*(Projects submitting final reports after 1 January 2014 must use this format.)*

LIFE Project Number

# LIFE10 ENV/CZ/651

# FINAL Report

## Covering the project activities from 01/09/2011 to 31/08/2016

Reporting Date

## 28/02/2017 (revised version)

LIFE+ PROJECT NAME or Acronym

# MEDETOX

Project Data

| | |
|---|---|
| **Project location** | Prague |
| **Project start date:** | 01/09/2011 |
| **Project end date:** | 31/08/2016 |
| **Total Project duration (in months)** | 60 months |
| **Total budget** | 1,223,524 € |
| **Total eligible budget** | 611,762 € |
| **EU contribution:** | 611,762 € |
| **(%) of total costs** | 50 |
| **(%) of eligible costs** | 50 |

Beneficiary Data



| | |
|---|---|
| **Name of Beneficiary** | Institute of Experimental Medicine AS CR |
| **Contact person** | Mr. Jan Topinka |
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| **Project Website** | http://www.medetox.cz |

# 1. Table of contents

# 2. Executive Summary

## 2.1. Project objectives

The MEDETOX project aimed to demonstrate innovative methods of monitoring toxicity of diesel engine exhaust emissions during real urban driving, with a detailed focus on the situation on the ring road of Prague.

The project was focused on the following topics:
- The construction and validation of several innovative emissions monitoring and/or sampling systems for assessment of emissions on the road.
- The preparation and validation of simplified toxicity assays which could be carried out using the collected samples.
- An investigation of real-world emissions and of the toxicity of particle-bound organic compounds collected during real-world operation and during its simulation in the laboratory, with a particular focus on urban driving and on the effects of congestion.
- An investigation of real-world emissions and of the toxicity of particle-bound organic compounds collected during the operation of engines on various candidate replacement fuels.

## 2.2. Key deliverables and outputs

The following key technologies and methods were demonstrated within the project:
- Miniature and low-cost portable on-board systems for vehicle emissions monitoring.
- A miniature ultra-low-cost detector of particle length, used for vehicle emissions monitoring, and also tested within the European Metrology Program as a novel tool for periodic emissions inspections of motor vehicles.
- A portable FTIR (Fourier Transform Infra-Red) spectrometer for measurement of unregulated pollutants of interest, such as nitrogen dioxide, ammonia, formaldehyde, acetaldehyde, and the greenhouse gases methane and nitrous oxide.
- Particle size distributions and particle counts were measured in vehicle exhaust and in ambient air near roadways using instruments mounted on hand carts.
- Acellular tests of DNA adducts and oxidative DNA damage were demonstrated as, relative to the toxicological tests in general, relatively easy, fast, inexpensive screening tests, requiring a relatively small amount of material.
- The standardized protocols for sampling and toxicity testing of diesel emissions under various real traffic conditions as tools for hazard identification and risk assessment, based on toxic events of vehicle emissions.
- These tests are described in detail on www.medetox.cz/methods and were reported in the Mid-term Report and include:
  - Sampling
  - Analysis of cytotoxicity
  - Analysis of DNA adducts
  - Micronucleus test
  - Oxidative stress
  - Comet assay

**The results of this project should be used for the improvement of legislation relevant to the regulation of vehicle emissions in the European Union.**

# DELIVERABLES OF THE PROJECT

| Name of the Deliverable | Code of the associated action | Planned achievement | Achieved |
|---|---|---|---|
| Establishment of the Project Manager and the Steering Committee | A1 | 30/09/2011 | 17/9/09/2011 |
| Notice boards describing the project for relevant public places | A10 | 30/11/2011 | 22/04/2012 |
| Creation of the project website | A10 | 31/12/2011 | 30/12/2011 |
| Finalization of monitoring protocol for actions A3, A4. | A3, A4 | 31/05/2012 | 31/05/2013 |
| An article in the Czech magazine “Vesmir” (“Espace”) will be published in 2012. The paper will explain to the general public the aims of the project and will describe the problems targeted by MEDETOX. | A10 | 31/12/2012 | 30/04/2013 |
| Special issue of the Czech popularization journal “Ochrana ovzduší” (“Environment Air Protection”) will be delivered in 2013, to communicate the running results of the project to the broader public. | A3, A4, A5, A10 | 31/10/2013 | 31/12/2012 |
| At least 3 manuscripts submitted in peer reviewed impacted journals demonstrating the outputs of Actions A3, A4, A5 | A3, A4, A5, A10 | 31/10/2013 | 31/12/2012 |
| Finalization of monitoring protocol for action A6 | A6 | 30/06/2014 | 30/06/2014 |
| Report on the comparison between laboratory and real traffic sampling of diesel exhaust on the Prague ring | A5 | 31/10/2014 | 31/10/2014 |
| Report on the comparison between toxicity testing in the laboratory and in real traffic on the Prague ring | A5 | 31/10/2014 | 31/10/2014 |
| Completion of the prototype | A3, A5 | 31/10/2014 | 18/12/2015 |
| Database with a complex overview of the toxicity of diesel exhaust under real traffic on the Prague ring | A6 | 31/07/2015 | 15/06/2015 (outputs 2015-No.19,20) |
| An article in the Czech magazine “Vesmír” (“Espace”) will be published in 2014. The paper will explain to the general public the running results of the project. | A10 | 28/02/2015 | 15/04/2013 |
| Technical publication of the results of the toxicity of diesel exhaust under real traffic on the Prague ring | A6 | 30/06/2015 | 30/06.2015<br>2015-19<br>2015-20 |

| | | | |
|---|---|---|---|
| A special issue of the Czech popularization journal "Ochrana ovzduší" ("Environment Air Protection") will be delivered in 2016, and will communicate the final results of the project to the broader public.<br>The journal ceased to be published. The following alternatives were chosen: a) two summary presentations at "Ovzduší" conference, and one summary presentation at the "Ovzduší a zdraví" (Atmosphere and Health) conference attended by many readers of the journal, b) an additional article in "Vesmír" (1/2016), c) two articles in popular journal „Respekt" (1/2015), d) two articles in „Autoexpert" popular journal, e) a lecture at a large national automotive technicians workshop, f) additional coverage on TV | A3, A4, A5, A7, A10 | 30/06/2016 | 30/06/2016<br>Outputs:<br>a) 2015-09,10,11,12,13<br>b) 2016-01<br>c) 2015-01,02<br>d) 2016-03<br>e) 2016-07,19<br>f) 2015-03, 29,30,32,35,36 |
| At least 3 manuscripts submitted in peer reviewed impacted journals demonstrating the outputs of Actions A6, A7 | A6, A7, A10 | 31/07/2016 | 31/07/2016<br>Atm Env 2015<br>Atmosphere 2015<br>Fuel 2016 |
| At least 5 public media outputs (Czech public TV, radio newspapers) to explain to the general public the final results of the project | A10 | 31/08/2016 | 31/08/2016<br>Czech TV 2014-04<br>Czech TV 2014-18<br>Czech TV 2015-03<br>Czech TV 2015-29 (3x)<br>BBC documentary 2015-32<br>Echo journal 2015-30<br>German TV WRD 2015-35 |
| Technical publication on the results of toxicity testing of the effects of fuel additives | A7 | 31/08/2016 | 31/08/2016<br>IJMS 2016 |
| Layman`s report on the project results | A10 | 31/08/2016 | 31/08/2016 |
| Monograph on the project`s data | A10 | 31/08/2016 | 31/08/2016 |
| Audit | A8 | 30/11/2016 | 20/11/2016 |

# MILESTONES OF THE PROJECT

| Name of the Milestone | Code of the associated action | Deadline Achieved/Updated |
|---|---|---|
| KickOff Meeting | A1 | 30/09/2011 |
| Start of the optimisation of on-board sampling of diesel exhaust | A3 | 30/11/2011 |
| Start of the optimisation of toxicity assays in on-board samples of diesel exhaust | A4 | 31/12/2011 |
| Completion of the preparatory phase of the projects (projects for networking, relevant stakeholders, and audiences for dissemination of results identified) | A9, A10 | 29/02/2012 |
| Start of the comparison of laboratory and on-board sampling and toxicity testing | A5 | 01/05/2012 |
| Workshop for proper stakeholders to explain the aims and expected outputs of the project (Brno, May 2012) | A10 | 30/06/2012 |
| Start of the sampling and analysis of toxicity of diesel exhaust under various traffic conditions in Prague | A6 | 01/01/2013 |
| Completion of the optimisation of on-board sampling of diesel exhaust | A3 | 31/12/2014 |
| Completion of the optimisation of toxicity assays in on-board samples of diesel exhaust (database of results) | A4 | 01/10/2013 |
| Workshop for proper stakeholders to communicate the running results of the project (Brno, May 2014). | A3, A4, A5, A10 | 30/06/2014 |
| Completion of the comparison of laboratory and on-board sampling and toxicity testing (database of results) | A5 | 01/10/2014 |
| Start of the sampling and analysis of toxicity of diesel exhaust – the effects of various fuel additives | A7 | 01/02/2015 |
| Completion of the sampling and analysis of the toxicity of diesel exhaust under various traffic conditions in Prague (database of results) | A6 | 01/07/2015 |
| Workshop for stakeholders to communicate the final results of the project (Brno, May 2016) | A3, A4, A5, A7, A10 | 01/07/2016/31/05/2016 |
| Completion of the sampling and analysis of the toxicity of diesel exhaust – the effects of various fuel additives (database of results) | A7 | 01/09/2016 |
| Completion of the After LIFE communication plan | A10 | 01/09/2016 |
| Final measurement protocols delivered | A10 | 01/09/2016 |
| Final meeting of participants | A1 | 01/09/2016 |

**All activity reports were submitted on time.**

# ACTIVITY REPORTS

| Type of report | Deadline |
|---|---|
| Inception Report (delivered) | Month 9 – 31/05/2012 |
| Mid-term Report with payment request (delivered) | Month 27 – 30/11/2013 |
| Progress Report (current report) | Month 45 – 31/05/2015 |
| Final Report | Month 63, 3 months after the end of the project – 30/11/2016 |

# 3. Introduction

## 3.1. Environmental problem addressed

Diesel engine emissions represent one of the major sources of air pollution worldwide. Nowadays, it is generally accepted that these emissions are, under real urban traffic conditions, substantially higher than when detected in a testing laboratory using various testing cycles.

## 3.2. Major objectives of this project:

1. To demonstrate innovative methods to assess the possible health risks connected with the exposure of the general population to diesel exhaust particles under real traffic conditions.
2. To disseminate the methodologies to relevant government and national/international regulatory authorities and other potential users.
3. To identify health risks related to emerging fuels and fuel additives, and to demonstrate the use of standardized tests of toxicity as an appropriate tool for regulatory and other environment policy decisions.
4. To build an effective interdisciplinary network targeted at the holistic assessment of the health risk potential of engine exhaust, during real-world operation of road vehicles and mobile machinery, and the monitoring of the effects of various policy decisions. This is accomplished by a well-balanced team of experts in engines and emissions (TUL – Technical University Liberec), toxicity assessment (IEM – Institute of Experimental Medicine) and public policy (ME – Ministry of the Environment).

## 3.3. Technical and methodological solution

Following the above mentioned objectives, was a major part of the project. It included several actions (particularly A3-A7) aimed at the standardized methodology of diesel exhaust

sampling and toxicity testing. The project was built on existing methods of measurement of vehicular emissions, both in the laboratory and in real-world operation, and on existing toxicological assays methods. These methods were applied to a real-world assessment of diesel exhaust toxicity in urban operation, on a scale far exceeding the normal, and very limited, vehicular emissions monitoring studies.
The methodology (it includes A9-11) was followed predominantly by organizing multiple special seminars for stakeholders and potential users, and by distribution of the project deliverables. The project news was regularly published on the project website. The results of the project were further communicated to experts via active participation at top international conferences, by papers in highly impacted international journals, and by preparation of a special project monograph. For the broader public including governmental and regulatory authorities, the Layman`s report and the notice boards were prepared.
The standardized methodologies of diesel exhaust sampling and toxicity testing were adopted to analyze the toxic effects of various emerging fuels and fuel additives. The innovative methodology of toxicity testing gained within MEDETOX was demonstrated on monitoring the effects of operating conditions and traffic density, and of the most commonly used and/or most promising fuels and fuel additives.

## 3.4. Expected longer term results

The results of this monitoring might be used in current and future public environmental policy decisions. Long-term cooperation within MEDETOX, has built the interdisciplinary network which might be useful for future projects in the area of regulation of complex environmental mixtures.

**Expected longer term results**

- Project results and project staff have contributed to the formation of the current plans to introduce the monitoring of real driving emissions of nitrogen oxides and particulate matter in the EU.
- Portable on-board emissions monitoring systems (PEMS) for vehicle emissions measurements were used to uncover excess emissions during real-world operation, including the first "DieselGate" involving heavy-duty vehicles and excess $NO_x$ emissions in the United States two decades ago, and including the "current" DieselGate affair with diesel automobiles.
- Demonstration of a miniature PEMS serves as an enabling tool to extend real driving emissions measurements established in EU legislation for heavy-duty vehicles and is planned for automobiles, motorcycles and other smaller vehicles within the EU legislation.
- Demonstration of online, on-road measurements of ammonia, nitrous oxide, and other pollutants of interest with a portable FTIR, serves as an enabling tool for real driving emissions limits of such compounds within the EU legislation.
- Demonstration of a low-cost miniature particle sensor serves as an enabling tool for the replacement or supplementation of the current opacity measurements during periodic vehicle inspections, with another method capable of assessing the functionality of a diesel particle filter.
- Project findings and background information gathered during the project have been used in citizen actions against unwise land-use choices that would have likely resulted in the deterioration of air quality, including the construction of a large shopping centre which would have brought large amounts of traffic into a historical city centre.

