exposure to particulate air pollution in the sub-micrometer range. Results also indicate that locally produced smaller particles and regionally transported particles may exhibit different effects.

3.5. Denmark

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

At the Aarhus University there is a study about comparing different exhaust and wood smoke particles in a whole blood assay. There also are plans of preparing an experimental exposure study of volunteers in a climate chamber with new Diesel engine exhaust. Both groups (Aarhus & Copenhagen) have projects related to air pollution and are interested in developing and using relevant toxicity tests.

3.6. Norway

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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.

The main treated questions and submitted publications are:

1. What groups of compounds in diesel exhaust particles have the greatest impact on inflammatory responses of lung?
   Both native DEPs and DEP-extract, but not residual DEPs, induced marked mRNA expression of COX-2, IL-6 and IL-8, as well as cytotoxicity and release of IL-6. However, CYP1A1 was primarily induced by the native and residual DEPs. Overall, the results of near-edge X-ray absorption fine structure (NEXAFS) spectroscopy and gas chromatography with mass spectrometry (GC/MS) analysis of DEP-extracts indicated that the majority of the analyzed PAHs and PAH-derivatives were extracted from the particles, but that certain PAH-derivatives, probably their carboxylic isomers, tended to be retained on the residual DEPs. Moreover, it appeared that certain components of the methanol extract may suppress CYP1A1 expression. These results provide insight into how different components of the complex DEP-mixture may be differently involved in DEP-induced pro-inflammatory responses and underscore the importance of identifying and clarifying the roles of active DEP-components in relation to different biological effects.
   These results have been extended (Totlandsdal et al., submitted) by further fractionation of the extracts. The preliminary results indicate that the polar fraction has a greater impact on the responses than the other fractions analyzed.
   However, the oxo-and nitro-PAH may not be the most important components to elicit effects.
2. Does the addition of biodiesel and use of DPF have an effect on the toxicity of DEP? (Gerlofs-Nijland et al., submitted) This study shows that PM mass reduction by technological or fuel interventions will not by definition result in decreased hazard of engine emissions. We suggest that the biological effects of vehicle PM interventions are thoroughly investigated before being implemented on a large scale.
3. A review of the effects of DEP in lung cells has also been submitted.

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

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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. There is participation on the activities of harmonization the methodology of risk assessment RIVM & EngToxNet. Further information, about the actual projects of engines and toxicology, see annex A13.

3.8. 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:

During this year we have focused our efforts on validation and testing our new multidisciplinary laboratory. Shortly, we are testing different cell types and exposure conditions in our air liquid interface exposure system. We started with silver nanoparticles and now the very first experiments on diesel emissions are going on.

In addition, one paper on cytotoxic and inflammatory potential of emission particles from fossil and biodiesel fuels was published Oct. 2012 in Particle and Fiber Toxicology, [12]. In this study traditional cell exposure method with collected particles was used.

3.9. Greece

(contacts: 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 2012 as follows:

- We have submitted a proposal for the study of toxicity of engineered catalytic nanoparticles employed in emission control.
- We have initiated an exposure study of living tissues to nanoparticles with in situ monitoring of the nanoparticle incorporation and localization in the tissue.
- We have completed analysis and design studies for the upgrade of our 24-cell culture exposure chamber to a 36-cell culture exposure chamber with a much smaller footprint.
- We have developed methods for direct dose estimation, on line particle sampling as well as on line Reactive Oxygen Species (ROS) evaluation in the exposure chamber.
- A collaboration with IST Lausanne, Switzerland has been initiated employing our exposure chamber for assessing the impact of nanoparticles on cell cultures.

3.10. USA

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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 running projects at the University of Southern California is given in [1], (annex A18).

Information and references from EPA, Chapel Hill, 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).

In both institutions there are competences for the research with all kinds of exposure.

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.

In October 2011, a study about exposure of miners and lung cancer was published by US National Cancer Institute and NIOSH, [13], fragments see annex A14. Statistically significant increasing trends in lung cancer were observed with increasing cumulative and average breathable elemental carbon.

In April 2012 a study of engine industry was published, [14], in which the authors show an impressive reduction of legislated and non-legislated emissions by means of new technologies. It is postulated, that the carcinogenic hazards of Diesel exhaust shall be regarded separately for the new and for the traditional technologies. Some fragments of this work (with 177 references) are given in annex A15.