- Project findings on the reality of motor vehicle emissions have been disseminated in many public lectures, including lectures at many conferences, but also at the Committee for Sustainable Transportation of the Czech Government, at political meetings, and at meetings and seminars for automobile repair technicians.
- Toxicity assays demonstrated within the project have, along with different types of laboratory toxicity assays carried out by several other groups, contributed to the increase in cooperation between engineering and toxicology groups in the field of a more direct assessment of the effects of new fuels and technologies on human health.
- Ambient air measurements have sparked an interest from local authorities and citizen groups to assess air quality on a local (microscale) basis.
- Technologies and methods demonstrated within the project are directly applicable to small home heating appliances; another distributed source of air pollution responsible for a large fraction of the total particulate matter in the air.
- Miniature and low-cost portable on-board monitoring systems are an enabling technology for the extension of the monitoring of real driving emissions to small engines, and to small or specialized non-road mobile machinery, where "classic" portable on-board monitoring systems are too bulky.
- Mini particle length detectors offer an inexpensive way to detect malfunctions of diesel particle filters.
- Portable FTIR is an enabling technology for extension of the monitoring of real driving emissions to specific non-regulated pollutants of interest, arising out of new fuels and technologies.
- Mobile, time-resolved measurements of particle size distributions and particle number concentrations in ambient air allow for a more realistic assessment of particle-related health hazards, than particle mass measurements used to date.
- Acellular tests of DNA adducts and oxidative damage, may be used in the future for high throughput analyses of the toxic effects induced by engine emissions.
- Application and extension of the methods used, from heavy diesel vehicles, gasoline engines, to small and non-road engines, and to home heating appliances have been planned in detail, and are the subject of several currently proposed projects.

# 4. Administrative section

## 4.1. Description of the management system

Since no substantial changes in the project were required, there were no amendments to the Grant Agreement. As already reported in the Mid-term Report (08/07/2015), it has been discovered that the issue at hand is so complex that an iterative, rather than a sequential approach, is mandated from the beginning of the project. Therefore, it is difficult to discriminate strictly, the efforts and budget dedicated to all experimental Actions (A3-A7).

This fact is clearly seen from the time schedule shown in the following table:

**Time schedule of the project Actions**

| | 2011 | | 2012 | | | | 2013 | | | | 2014 | | | | 2015 | | | | 2016 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Number of action** | Sept | IV | I | II | III | IV | I | II | III | IV | I | II | III | IV | I | II | III | IV | I | II | 7-8 | 9-11 |
| A1 | x | x | x | X | x | x | x | x | x | x | x | x | x | x | X | x | x | x | x | x | x | x |
| A2 | x | x | x | X | x | x | x | x | x | x | x | x | x | x | X | x | x | x | x | x | x | |
| A3 | | x | x | X | x | x | x | x | x | x | x | x | x | | | | | | | | | |
| A4 | | x | x | X | x | x | x | x | x | | | | | | | | | | | | | |
| A5 | | | | X | x | x | x | x | x | x | x | x | x | | | | | | | | | |
| A6 | | | | | | | x | x | x | x | x | x | x | x | X | x | | | | | | |
| A7 | | | | | | | | | | | | | | | X | x | x | x | x | x | x | |
| A8 | | | | | | | | | | | | | | | | | | | | | | x |
| A9 | x | x | x | X | x | x | x | x | x | x | x | x | x | x | X | x | x | x | x | x | x | |
| A10 | x | x | x | X | x | x | x | x | x | x | x | x | x | x | X | x | x | x | x | x | x | x |
| A11 | | | | | | | | | | | | | | | | | | | | | x | x |

The time schedule of the project Actions remains unchanged as reported in the Mid-term Report, and all Actions were completed on time.

The project actions were organized by the leaders of the project teams, who frequently communicated with each other, and managed actions within their respective project teams. Steering Committee (SC: Ing. J. Topinka, DrSc. – chairman, Ing. M. Vojtíšek, PhD., Mgr. P. Gruntorád) organized 15 regular meetings during the project period, solving actual problems.

The most important problems solved by SC in the period between the Progress report and the Final report (01/06/2015 – 31/08/2016) were:

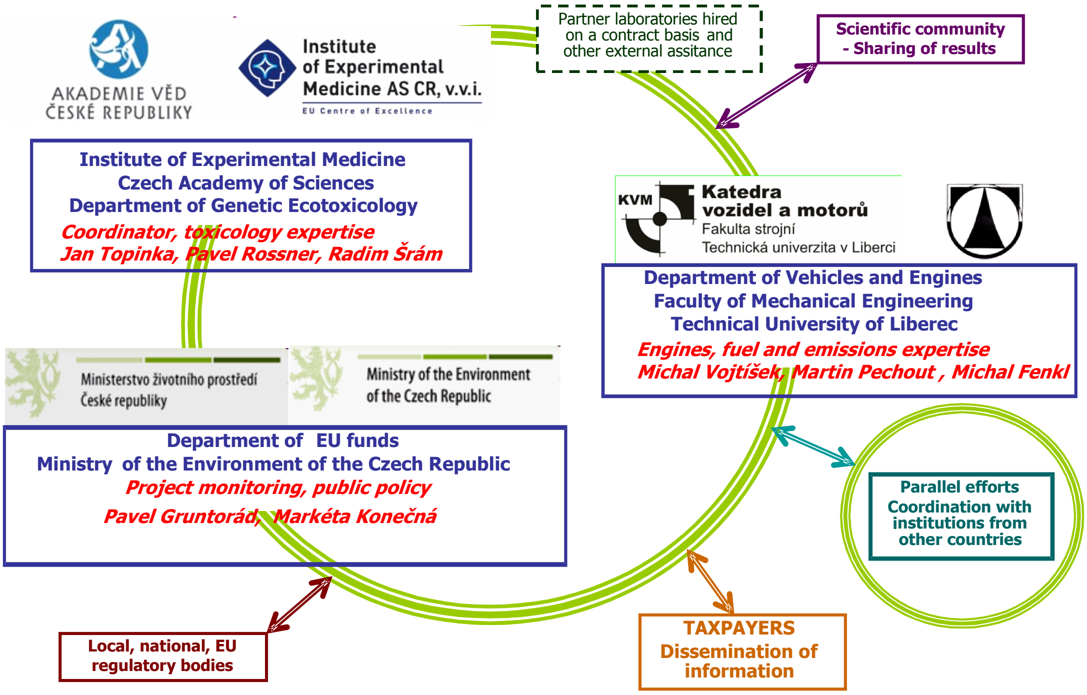
- The connection between the originally planned financial items and the actual expenses should be clarified. Particularly, all personal costs should be accompanied by payment slips.

- All expenses should be directly related to the MEDETOX project; otherwise they should be removed from the tables.
- Some details on travel expenses should be added into Travel expenses.
- Preparation of the Layman`s Report, the MEDETOX monograph and the After LIFE communication plan is needed.
- Some transfers of budget between partners and categories.
- In addition, the complexity of technical and scientific issues required in-depth technical discussions, fostering of mutual understanding of the surrounding issues, and close collaboration in the preparation of dissemination materials in order to address both toxicology and engine aspects. Dr. Topinka and Dr. Vojtíšek have been working together very closely on these tasks, with meetings **every one to two weeks throughout the whole project**. During these meetings, operational and management issues were also addressed.

  Since the start of the project, three reports have been delivered: Inception Report (23/05/2012, Month 9), Mid-term Report (20/11/2013, Month 27) and Progress Report (08/07/2015, Month 45).

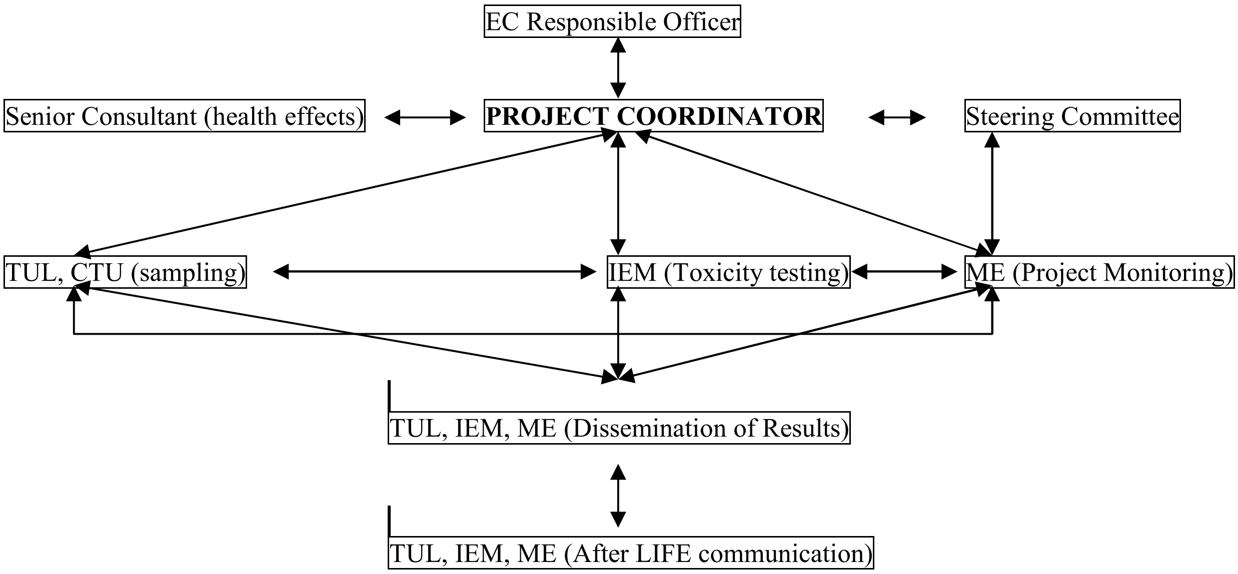
**Multiple seminars, workshops and meetings were organized, as listed in Outputs and Indicators (see Annex 7.4.)**

**Organigramme of the project team and the project management structure -** as envisioned in the project proposal and in the Mid-term and Progress Reports; we keep the same team and project management structure during the whole project:



**Structure of the project management:**

Czech Technical University in Prague (CTU), was involved as the sampling location.




**There were no changes due to amendments of the Grant Agreement.**

Partnership agreements were submitted to the Commission with the Inception Report (23/5/ /2012). Amendment No.1 to the contract between IEM and TUL was reported in the previous report (Progress Report, 8/7/2015) as Annex 7.5.6.

## 4.2. Evaluation of the management system – project monitoring (A2)

The project management was generally very efficient. Regular meetings (a total of 15 regular SC meetings were organized) of SC resolved any actual problems connected with the project. Moreover, as mentioned above, regular (every 1-2 weeks) informal meetings of key partners (mostly Jan Topinka and Michal Vojtisek) solving actual problems of the project progress were organized. The project was interdisciplinary, and a very efficient collaboration between engine specialists (represented by TUL employees) and toxicologists (IEM employees) was established.

Project monitoring (Action 2) was ensured by Pavel Gruntorád, SC member. He was focused on the monitoring of the realization of the project. Attention was paid to project actions and their harmonograms and financial issues. The overview of indicators was defined, which served as a tool for the monitoring of the realization of individual activities.

Within Action 2, a plan of activities for the next period was created. The rules were defined for the transfer of information on the realization of the project. Information was summarized in individual monitoring protocols (see table below), as well as in the Summary protocols (see Annexes 7.2.3., 7.2.4. and 7.2.5). Regular evaluation of the project progress took place at the SC meetings. Project management informed everyone regularly on the realization of the project. All changes within the project plans were consulted with the external monitor.

| Protocol No. | Covering Period |
|---|---|
| 1 | 01/09/2011 – 31/03/2012 |
| 2 | 01/04/2012 – 31/12/2012 |
| 3 | 01/01/2013 – 31/05/2013 |
| 4 | 30/06/2013 – 31/12/2013 |
| 5 | 01/01/2014 – 30/06/2014 |
| 6 | 01/07/2014 – 31/12/2014 |
| 7 | 01/01/2015 – 30/06/2014 |
| 8 | 01/07/2015 – 31/01/2016 |
| 9 | 01/02/2016 – 31/08/2016 |

In summary, the project fulfilled all of the planned activities. The extent of dissemination activities is much higher than required by the project indicators. Publicity of the project was also very successful in the Czech Republic as well as in EU.

Regular visits of Mr. Daniel Svoboda, representing the external monitoring team, contributed to the quality of the project outputs and their communication to stakeholders and the general public. These visits, as well as communication with the Commission (including 1 visit of Commission representatives) contributed to the improvement of financial reporting.

# 5. Technical section

## 5.1. Technical progress, per action

The following sections are dedicated to technical/experimental Actions A3-A7, networking (A9)as well as audit (A8) and After LIFE communication plan (A11)

**5.1.1. Action 3: Optimisation of on-board monitoring and sampling apparatus and methods in real traffic in Prague** (1/10/2011 – 30/09/2013):

The goal of this action was to prepare portable on-board emissions monitoring and a sampling system for subsequent demonstration. This action was completed, and its outcomes were described in full in the Mid-term Report and also in the project monograph. Therefore, only a review is given here.

The basic concept of a portable, on-board emissions monitoring system (PEMS), developed in part by Michal Vojtisek twenty years ago and currently used in EU and U.S., has been expanded to the following technologies that have been demonstrated:

1. A miniature version of PEMS for the measurement of basic pollutants (HC, CO, $CO_2$, $NO_x$, particulate matter)
2. Expanded PEMS, using an FTIR analyzer for assessment of non-regulated pollutants: greenhouse gases methane ($CH_4$) and nitrous oxide ($N_2O$), reactive nitrogen species ammonia ($NH_3$), nitrogen oxide (NO) and dioxide ($NO_2$), hazardous pollutants such as formaldehyde, acetaldehyde, and others, and a particle size classifier for evaluation of particle size distributions
3. Portable proportional sampling system

And in addition to the original plan:

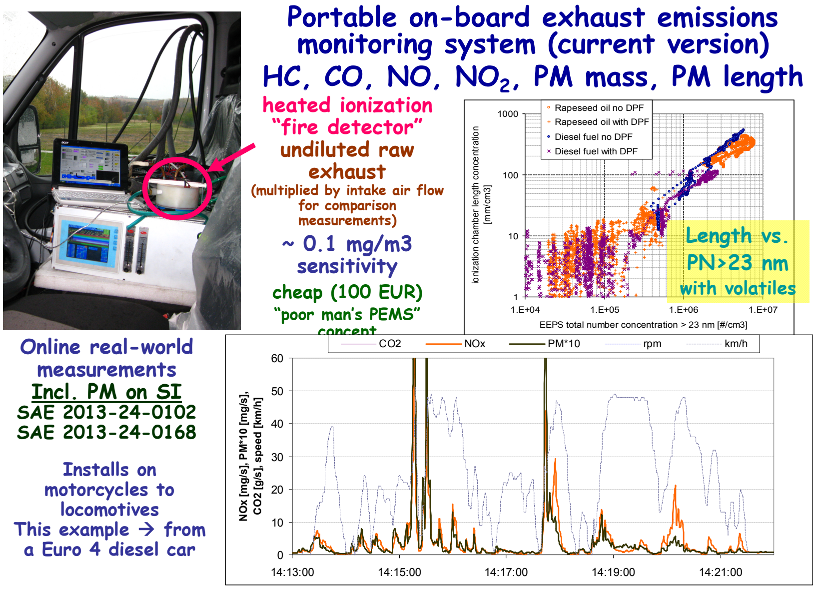
4. A miniature, low-cost nanoparticle detector based on commercial smoke detector technology, which has also been proposed as a tool to detect malfunction of the absence of a diesel particle filter
5. Tandem high-volume samplers for collecting large amounts of material in laboratory settings
6. Use of portable particle monitoring systems for ambient air quality assessment, and for the measurement of combustion generated nanoparticles in the streets.

Selected papers in peer-reviewed journals:

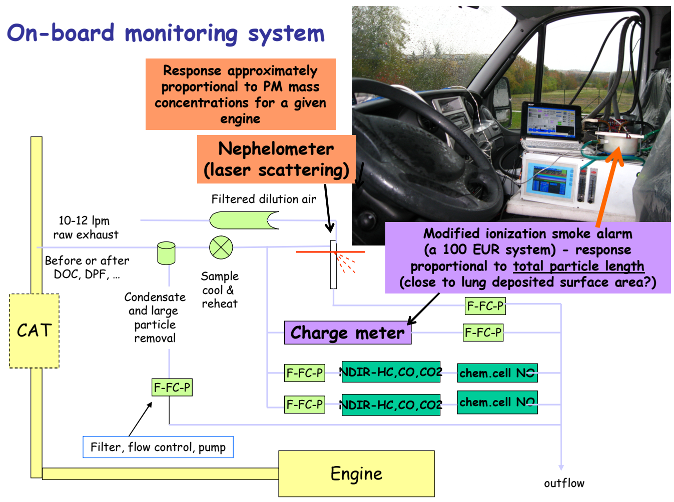
- Vojtisek-Lom M.; Czerwinski J.; Leníček J.; Sekyra M.; Topinka J.: Polycyclic aromatic hydrocarbons (PAHs) in exhaust emissions from diesel engines powered by rapeseed oil methylester and heated non-esterified rapeseed oil. Atmospheric Environment, 60, 2012, 253-261. (jointly with Action A4, copy delivered with Mid-term Report).
- Vojtisek-Lom, M.: Inference of steady-state non-road engine exhaust emissions values from non-stabilized data. **Society of Automotive Engineers Technical Paper Series**, ISSN 0148-7191, 2012-01-1673.
- Vojtisek-Lom, M.: Assessment of Low Levels of Particulate Matter Exhaust Emissions Using Low-Cost Ionization-Type Smoke Detectors. Society of Automotive Engineers

Technical Paper Series, ISSN 0148-7191, 2013-24-0168, doi: 10.4271/2013-24-0168, 2013 (jointly with Action A5).

**A miniature version of PEMS for the measurement of basic pollutants (HC, CO, $CO_2$, $NO_x$, particulate matter)**



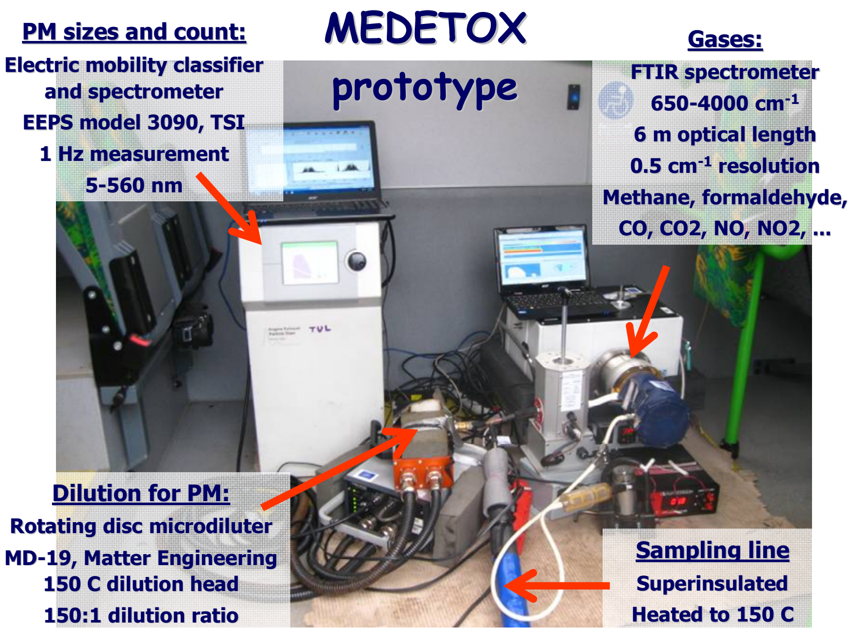

*Miniature PEMS with particle measuring capability*




*On-board portable monitoring system schematics*

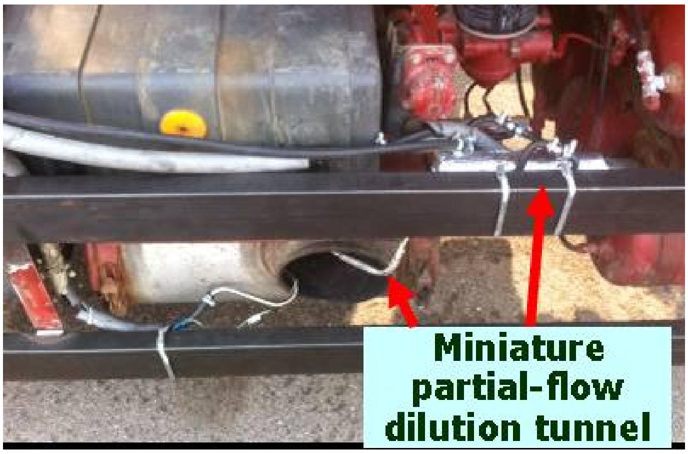
**Expanded PEMS – Fourier Transform Infra-Red (FTIR) spectrometer for gaseous pollutants, a particle size classifier for particle emissions characterization**


*FTIR system mounted on an older passenger car operated on alcohol fuels within a student research project [Vojtisek-Lom et al., 2013; Vojtisek-Lom et al., 2015; Pechout et al., 2015]*



*FTIR as part of a prototype for gaseous and particulate emissions measurements*

**Proportional sampling system**

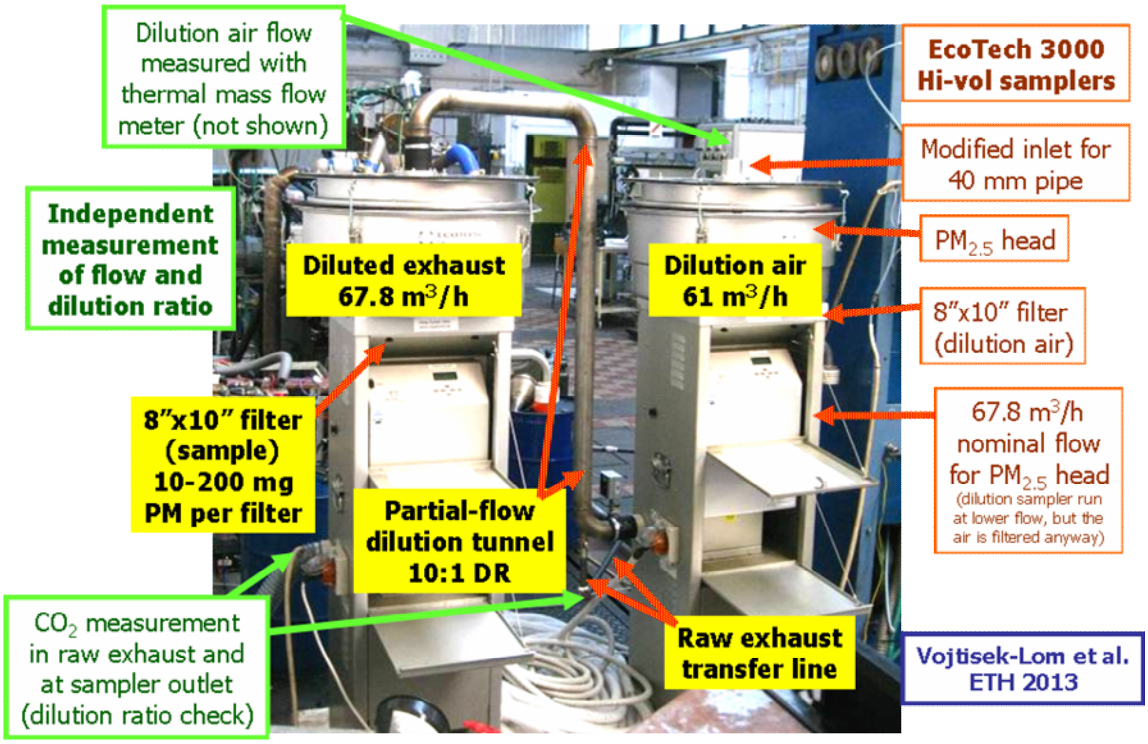



*Iveco Trakker – installation of the sampling system (left, in foreground) in the cab, and of the dilution tunnel at the tailpipe (right)*



*Iveco Trakker at the new engine laboratory of the Czech Technical University in Prague in Roztoky – presentation of the sampling system during an on-board emissions monitoring workshop in December 2012*

**High-volume sampling system using a pair of high-volume samplers to collect large amounts of particulate matter from engine exhaust during laboratory tests**




*Laboratory sampling apparatus using high-volume atmospheric samplers, employed in a laboratory simulation of a congested area operation*

Envisioned future use of the prototype developed within A3

After the end of the project, the prototype has been used in a student project to measure exhaust emissions from a diesel locomotive, and in another student project to measure exhaust emissions from automobiles powered by n-butanol and isobutanol. Both projects were funded by public funds which have covered the operating costs of the testing, with no profit being realized. The prototype was also used briefly to investigate the excess emissions of nitrogen oxides from passenger cars with diesel engines, with operating costs funded by a non-profit environmental group.

In the immediate future, the prototype is likely to be used for measurement of emissions from small spark ignition engines. If this measurement will take place, it will also be funded by public funds. Another envisioned use is the application of the prototype or its parts to the assessment of realistic in-use emissions from local heating appliances.

Since the prototype is primarily intended for measurement and sampling of emissions of pollutants many of which are not subject to any legislative limits, and under realistic operating conditions which are not subject to any legislative limits, and given the interests in the automotive industry to limit their own research and development testing to the pollutants and conditions covered by the legislation, it is unlikely that the prototype will be used in commercial for-profit research.

Rather, it is envisioned that it will be used to support public decision making (evaluation of realistic effects of: new technology vehicles, emerging engine and aftertreatment technologies, currently promoted and newly developed alternative fuels, transportation engineering measures, etc.) and to support emerging legislation (i.e., expansion of real driving emissions and in-use compliance testing, currently legislated for nitrogen oxides, to additional pollutants). Typically, such projects are funded primarily by public funds, and to a lesser extent by non-profit groups.

Also, all beneficiaries of the project are non-profit public entities, the involvement of which in commercial activities is generally limited to a minor part of their revenues, and is also subject to limitations imposed by the law.

**5.1.2. Action 4: Optimisation of toxicity assays** (01/09/2011 – 30/09/2013):

This action was completed. All planned activities within this Action were fulfilled, and standardized operational protocols (SOPs) for toxicity assays were delivered and described in the Mid-term Report.