Further information about this study, as well as about the activities of Health Effects Institute (HEI … www.healtheffects.org) and activities of Manufacturers Emissions Control Association (MECA … www.meca.org) is given in a MECA's special report from May 2012, annex A16.

3.11. Canada

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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.

To mention is a recent study from Montreal, which confirms the increased risk of lung cancer due to the occupational exposure to Diesel engines emissions, [15], fragments see annex A17.

3.12. WHO

The International Agency for Research on Cancer (IARC), which is a part of the World Health Organization (WHO), classified the Diesel engine exhaust as "carcinogenic to humans (group 1)". This is based on the research results from last years and on the sufficient evidence, that exposure is associated with an increased risk for lung cancer.
The WHO Press Release No. 213, from June 2012 is given in annex A18 and the Monograph WHO Working Group members, see annex A19.

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.

An extensive study about physico-chemical characterization of Diesel emissions with Swedish "Environmental Class 1" fuel was performed at AVL MTC AB with support of the Swedish Transport Administration, [16].
Beside the deepened analysis of nonlegislated components, like Aldehydes, Alkenes, PAH, particle mass & counts bacterial mutagenicity tests Ames were used as a health effect indicator (abstract see annex A20).
A lower emission level and lower mutagenic potential with the Swedish special Diesel fuel were confirmed.
No activities with aerosol exposure of cells or tissues were reported.

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

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 A21 shows the presentations of the sessions "Health effects" from the last year 2012. Further information can be asked and a CD can be ordered at: ttm.a.mayer@bluewin.ch.

At the last Conference, June 2012, a special Focus Event was organized about the possibilities and necessity of more appropriate valuation of atmospheric aerosol pollution, see annex A22.

From the theses of this Focus Event 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.

In Information Newsletter March – April 2012 of AECC (Association for Emission Control by Catalyst, www.aecc.be) several studies about health effects are mentioned, see annex A23.

4.2. 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.efca.net	… European Federation of Clean Air and Envrionmental 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 a 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 spreaded – 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.

New look on particle toxicity 2012

- Classification of Diesel engines exhaust by WHO in the group 1 "carcinogenic" has taken place in June 2012 , which is expected to modify several local antipollution legislations,
- The recent knowledge about: nanoparticles, their penetration in the living organisms and their adverse health effects as well as their impact to global warming is nevertheless not yet entirely taken into consideration by regulations, therefore further restrictions to the other nanoparticle emission sources (like DISI, or 2-strokers) might be expected soon.

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.

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7. REFERENCES

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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
CVS	constant volume sampling
DEP	Diesel exhaust particles
DMA	differential mobility analyser
DOE	US Department of Energy
DPF	Diesel Particle Filter
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
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
HEI	Health Effects Institute
I & M	NL Ministry of Infrastructure & Environment (www.government.nl/ministries)
IA	Implementing Agreement
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
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
VSS	Verband der Schweizerischen Schmierstoffindustrie (www.vss-lubes.ch)
VTT	Technical Research Center of Finland
WHO	World Health Organisation

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 [2], review, epidemiology, diesel-lung cancer hypothesis, 2012

A 5 Reference [3], exposure to Diesel & Gasoline engine emissions and risk of lung cancer, 2005

A 6 Reference [4], exposure to Diesel engine emissions and risk of lung cancer 2010

A 7 Reference [5], exposure to Diesel engine emissions and risk of lung cancer 2012

A 8 Reference [7], policy, wear emissions from road transport, 2012

A 9 Swiss SAPALDIA Study & Health Institutes

A 10 Project BioToxDi, report 2012

A 11 EMPA Note: Diesel soot reclassified, Aug. 2012

A 12 Reference [11], correlation: particulate air pollution and cardiovascular mortality in Beijing

A 13 Information from Czech Republic

A 14 Reference [13], the Diesel Exhaust Miners Study, Oct. 2011

A 15 Reference [14], carcinogenic hazards & revolutionary changes in Diesel technology, Apr. 2012

A 16 MECA Executive Bulletin: HEI Annual Conference, Chicago, April 2012

A 17 Reference [15], exposure to Diesel emissions and risk of lung cancer, July 2012

A 18 WHO Press Release no 213, June 2012

A 19 Monograph WHO Working Group Members, July 2012

A 20 Reference [16], fuel impact on Diesel exhaust emissions and bacterial mutagenicity, 2012

A 21 Sessions “Health Effects” of the NPC (Nanoparticle Conference), Zürich, June 2012

A 22 NPC 2012 Focus Event

A 23 AECC Newsletter March-April 2012