Thirty samples of particulate engine emissions were tested under various test conditions for various toxicity markers (cytotoxicity, DNA adducts in acellular test and in model human lung cells A549, DNA strand breaks, oxidative damage and micronuclei in A549 cells). The SOPs and monitoring protocols for individual tests of toxicity adopted for on-board sampled diesel exhaust resulted from this activity.

The most important results of the Action:

- Milligram quantities of collected particulate engine emissions are necessary to analyze their toxic effects.
- DNA adducts in the acellular assay is the most sensitive parameter to analyze genotoxic effects of the engine emissions.
- The highest genotoxicity of particulate emissions is observed for a full-load engine, following immediately after a low-load period (deposit burn-off).

The most important outputs of the Action:

- The standardized protocols for individual toxicity assays are reported both in Czech and in English (delivered within the Midterm report)
- The paper in impacted journal: Topinka J., Milcová A., Schmuczerová J., Mazač M., Pechout M., Vojtíšek M.: Genotoxic potential of organic extracts from particle emissions of diesel and rapeseed oil powered engines. Toxicology Letters, 212, 2012, 11-17. (copy delivered with the Mid-term Report).
- The paper in impacted journal: Vojtisek-Lom M.; Czerwinski J.; Leníček J.; Sekyra M.; Topinka J.: Polycyclic aromatic hydrocarbons (PAHs) in exhaust emissions from diesel engines, powered by rapeseed oil methylester and heated non-esterified rapeseed oil. Atmospheric Environment, 60, 2012, 253-261. (jointly with Action A3, copy delivered with the Mid-term Report).

**5.1.3. Action 5: Verification of optimized sampling and toxicity assays** (01/06/2012 – 30/09/2014):

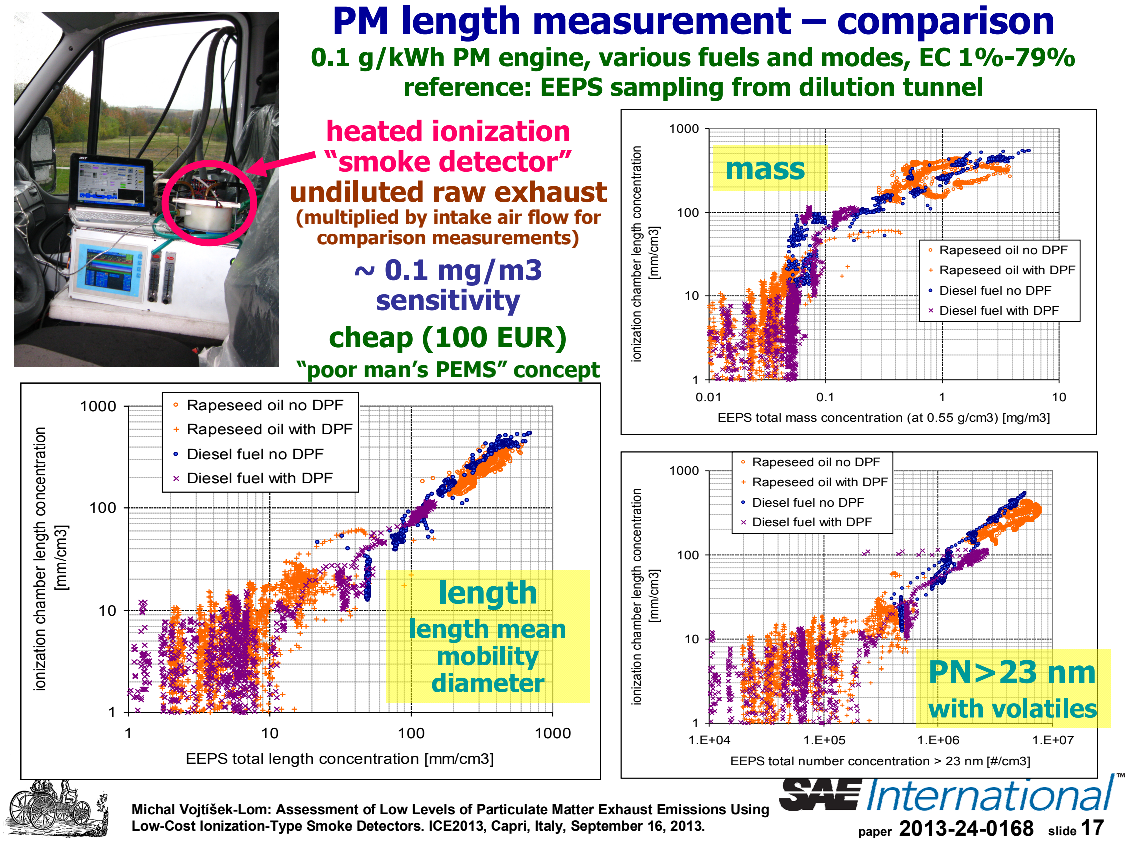
We recognized that for technical reasons preliminary on-board samplings should be accompanied with measurements within A3. Therefore, action started earlier than originally planned (01/06/2012 instead of 01/03/2013).
The early version of a portable, on-board proportional sampling system was compared during transient tests during several occasions of ongoing measurements at the Czech Technical University in Prague. Results of this comparison are generally positive, with some caveats and room for improvement. The portable measuring ionization chamber, used as a small, simple, inexpensive tool to measure particle length concentration, has undergone preliminary comparison tests in two laboratories, the results of which have been published in SAE 2013-24-0168. The device has been also enlisted in the European metrology program.

The most important outputs of the Action:

- Multiple rounds of verification of the portable proportional sampling system.
- Multiple rounds of verification of the low-cost portable system for measuring total particle length.

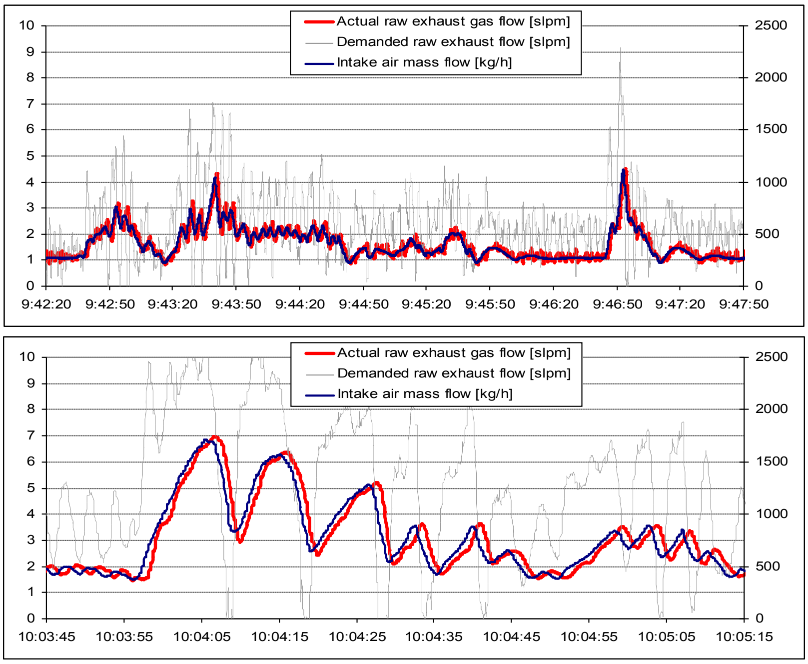
Verification of the portable proportional sampling system

As an example, correlation between the low-cost system and particle number and particle mass measurement are given below (slide from a presentation at a Society of Automotive Engineers conference).

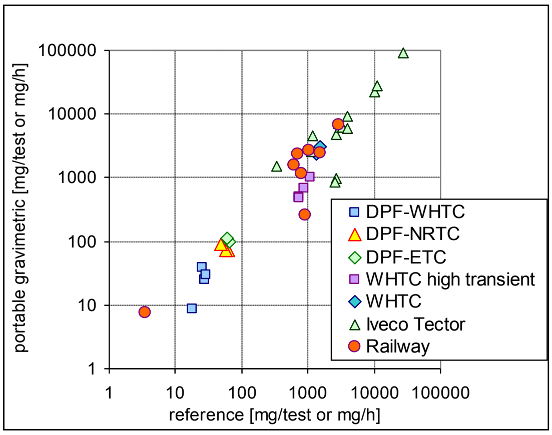



Verification of the portable proportional sampling system

Several series of comparison tests were conducted on production and prototype diesel engines of various emission levels. These tests were summarized in an article in the MECCA peer-reviewed automotive journal. The following graphs show an example of data from comparison testing, and aggregate results of all tests. As the duration of these tests spanned from minutes to tens of minutes, the results are reported on a grams per hour basis.




*Comparison of the actual raw exhaust gas flow into the sampling system, with the actual engine intake air flow during three subsequent runs of the WHTC cycle*




*Comparison of the PM mass emissions measured by the experimental system (vertical axis) against the respective reference measurements for all tests described here*

Articles in peer-reviewed journals:

- Vojtíšek M., Kotek M.: Estimation of Engine Intake Air Mass Flow Using a Generic Speed-Density Method. MECCA, 2014, 1, 7-15, DOI: 10.2478/mecdc-2014-0002.
- Vojtíšek M., Pechout M.: Assessment of a Low-Cost Portable Proportional Exhaust Sampling System for Gravimetric Particulate Matter Emissions Measurement. MECCA, 2013, 2, 22-28, doi 10.2478/mecdc-2013-0009
- Vojtisek-Lom, M.: Assessment of Low Levels of Particulate Matter Exhaust Emissions Using Low-Cost Ionization-Type Smoke Detectors. Society of Automotive Engineers Technical Paper Series, ISSN 0148-7191, 2013-24-0168, doi: 10.4271/2013-24-0168, 2013 (jointly with Action A3).

**5.1.4. Action 6: Measurements under real traffic conditions** (1.1. 2013 – 30.6. 2015):

For technical reasons, the measurements under real traffic conditions under A6 had to start earlier (1.1. 2013 instead of 1.1. 2014).

Several sets of tests were conducted as a part of the optimization of the system on heavy-duty trucks in Prague. Most of these tests yielded valuable insights and qualitative observations about engine operating conditions and emissions. One series of tests was conducted in April 2013 on an Iveco Trakker heavy-duty truck. A total of approximately 18 mg of particulate matter was collected in over one hundred sampled filters. The results have revealed that extended idling has caused increased emissions of particulate matter not only during idling, but also during subsequent operation at load. Further, DNA adducts tests have indicated that not only PM emissions are increased, but that the particles are more prone to cause damage to the DNA.

For this reason, a series of follow-up tests were conducted in a laboratory under conditions mimicking the situation where trucks spend a longer time in stop-and-go traffic or "creep" , followed by acceleration uphill at full load. This study has confirmed that extended idling increases both the absolute amount of particulate matter and its genotoxic potency, with such an increase taking place not only during idling but also during subsequent operation at higher loads.

Additionally, a series of ambient measurements with parts of the measurement system mounted on hand carts were conducted in the neighbourhood of Spořilov, intersected by three major sections of the national freeway network. It is relatively common for heavy-duty trucks to spend longer times in congestion prior to entering the neighbourhood, and to use full engine power to accelerate onto a freeway and to climb about 75 vertical meters through the neighbourhood. This study has revealed that despite vehicles being only one source of particulate matter, virtually all nanoparticles detected in the streets at breathing levels were from internal combustion engines (mostly vehicular traffic, but also garden and construction equipment). The highest concentrations – often two orders of magnitude above the "urban background" were on a pedestrian overpass over the sections where trucks accelerate onto the freeway.

Following an immense interest from citizen groups, similar measurements were conducted at other locations, including the smaller peripheral towns of Líbeznice and Čelákovice.

Also, the measurements were demonstrated during a science day at the Sion Elementary School in Hradec Králové, where students from the first and third grade measured concentrations of nanoparticles in the air around the school and in the surrounding areas.

Additional tests in real driving conditions have been conducted on diesel-powered rail vehicles, with the finding that even older engines yield to very low emissions of particulate matter per seat compared with road vehicles.

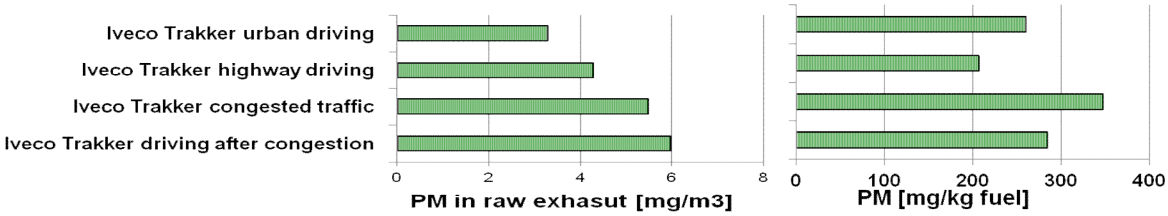
High concentrations measured in areas where garden machinery was used have inspired a "spin-off " study of the emissions of smaller engines, which are not subject to any legislative limits on particulate matter emissions. It was confirmed that particle emissions from garden machinery can be relatively very high.

### 5.1.4.1. Case study #1: Genotoxicity of diesel emissions in real world driving: Effects of cold starts, congestion, and DPF


The genotoxicity of organic extracts of particulate matter, collected during real driving of two diesel trucks was evaluated in this study. A Euro 3 Iveco Trakker with no after-treatment and a Euro 5 Iveco Daily truck with DOC+DPF were driven around Prague, each for two days. (Photos from top to bottom: Sampling system mounted on a DAF Euro 5 truck with a SCR NOx reduction catalyst; Vehicle #1, Iveco Daily Euro 5 with a diesel particle filter (DPF); Vehicle #2, Iveco Trakker, Euro 3, no exhaust after-treatment.)

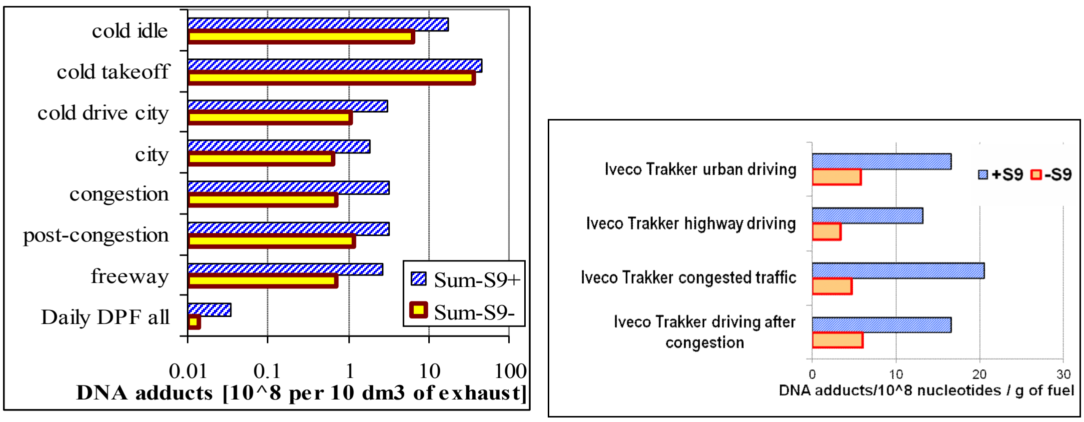

Exhaust emissions were measured by a portable on-board emissions monitoring system. Particulate matter was sampled on Pall TX40HI20-WW filters by a miniature on-board proportional sampling system.

Mean concentrations of particles in the exhaust and mean particle emissions per kg of fuel on the Iveco Trakker are shown below:




The analysis of PAHs in the exhaust of Iveco Trakker indicate significant concentrations of several cPAHs – benzo[a]pyrene, benzo[a]anthracene and chrysene during cold idle, cold takeoff and cold city drive, while no PAHs were detected for any driving conditions in Iveco Daily Truck equipped with a diesel oxidation catalyst and a diesel particle filter (DOC+DPF). We further studied genotoxicity (DNA adducts) and oxidative damage (8-oxo-deoxyguanosine, 8-oxo-dG) induced by organic compounds bound to exhaust particles. In agreement with cPAH content, the results show that in an engine without after-treatment, genotoxicity of emissions was significantly elevated during the cold start and cold operation of the engine.

During the warmed-up phase, exposure to an equivalent of 10 $dm^3$ of undiluted exhaust collected during congestion and during operation following the congestion, has lead to more DNA adducts than an "ordinary" urban and freeway operation.




*Left: Induction of DNA adduct levels in DNA treated with extractable organic matter (EOM), mass corresponding to 10 $dm^3$ of undiluted engine exhaust for 24 h. Results for various engine regimens are shown for samples with metabolic activation of PAHs by rat liver microsomal fraction S9 (+S9) and without metabolic activation (-S9). The values represent the mean from two replicates varying by <±15%*

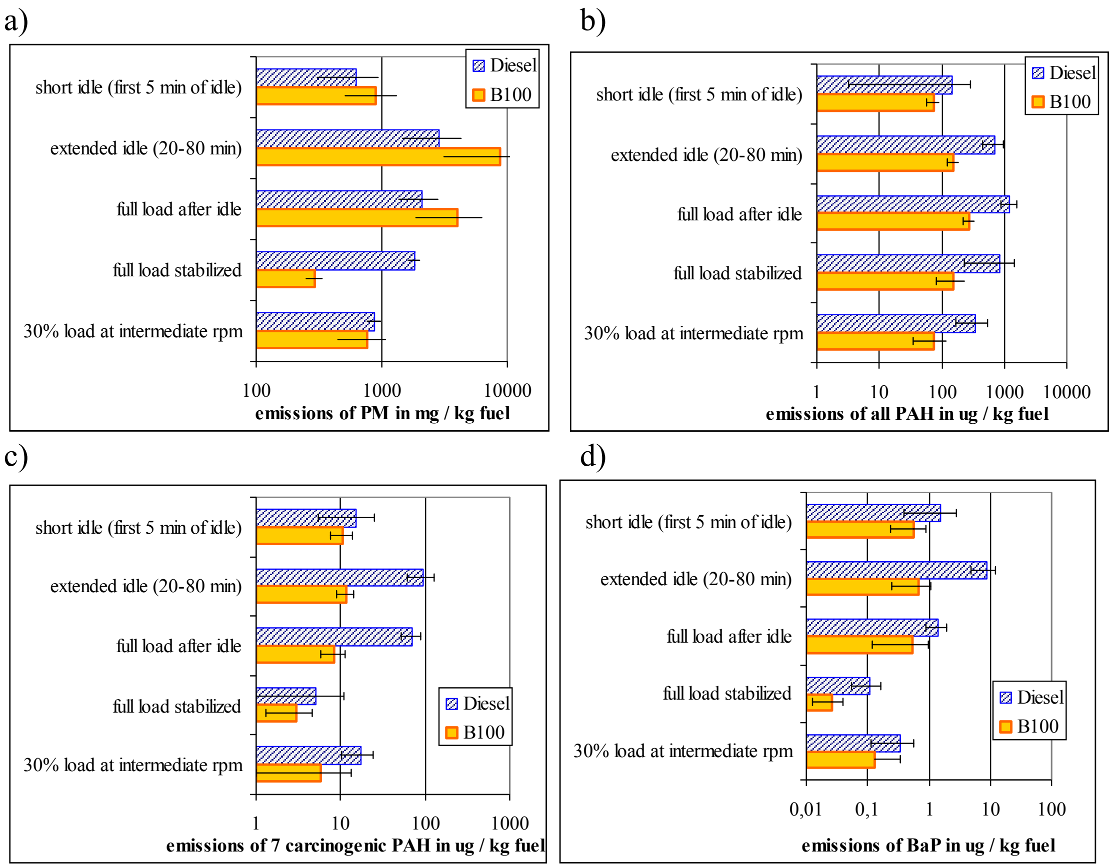
*Right: DNA adducts expressed per volume of raw exhaust and per kg of fuel consumed during the warmed-up operation of the Iveco Trakker Euro 3 truck, with no exhaust after-treatment.*

Exhaust from the truck equipped with DOC+DPF had less DNA adducts, by approximately two orders of magnitude, compared to the truck without after-treatment. In contrast to non-detectable PAHs in Iveco Daily truck emissions, we observed detectable DNA adducts induced by exhaust from this truck. This observation suggests a high sensitivity of DNA adducts as a marker of genotoxic effects of engine emissions. The levels of 8-oxo-dG suggest a weak effect of particle bound organic compounds on the extent of oxidative damage of DNA.

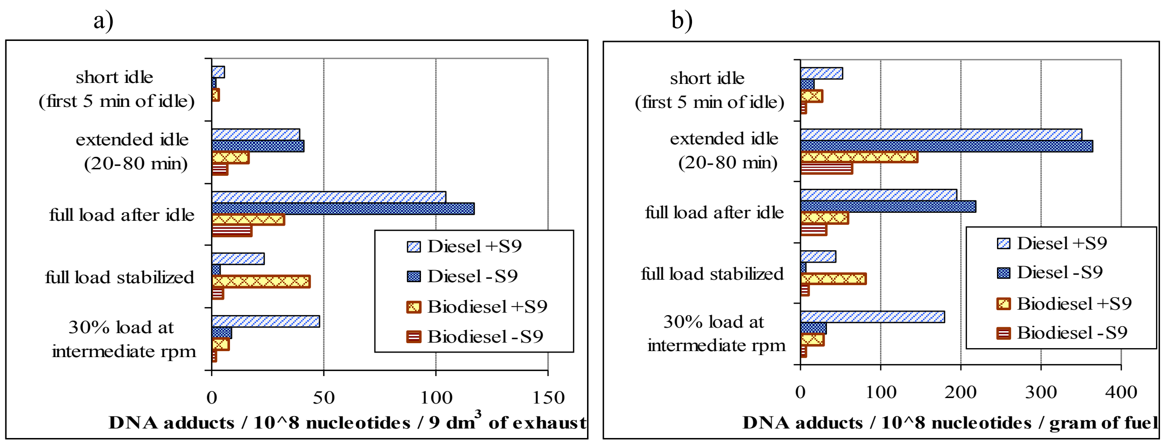
The results confirm that the benefits of DPF, observed in the reductions of particle mass and particle number emissions, also extend to the reduction of genotoxicity. The results suggest that a cold start, cold operation and congestion increase the genotoxicity of exhaust emissions.

#### 5.1.4.2. Case study #2: Simulation of extended idling in the laboratory: Polycyclic aromatic hydrocarbons (PAH) and their genotoxicity in exhaust emissions from a diesel engine during extended low-load operation on diesel and biodiesel fuels.

This case study investigates the effects of emissions including carcinogenic polycyclic aromatic hydrocarbons (cPAH) of a conventional diesel engine without a particle filter. Experiments were carried out during extended idle, and during a loaded operation immediately following the extended idle. Extended low-load operation of diesel engines due to idling and creep at border crossings, loading areas and during severe congestion have been known to deteriorate the combustion and catalytic device performance, and to increase the emissions of particulate matter (PM). A conventional diesel engine was coupled with a dynamometer and operated on diesel fuel and neat biodiesel, alternately at idle speed and 2% of rated power, and at a 30% and 100% load at intermediate speed. The exhaust was sampled on fibre filters, from which the content of elemental and organic carbon and polycyclic aromatic hydrocarbons (PAH), including cPAH and benzo[a]pyrene (B[a]P) were determined. The emissions of cPAH and B[a]P increased 4-6 times on diesel fuel and by 4-21% on biodiesel during extended idling, relative to a short idle and 8-12 times on diesel fuel, and 2-20 times during the subsequent operation at full load relative to stabilized operation at full load. The total “excess” cPAH emissions after the transition to full load were on the same order of magnitude as the total “excess” cPAH during extended idling. The absolute levels of PAH, cPAH and B[a]P emissions under all operating conditions were lower on biodiesel compared to diesel fuel. The genotoxicity of organic extracts of particles was analysed by acellular assay with calf thymus DNA (CT-DNA), and was consistently higher for diesel than for biodiesel. The exhaust generated during extended idle and subsequent full load, exhibited the highest genotoxicity for both fuels. These two regimes are characterized by significant formation of cPAH as well as other DNA reactive compounds, substantially contributing to the total genotoxicity. Oxidative DNA damage by all tested extracts was negligible.
Major results of the study are depicted in the figures below.




*Comparison of fuel-specific emissions of a) total PM mass, b) all PAH, c) carcinogenic PAH and d) benzo[a]pyrene, across operating modes and between diesel fuel and neat biodiesel*




*Induction of DNA adduct levels in CT-DNA treated with extractable organic matter (EOM) corresponding to 9 dm$^3$ of undiluted engine exhaust for 24 h. Results for the diesel and biodiesel various operating modes are shown for samples with metabolic activation by rat liver microsomal fraction S9 (+S9), and without metabolic activation (-S9). DNA adduct levels per volume of undiluted exhaust (**a**) and gram of fuel (**b**) are shown. The values represent the mean from two replicates varying by < ±15%*

Selected relevant presentations at international conferences:

- Vojtisek-Lom, M.: Consideration of congested urban traffic in exhaust toxicity assessment. Proceedings of the 16th ETH Conference on Combustion Generated Nanoparticles, Zurich, Switzerland, June 24-27, 2012.
- Topinka J., Rössner P., Milcová A., Schmuczerová J., Švecová V., Štolcpartová J., Fenkl M., Pechout M., Vojtíšek M.: Genotoxicity of Diesel Emissions in Real World Driving: Effects of Cold Starts, Congestion, DPF. 19th Conference on Combustion Generated Nanoparticles, 28.6.-1.7. 2015, Zurich, Switzerland.
- Vojtíšek M., Dittrich L., Fenkl M.: Measurement of emissions from independent bus heaters. 19th Conference on Combustion Generated Nanoparticles, 28.6.-1.7. 2015, Zurich, Switzerland.
- Vojtíšek M., Jirku M., Pechout M.: Real driving emissions from a diesel-hydraulic rail vehicle. 19th Conference on Combustion Generated Nanoparticles, 28.6.-1.7. 2015, Zurich, Switzerland.
- Topinka J., Líbalová H., Milcová A., Schmuczerová J., Rösner P. Jr., Pavlíková J. a Hovorka J. Evaluation of mass-dependent toxicity of PAHs and their derivatives extracted from size segregated urban aerosol in A549 cells. International Symposium on Polycyclic Aromatic Compounds, 13. – 17.9. 2015, Bordeaux, Francie.
- Rossner, P.: Chemical characterization and genotoxicity of emissions from diesel and gasoline fuels. Presentation at the Central and Eastern European Conference on Health and Environment (CEECHE 2016).
- Vojtíšek M., Pechout M., Beránek V.: Measurement of late-model diesel automobile real driving emissions of reactive nitrogen compounds with on-board FTIR. Transport and Air Pollution Conference, Lyon, France, May 2016.

Papers in peer-reviewed journals:

- Vojtíšek M., Pechout M., Dittrich L., Beránek V., Kotek M., Schwarz J., Vodička P., Milcová A., Rossnerová A., Ambrož A., Topinka J.: Polycyclic aromatic hydrocarbons. (PAH) and their genotoxicity in exhaust emissions from a diesel engine during extended low-load operation on diesel and biodiesel fuels. Atmospheric Environment 109, 2015, 9-18. (jointly with Action 7)
- Stolcpartova J., Pechout M., Dittrich L., Mazac M., Fenkl M., Vrbova K., Ondracek J., Vojtisek-Lom M.: “Internal Combustion Engines as the Main Source of Ultrafine Particles in Residential Neighborhoods: Field Measurements in the Czech Republic”, Atmosphere, 6(11), 2015, 1714-1735; doi: 10.3390/atmos6111714
- Vojtíšek, M., Pechout, M., and Fenkl, M.,: Measurement of Exhaust Emissions of Small Gasoline Engines Under Real-World Driving Conditions, SAE Technical Paper 2014-01-2811, 2014, doi: 10.4271/2014-01-2811.
- Rossner P., Strapacova S., Stolcpartova J., Schmuczerova J., Milcova A., Neca J., Vlkova V., Brzicova T., Machala M., Topinka J. Toxic Effects of the Major Components of Diesel Exhaust in Human Alveolar Basal Epithelial Cells (A549). Int J Mol Sci. 2016 Sep; 17(9): 1393.

European Aerosol Conference, Milano, September 6-11, 2015 - **1COA_P016**

# Internal combustion engines are still the dominant source of nanoparticles in residential neighborhoods


Michal Vojtisek-Lom[1,2], Martin Pechout[1], Luboš Dittrich[1], Jitka Štolcpartová[3]

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Czech Technical University in Prague Faculty of Mechanical Engineering
TECHNICAL UNIVERSITY OF LIBEREC Faculty of Mechanical Engineering
MEDETOX
INVESTMENTS IN EDUCATION DEVELOPMENT

Nanoparticles ($d_p$<100 nm) are more detrimental to human health than equivalent mass of larger particles.
In order to reduce their concentrations in air, we should know their sources, just like we did with PM mass.
Emissions of nanoparticles from internal combustion engines are strongly dependent on current and prior engine operating conditions and their distribution is therefore highly non-uniform (in time, in space, among vehicles, etc.).

**Goal:** Compact, mobile, size-resolved measurement of nanoparticles in ambient air in residential neighborhoods.

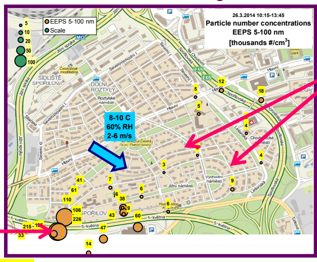
## Experimental setup:

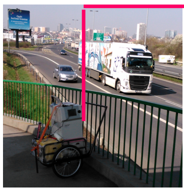
Portable vehicle emissions monitoring instrumentation used for ambient measurement:
Fast mobility spectrometer (Engine Exhaust Particle Sizer, TSI Inc.), condensation counter (UF-CPC, Palas), notebook, GPS, batteries mounted on hand carts (or a baby carriage).
Study designed, measurements done, and data interpreted by an interdisciplinary team of engine – combustion – aerosols – toxicology specialists.

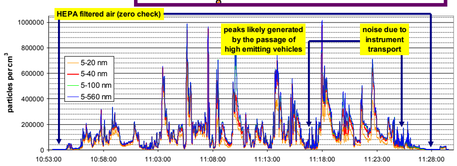
## Spořilov neighborhood "instrumented walking tour"

Quantitative measurements taken during 1-5 minute stops, qualitative assessment during walking.

**"Spořilov hotspot":**
After low-speed travel through congested area of Prague, heavy trucks accelerate onto a freeway and climb a hill – "reentrainment" of material deposited in the exhaust system.

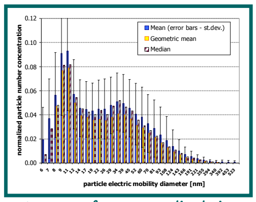





## Absence of larger particles & absence of higher concentrations in the inner neighborhood:

Assuming that home heating appliances are evenly distributed throughout the neighborhood, why don't we see anything upwind of the road?

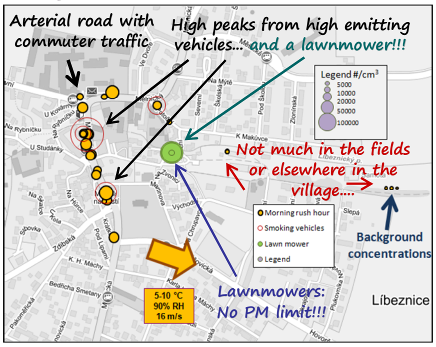



Average of 40 normalized size distributions at various locations

## Size matters:

**Large peaks around 10 nm, with a second peak in tens of nm, correspond to engine exhaust particle size distribution.**
Particles around 10 nm can be missed if measurements start around 15-20 nm (ambient studies) or 23 nm (vehicle emissions type approval – PMP).

## Village of Líbeznice "instrumented walking tour"

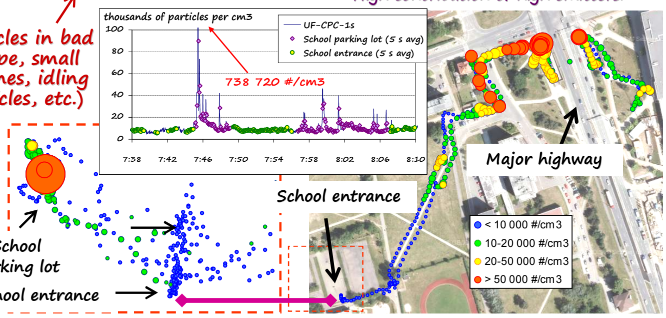



## Highest concentrations: High emitting engines

(vehicles in bad shape, small engines, idling vehicles, etc.)

## Sion High School "instrumented walking tour"

Highest concentrations at parking lot.
High contribution of high emitters.




**Concluding remarks:** Examples of data from several instrumented neighborhoods walking tours suggest that internal combustion engines remain the dominant source of nanoparticles in the Czech Republic.
Nanoparticles are concentrated where they are expected based on knowledge of internal combustion engine emissions: in the vicinity of high emitting vehicles, idling vehicles, congested areas, intersections.
Large peaks around 10 nm, with a second peak in tens of nm, correspond to engine exhaust patterns. Large concentrations are generally absent away from engines, but where one would expect operational home heating appliances (all measurements done in winter).


Acknowledgments:
The work was funded by the EU LIFE+ program, project MEDETOX – Innovative Methods of Monitoring of Diesel Engine Exhaust Toxicity in Real Urban Traffic (LIFE10 ENV/CZ/651) and by the Czech Science Foundation, project BIOTOX – Mechanisms of toxicity of biofuel particulate emissions (13-01438S). The salary of the first author was funded by ESF project CZ.1.07/2.3.00/30.0034, Support of Research Teams at Czech Technical University in Prague.
Note: Detailed report submitted to Atmosphere (ISSN 2073-4433)

**5.1.5. Action 7: Effects of fuel additives** (01/01/2015 – 31/08/2016)**:**

The effects of biofuels for diesel engines were taken into consideration from the beginning of the project, resulting in publications of selected findings during Actions 3 and 4, including the following articles in peer-reviewed journals ( already listed in the respective actions, and reported in the Mid-term and Progress reports):

- Vojtíšek, M., Pechout, M., Barbolla, A.: Experimental investigation of the behaviour of non-esterified rapeseed oil in a diesel engine mechanical fuel injection system. Fuel, 97, 2012, 157-165.
- Topinka J., Milcová A., Schmuczerová J., Mazač M., Pechout M., Vojtíšek M.: Genotoxic potential of organic extracts from particle emissions of diesel and rapeseed oil powered engines. Toxicology Letters, 212, 2012, 11-17.
- Vojtíšek M.; Czerwinski J.; Leníček J.; Sekyra M.; Topinka J.: Polycyclic aromatic hydrocarbons (PAHs) in exhaust emissions from diesel engines powered by rapeseed oil methylester and heated non-esterified rapeseed oil. Atmospheric Environment, 60, 2012, 253-261.

The effects of biodiesel and second generation biofuels on exhaust toxicity were addressed primarily during laboratory studies. These results were presented at international conferences and also submitted for publication in international peer-reviewed journals.

Submitted manuscripts:

- Vojtíšek M., Beránek V., Mikuška P., Křůmal K., Coufalík P., Sikorová J.: Blends of butanol and hydrotreated vegetable oils as drop-in replacement for diesel engines: Effects on combustion and emissions. (Fuel journal, in review)
- Cervena, T., Rossnerova, A., Sikorova, J., Beranek, V., Vojtisek-Lom, M., Ciganek, M., Topinka J., Rossner, P. (2016). DNA Damage Potential of Engine Emissions Measured In Vitro by Micronucleus Test in Human Bronchial Epithelial Cells. Basic & Clinical Pharmacology & Toxicology (accepted in October 2016, 10.1111/bcpt.12693).
- Libalova, H., Rossner, P., Vrbova, K., Brzicova, T., Sikorova, J., Vojtisek-Lom, M., Beranek V., Klema J., Ciganek M., Neca J., Pencikova K., Machala M., Topinka J. (2016). Comparative Analysis of Toxic Responses of Organic Extracts from Diesel and Selected Alternative Fuels Engine Emissions in Human Lung BEAS-2B Cells. International Journal of Molecular Sciences, 17(11), 1833. (published November 2016)

Conference presentations:

- Novotna, B.: Genotoxicity of organic extracts from truck emissions produced at various engine operating modes using diesel or biodiesel (B100) fuel. Presentation at the Central and Eastern European Conference on Health and Environment (CEECHE 2016).
- Libalova, H.: Comparative analysis of the toxic responses of organic extracts from diesel/biodiesel engine emissions in human lung BEAS-2B cells. Presentation at the Central and Eastern European Conference on Health and Environment (CEECHE 2016).
- Libalova, H., Vrbova, K., Brzicova, T., Sikorova, J., Milcova, A., Vojtisek-Lom, M., ... & Machala, M. (2016). Comparative analysis of the toxic responses of organic extracts from diesel/biodiesel engine emissions in human lung BEAS-2B cells. Toxicology Letters, (258), S281-S282.

Additionally, with the interest in particulate matter shifting towards spark ignition engines, several tests were conducted on gasoline powered cars, where ethanol, n-butanol and isobutanol were used in various concentrations as fuels. These currently used (ethanol) and candidate (n-butanol and isobutanol) alcohol fuels, can be considered either "first generation" or "advanced" /""second generation" depending on the feedstock and the fuel production process. These results were also presented at international conferences and published in international peer-reviewed journals.

Technical papers and presentation at the Society of Automotive Engineers conferences:

- Vojtisek-Lom, M, Pechout, M., Mazac, M.: Real-world on-road exhaust emissions from an ordinary gasoline car operated on E85 and on butanol-gasoline blend. Society of Automotive Engineers Technical Paper Series, ISSN 0148-7191, 2013-24-0102, doi: 10.4271/2013-24-0102, 2013.
- Pechout M., Dittrich A., Mazač M., and Vojtíšek M., "Real Driving Emissions of Two Older Ordinary Cars Operated on High-Concentration Blends of N-Butanol and ISO-Butanol with Gasoline," SAE Technical Paper 2015-24-2488, 2015, doi: 10.4271/2015-24-2488.
- Vojtíšek M., Beránek V., Štolcpartová J., Pechout, M. et al., "Effects of n-Butanol and Isobutanol on Particulate Matter Emissions from a Euro 6 Direct-injection Spark Ignition Engine During Laboratory and on-Road Tests," SAE Int. J. Engines 8(5),2015, doi: 10.4271/2015-24-2513

Conference with non-reviewed proceedings only:

- Vojtíšek M., Beránek V., Klír V., Pechout M., Mazač M., Dittrich L.: Effect of alcohol blending on real driving emissions of particulate matter from ordinary gasoline automobile engines: A comparison among ethanol, n-butanol and isobutanol. Presentation at conference BIOTRETH, Krakow, Poland, 25.-26.11.2015.

20th ETH Conference on Combustion Generated Nanoparticles, Zurich, June 13-16, 2016

# Experimental investigation of particles produced by combusting blends of "high-quality" and "cost-competitive" biofuels in a tractor engine

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## Background

- Biodiesel (n-alkyl-esters of fatty acids) and non-esterified vegetable oil popular drop-in fuels for agricultural machinery and road vehicles. Downside: cold performance, fuel stability, poor combustion at low loads, potential conflict with food production.
- HVO and synthetic fuels use a wider variety of feedstocks, but their present downside is their high cost.
- Alcohols can be produced inexpensively and with low embedded fossil energy from different agricultural residues. Downside: low cetane number and the resulting deterioration in combustion.
- Oxygenated fuels (alcohols, biodiesel, vegetable oils) offer a reduction in particulate matter due to oxygen content.
- Two of four butanol isomers - n-butanol and iso-butanol - are mentioned as a suitable fuels for spark ignition engines (for CI only when small share of butanol is used), can be produced from biomass at cost comparable to ethanol [Tao et al., Biofuels Bioprod. Biorefin. 8(1): 30-48, 2014], higher cetane number, lower hygroscopicity, lower aggressivity to materials compared to ethanol.

**Goal: To explore the effects of introducing n-butanol, iso-butanol, HVO and rapeseed oil and their mixtures a fuel into a tractor engine on combustion, performance, and emissions.**
**This poster focuses on particulate matter emissions.**

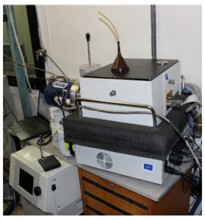
**Acknowledgments:** This work was sponsored by the EU LIFE+ programme, project LIFE10 ENV/CZ/651 – MEDETOX, Innovative Methods of Monitoring of Diesel Engine Exhaust Toxicity in Real Urban Traffic

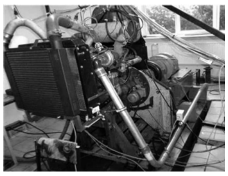
## Experimental

- A Zetor 1505 (EU Stage III) has been tested during 12 loaded points steady state operation (also including regimes of NRSC), idle and extended idle
- Parameters of the engine: displacement 4,016 dm³, max. torque: 525 Nm @ 1500 rpm, power: 90 kW @ 2200 rpm, compression ratio 17.0 : 1, turbocharged with intercooler, in-line four cylinder, in-line injection pump
- The engine was operated on idle, extended idle (1200 rpm and 20Nm) and four load levels (25%, 50% and 75% of torque when operated on diesel fuel) and full load at rated rpm (2200) and maximum torque rpm (1500).
- Exhaust gas pollutant were investigated by FTIR (Midac, $CO_2$, CO, NO, $NO_2$, $N_2O$, $NH_3$, acetaldehyde and formaldehyde)
- Size distribution spectra of particulate matter were analyzed in sample of exhaust gas taken from partial dilution tunnel by EEPS TSI 3090 without removing of volatile fraction
- Gravimetric production was evaluated using a proportional particulate matter sampler

Fuels tested

| | |
|---|---|
| **Diesel** | **Diesel fuel (reference)** |
| E10 | 10% ethanol |
| nBu16 | 16% n-butanol |
| iBu16 | 16% i-butanol |
| nBu16-B10 | 16% n-butanol, 10% biodiesel (FAME) |
| **Diesel** | **Diesel fuel (reference)** |
| iBu16-B10 | 16% i-butanol, 10% biodiesel (FAME) |
| E10-B10 | 10% ethanol, 10% biodiesel |
| HVO (100) | Hydrotreated veg. oil (NExBTL) |
| RO (100) | Rapeseed oil (fuel-grade) |
| RO-nBu16 | 16% n-butanol, rapeseed oil |
| RO-nBu16-B20 | 16% n-butanol, 20% biodiesel, RO |
| **Diesel** | **Diesel fuel (reference)** |
| HVO5-B5 | 5% NExBTL, 5% biodiesel |
| E10-B10 | 10% ethanol, 10% biodiesel |
| nBu15-B16 | 16% n-butanol, 15% biodiesel (FAME) |
| HVO50 | 50% NExBTL |
| HVO50-B50 | 50% NExBTL, 50% biodiesel |
| **Diesel** | **Diesel fuel (reference)** |
| RO-iBu16 | 16% isobutanol, rapeseed oil |
| RO-iBu16-B20 | 16% isobutanol, 20% biodiesel, RO |

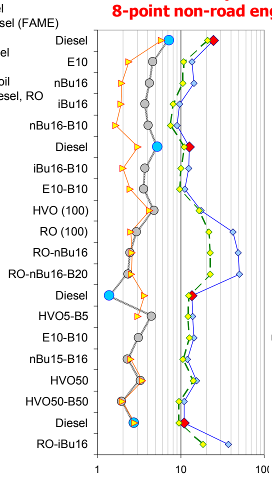


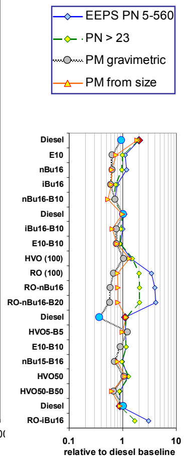


## Results and Discussion

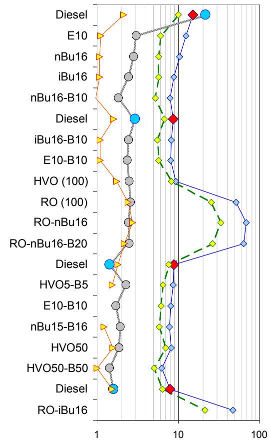
- Addition of alcohols into diesel increased count of the nucleation mode particles and decreased the accumulation mode
- The nucleation mode concentrations were lower for iso-butanol blends compared to n-butanol blends
- All alcohols decreased PM production, i-butanol most effectively
- Utilization of HVO caused increase of accumulation mode particle raising with lowering of load with weak effect on nucleation mode
- RO utilization generally decreased PN accumulation mode at higher loads but increased PM at lower loads and idle
- Addition of alcohols to rapeseed oil did not improve the problem of high particle emissions at low loads
- Nearly no effect of both butanols on nucleation mode has been observed, i-butanol exhibited lower concentrations
- Total particle production on alcohol blends was comparable or significantly decreased depending on used weight factors

**ISO 8178 C-1 (NRSC) averages**
**8-point non-road engine test**

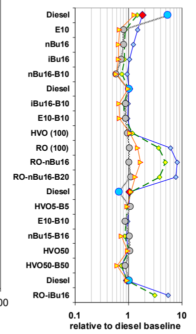


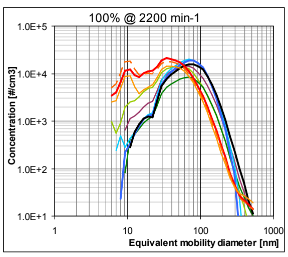



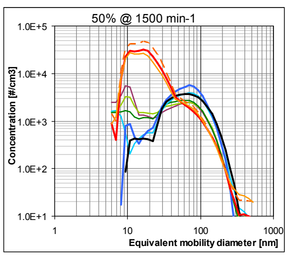

**ISO 8178 C-2 averages**
**(accent on lower loads)**

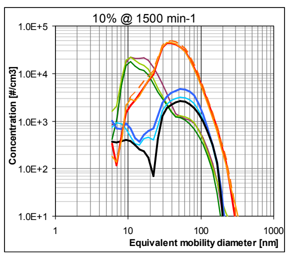


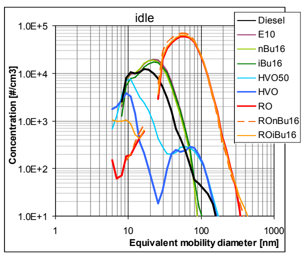

| engine rpm | torque [%] | weight factors C1 [%] | C2 [%] |
|---|---|---|---|
| 2200 | 100 | 15 | |
| 2200 | 75 | 15 | |
| 2200 | 50 | 15 | |
| 2200 | 25 | | 12 |
| 2200 | 10 | 10 | |
| 1500 | 100 | 10 | 2 |
| 1500 | 75 | 10 | 5 |
| 1500 | 50 | 10 | 32 |
| 1500 | 25 | | 30 |
| 1500 | 10 | | 10 |
| 780 | 0 | 15 | 15 |



### 5.1.6. Action 8: Project audit

- Project audit was performed by selected auditor: Ing. Pavel Antoš, auditor (official registration number 1416), office: AUDITORSKÁ A DAŇOVÁ KANCELÁŘ, s.r.o., Vodičkova 41, 110 00 Praha 1 (registration number: 181).
- On the basis of the financial control, in accordance with the programme, the auditor obtained reasonable assurance that the financial report of project gives a true and fair view of the expenses, income and investments incurred/made by the co-ordinating beneficiary Institute of Experimental Medicine AS CR, v.v.i., established in Czech Republic and of the associated beneficiaries Technical University Liberec, established in Czech Republic and Ministry of the Environment of' the Czech Republic, established in Czech Republic in connection with the abovementioned project within the time limit laid down by the Commission and in accordance with the LIFE+ Programme Common Provisions, the national legislation and accountings rules.

### 5.1.7. Action 9: Networking with other projects (01/09/2011 – 31/08/2016):

- ***Networking with the Swiss engine-toxicology group***, including a stay by Dr. Vojtisek as a visiting professor at Bern University of Applied Sciences in Biel, Switzerland, has resulted in a joint publication (Vojtisek et al., Atmospheric Environment, 2012, for details of all publications listed here see the list of outputs).
- ***Networking with Moscow State University*** has resulted in a joint publication (Popovicheva et al., 2014).
- Networking with the Josef Bozek Center for Vehicles and Sustainable Mobility at the ***Czech Technical University of Prague*** has resulted in the adoption of some real-world emissions measurement practices, and in ongoing work in the field by the group.
- ***Networking with the Czech University of Life Sciences*** has resulted in expansion of their scope of assessment of new fuels, primarily for non-road engines; by including advanced measurement of non-regulated pollutants or non-regulated metric of particulate matter.
- ***Cooperation with the University of Rouen (prof.Morin), CERTAM and the Council of French Region Haute Normandie (France)*** – common workshop in Rouen in March 2015 with 3 active presentations of project data and possibilities of future cooperation. http://www.medetox.cz/wp-content/uploads/2015/04/P4TA-workshop-agenda.pdf
    - ***Networking with the Prof. Morin's group at the University of Rouen*** (France) and vehicle emissions testing laboratory CERTAM (France), has resulted in the adoption of the exposure chamber developed by Prof. Morin and built by CERTAM by the Czech Technical University in Prague.
- ***Participation in LIFE+ seminar*** entitled "Alternative Future Urban Mobility, Berlin, November 21-22, 2013.
- ***A networking visit to RIVM in Bilthoven*** (The Netherlands) to share experiences relevant to engine exhaust toxicity measurements, was organized in December 2013.
- ***Cooperation with LIFE+ project Clean Air*** (LIFE11 ENV/DE/000495) was established (www.cleanair-europe.org) in the area of measurement of the effects of Eco-driving practices on motor vehicle emissions.
- ***Cooperation with the National Institute of Public Health, Oslo, Norway*** (Dr. Holme) – new assays to monitor toxic effects of engine emissions.

- ***Norwegian Institute of Air Research (NILU), Kjeller, Norway*** (Dr. Dušinská) – simple methods to measure the genotoxicity of engine emissions.
- ***Cooperation with the European Metrology Research Program*** project ENV02 PartEmissions: Emerging requirements for measuring pollutants from automotive exhaust emissions, http://www.ptb.de/emrp/PartEmission.html
    - Demonstration of the measuring ionization chamber as a small, simple and low-cost instrumentation to detect excessive particle emissions from modern diesel vehicles equipped with particle filters (DPF). Reports: (https://www.ptb.de/emrp/partemission-publications.html)
    - Field measurements with suitable instruments carried out under controlled conditions similar to Type1 type approval testing, also using additional test cycles not foreseen in the legislation. https://www.ptb.de/emrp/partemission-publications.html?&no_cache=1&cid=436&did=9547&sechash=afaeb51e
    - User's handling experience with the instrument selected, and testing results to analyse the instrument's long term stability and operational reliability.https://www.ptb.de/emrp/partemission-publications.html?&no_cache=1&cid=436&did=9548&sechash=badbac13
- ***Cooperation with the European Commission Joint Research Center in Ispra (Italy)***
    - Demonstration of the applicability of portable, on-board emissions monitoring system on scooters and motorcycles
    - Demonstration of the applicability of portable, on-board emissions monitoring system on small non-road engines (garden machinery)
    - Demonstration of the applicability of on-board FTIR for measurement of unregulated pollutants, both hazardous and greenhouse gases

### 5.1.8. Action 11: After LIFE communication plan

Detailed "After LIFE communication plan" was prepared (Annex 7.3.2.) and published on the Project website.

By the end of the MEDETOX project, a wide range of communication tools had been exploited in order to disseminate the outcomes to the stakeholders and other target audience. The most important dissemination methods are direct contacts with experts, seminars and conferences, newspaper articles, radio, television, Internet. Publications produced by the project are available for all in a PDF-format at the project's Internet site. These publications have been delivered to experts, different stakeholders etc. A monograph on the project MEDETOX was delivered at the end of the project.
Maintenance of the project website: The project Internet site www.medetox.cz demonstrates the project results, reports and publications including Layman`s Report and project monograph. Most of the materials are in pdf-format available for all. The project's Internet site will be available for at least 5 years of duration after the end of the project. After this majority of the information at the Internet site will be transferred into the websites of the project partners.
All data and publications are available for authorities of municipalities and the government.
Presentations in international meetings/conferences: A group of experts working in the project will be presenting the project's results in various national and international meetings. At least ten presentations is expected within next five years. Participation on such meetings should be covered from follow-up projects.
Popularisation and technical papers: Although the project MEDETOX produced already dozens of popularisation and technical papers (as demonstrated on project`s website), at least

twenty of manuscripts, articles, and similar materials will be further published within 5 years after the project deadline.
Continuing projects: Although the project has ended, several methods developed by the project, are used and will be used further in other projects. A wide group of experts will be participating in these projects. The experts have a close contacts with other professionals both in Czech Republic and abroad.

Already completed, ongoing or confirmed projects include
• A basic research project aimed at better understanding of mechanisms of toxicity of particles arising from the combustion of contemporary and advanced biofuels
• A work package within a larger Czech Ministry of Education funded project at the Czech Technical University aiming at the assessment of realistic effects of new fuels and technologies on emissions during real-world operating conditions and on the human health
• An industry-sponsored study on real driving emissions of ultrafine particles from modern automobiles, including vehicles powered by advanced fuels
• Fabrication and delivery of a miniature portable on-board emissions monitoring system

## 5.2. Dissemination actions (**Action 10**)

### 5.2.1. Objectives

Multiple dissemination activities are reported and documented on the project website www.medetox.cz and summarized in Annex 7.4. (Outputs and Indicators):

Dissemination of the results took place along several major lines:

1. Publications in peer-reviewed journals (**15x**)
2. Public presentations (**21x**)
3. Presentations at scientific conferences of international importance (**45x**)
4. Summary publications in a national peer-reviewed journal Ochrana ovzdusi (Environment Air Protection) and in a national popular magazine Respekt (**13x**)
5. Presentation at national conferences, workshops, meetings (**22x**)
6. Public presentations at citizen group meetings and interactions with citizen groups interested in the issue of the effects of motor vehicles on human health (**10x**).
7. Internet articles (**8x**)
8. Educational activities (**146 students involved**)

**Altogether, 142 publications, presentations, workshops and media outputs were delivered within the project (www.medetox.cz**, Annex 7.3.4.**).**

**Because of high interest in the MEDETOX project results among all stakeholders and the general public, the total number of outputs of each category is, in the end, much higher than originally planned in the project proposal.**

### 5.2.2. Dissemination: overview of major dissemination outputs per individual experimental actions

**Action 3:** Optimisation of on-board monitoring and sampling apparatus, and methods in real traffic in Prague

1. Vojtíšek, M., Pechout, M., Barbolla, A.: Experimental investigation of the behaviour of non-esterified rapeseed oil in a diesel engine mechanical fuel injection system. Fuel, 97, 2012, 157-165.
   Responsible beneficiary: TUL

2. Vojtíšek-Lom M., Pechout M., Mazač M.: Measurement of consumption rates of viscous biofuels. Fuel, Volume 107, May 2013, Pages 448–454.
   Responsible beneficiary: TUL

3. Pechout M., Dittrich A., Mazač M., and Vojtíšek M., "Real Driving Emissions of Two Older Ordinary Cars Operated on High-Concentration Blends of N-Butanol and ISO-Butanol with Gasoline," SAE Technical Paper 2015-24-2488, 2015, doi: 10.4271/2015-24-2488.
   Responsible beneficiary: TUL

4. Vojtíšek, M., Pechout, M., and Fenkl, M.,: Measurement of Exhaust Emissions of Small Gasoline Engines Under Real-World Driving Conditions, SAE Technical Paper 2014-01-2811, 2014, doi: 10.4271/2014-01-2811.
   Responsible beneficiary: TUL

5. Vojtisek-Lom, M.: Assessment of Low Levels of Particulate Matter Exhaust Emissions Using Low-Cost Ionization-Type Smoke Detectors. Society of Automotive Engineers Technical Paper Series, ISSN 0148-7191, 2013-24-0168, doi: 10.4271/2013-24-0168, 2013.
   Responsible beneficiary: TUL

6. Vojtisek-Lom, M, Pechout, M., Mazac, M.: Real-world on-road exhaust emissions from an ordinary gasoline car operated on E85 and on butanol-gasoline blend. Society of Automotive Engineers Technical Paper Series, ISSN 0148-7191, 2013-24-0102, doi: 10.4271/2013-24-0102, 2013.
   Responsible beneficiary: TUL

**Action 4:** Optimisation of toxicity assays to on-board sampled diesel exhaust in cell-free system and in cell cultures

1. The standardized protocols for individual toxicity assays are reported both in Czech and in English
   Responsible beneficiary: IEM

2. Topinka J., Milcová A., Schmuczerová J., Mazač M., Pechout M., Vojtíšek M.: Genotoxic potential of organic extracts from particle emissions of diesel and rapeseed oil powered engines. Toxicology Letters, 212, 2012, 11-17.
   Responsible beneficiary: IEM

3. Vojtisek-Lom M.; Czerwinski J.; Leníček J.; Sekyra M.; Topinka J.: Polycyclic aromatic hydrocarbons (PAHs) in exhaust emissions from diesel engines powered by rapeseed oil methylester and heated non-esterified rapeseed oil. Atmospheric Environment, 60, 2012, 253-261.
   Responsible beneficiaries: IEM and TUL

**Action 5**: Verification of optimized sampling and toxicity testing procedures – comparison of laboratory and real traffic conditions

1. Multiple rounds of verification of a portable proportional sampling system.
   Responsible beneficiary: TUL

2. Multiple rounds of verification of the low-cost portable system for measuring total particle length.
   Responsible beneficiary: TUL

3. Vojtíšek-Lom M., Pechout M., Mazač M.: Measurement of consumption rates of viscous biofuels. Fuel, Volume 107, May 2013, Pages 448–454
   Responsible beneficiary: TUL

4. Stolcpartova J., Pechout M., Dittrich L., Mazac M., Fenkl M., Vrbova K., Ondracek J., Vojtisek-Lom M.: "Internal Combustion Engines as the Main Source of Ultrafine Particles in Residential Neighborhoods: Field Measurements in the Czech Republic", Atmosphere, 6(11), 2015, 1714-1735; doi: 10.3390/atmos6111714.
   Responsible beneficiary: IEM and TUL

5. Vojtíšek M., Beránek V., Štolcpartová J., Pechout, M. et al., "Effects of n-Butanol and Isobutanol on Particulate Matter Emissions from a Euro 6 Direct-injection Spark Ignition Engine During Laboratory and on-Road Tests," SAE Int. J. Engines 8(5),2015, doi: 10.4271/2015-24-2513
   Responsible beneficiary: TUL

**Action 6**: Measurement of the toxic effects of diesel exhaust under various real traffic conditions in Prague

1. Rossner P., Strapacova S., Stolcpartova J., Schmuczerova J., Milcova A., Neca J., Vlkova V., Brzicova T., Machala M., Topinka J. Toxic Effects of the Major Components of Diesel Exhaust in Human Alveolar Basal Epithelial Cells (A549). Int J Mol Sci. 2016 Sep; 17(9): 1393.
   Responsible beneficiary: IEM

2. Vojtíšek M., Pechout M., Dittrich L., Beránek V., Kotek M., Schwarz J., Vodička P., Milcová A., Rossnerová A., Ambrož A., Topinka J.: Polycyclic aromatic hydrocarbons (PAH) and their genotoxicity in exhaust emissions from a diesel engine during extended low-load operation on diesel and biodiesel fuels. Atmospheric Environment 109, 2015, 9-18.
   Responsible beneficiary: IEM and TUL

**Action 7**: The effects of selected fuel additives (bio-fuels) on the diesel exhaust toxicity under real traffic conditions in Prague - pilot study

1. Vojtíšek, M., Pechout, M., Barbolla, A.: Experimental investigation of the behaviour of non-esterified rapeseed oil in a diesel engine mechanical fuel injection system. Fuel, 97, 2012, 157-165.
   Responsible beneficiary: TUL

2. Vojtíšek-Lom M., Pechout M., Mazač M.: Measurement of consumption rates of viscous biofuels. Fuel, Volume 107, May 2013, Pages 448–454.
   Responsible beneficiary: TUL

**Action 10**: Dissemination activities

1. BBC ONE, Panorama, The VW Emissions Scandal, 9th Dec 2015, 20:30. http://www.bbc.co.uk/programmes/b06q6nh2
   Responsible beneficiary: TUL

2. Vojtisek M., Innovative methods of combustion engines toxicity evaluation during city driving. Seminar at Ministry of Environment of the Czech Republic, 18.6. 2015
   Responsible beneficiary: TUL

3. 2016-06: MEDETOX presentation on VVI Brno Fairs and Exhibitions, 10.3.2016 https://www.sfzp.cz/soubor-ke-stazeni/56/16924-priorita_03_2016_web_v2.pdfHh
   Responsible beneficiary: TUL

4. Layman's Report
   Responsible beneficiary: IEM and TUL

5. Project's Monograph
   Responsible beneficiary: IEM and TUL

6. Workshop for stakeholders on the results of project, Brno NCO NZO, May 2012
   Responsible beneficiary: IEM

7. Workshop for stakeholders on the results of project, Brno NCO NZO, May 2014
   Responsible beneficiary: IEM

8. Workshop for stakeholders on the results of project, Terl4, Educational Center of Masaryk University, May 2016
   Responsible beneficiary: IEM

The list above contains only major dissemination outputs and deliverables. A complete list of dissemination activities is reported and documented on the project website www.medetox.cz, and in Annex 7.3.4. A complete list of all deliverables is reported in Section 2.2. (all deliverables were delivered as indicated).

LIFE logo was used for all parts of the prototype, for all presentations, posters and workshop materials. Notice boards on the project were erected in the main building of coordination, as well as associated beneficiaries. The website of the project (www.medetox.cz) was opened during the first year of the project, both in Czech and English. The website is regularly updated and contains all the relevant information on the project, its results and outputs. The website will be kept for 5 years after the end of the project.
The Layman's Report was delivered (see Annex 7.3.1.) both in Czech and English, in electronic (see project website), as well as in printed form (500 pcs). MEDETOX Monograph was delivered in English (500 pcs). Reports and monographs are distributed to all interested stakeholders during relevant workshops, roundtables, and scientific conferences. The distribution of these materials is one of the key activities within the After LIFEn communication plan. There are many press releases related to the project as summarized in Annex 7.3.4.

## 5.3. Evaluation of Project Implementation

### 5.3.1. Methodology applied

The project MEDETOX has clearly demonstrated character. The project aimed to demonstrate innovative methods of monitoring the toxicity of diesel engine exhaust emissions during real urban driving, with a detailed focus on the situation on the ring road of Prague. Existing technologies and toxicity methods were adopted and demonstrated to be used to assess possible adverse effects of diesel engine emissions under real traffic conditions. The project was focused on the following topics:

- Construction and validation of several innovative emissions monitoring and/or sampling systems for assessment of emissions on the road.
- Preparation and validation of simplified toxicity assays which could be carried out on the collected samples.
- Investigation of real-world emissions and of the toxicity of particle-bound organic compounds collected during real-world operation, and during its simulation in the laboratory, with particular focus on urban driving and on the effects of congestion.
- Investigation of real-world emissions and of the toxicity of particle-bound organic compounds collected during the operation of engines on various candidate replacement fuels.

The following key technologies and methods were demonstrated within the project:

- Miniature and low-cost portable on-board systems for vehicle emissions monitoring.
- A miniature ultra-low-cost detector of particle length, used for vehicle emissions monitoring, and also tested within the European Metrology Program, as a novel tool for periodic emissions inspections of motor vehicles.
- A portable FTIR (Fourier Transform InfraRed) spectrometer for the measurement of unregulated pollutants of interest, such as nitrogen dioxide, ammonia, formaldehyde, acetaldehyde, and greenhouse gases methane and nitrous oxide.
- Particle size distributions and particle counts have been measured in vehicle exhaust and in ambient air near roadways using instruments mounted on hand carts.
- Acellular tests of DNA adducts and oxidative DNA damage have been demonstrated as, relative to the toxicological tests in general, a relatively easy, fast, inexpensive screening test, requiring a relatively small amount of material.
- The standardized protocols for sampling and toxicity testing of diesel emissions under various real traffic conditions as tools for hazard identification and risk assessment based on toxic events of vehicle emissions.

These tests are described in details on www.medetox.cz/methods and include:

- Sampling
- Analysis of cytotoxicity
- Analysis of DNA adducts
- Micronucleus test
- Oxidative stress
- Comet assay

**Comparison of the results achieved (listed above) against the project's objective, clearly show that all the major aims were met.**

**Dissemination of the results was very efficient, and the idea that limits for engine exhaust emissions should be based on real driving conditions is now much more viable than at the beginning of the project.**

## 5.4. Analysis of long-term benefits

### 5.4.1. Impacts on Legislation and Decision Making

The project addresses priority objective 3 of the EU Environment Action Programme: To safeguard the Union's citizens from environment-related pressures and risks to health and well-being, and its article 44, which claims that "urban air pollution is set to become the primary environmental cause of mortality worldwide by 2050".

Direct / quantitative environmental benefits:

The primary long-term benefit of the project is the improvement in the evaluation of efforts leading to the reduction of the health effects of vehicle emissions. The project demonstrates three novel approaches, expansion of the concept of the monitoring of real-world emissions to just about any vehicle or mobile machinery powered by an internal combustion engine; a shift of attention towards urban operating conditions; and using relative toxicity as an alternative metric to particle mass and total number of volatile particles. Such monitoring is essential to proper decision making, as lessons learned from vehicle emissions research over the last several decades clearly demonstrate that any overlooked aspect – discrepancy between emissions during legislative cycles and during real-world operation (of which DieselGate is the only one, and the medially most known, example); high contribution of off-cycle emissions and of engines in "unsatisfactory technical conditions"; discrepancy between the changes in the metric prescribed by the legislation and the changes in health effects (reduction of total particle mass not leading to the commensurate decrease in health risk); and creation of new side effects (ammonia, nitrogen dioxide, and a potent greenhouse gas nitrous oxide, all resulting from exhaust after-treatment devices aiming at reducing $NO_x$ emissions).

Demonstration of availability of suitable technologies for the necessary tasks, including instrumentation suitable for on-board measurement, or toxicity assays that are relatively simple and economical to perform, are often a necessary and enabling step before appropriate legislation can be introduced. To give an example, real driving emissions legislation has been introduced in the EU about a decade after the sale of the first commercial PEMS designed by one of the members of the MEDETOX project team.

Contribution to the reduction of emissions and the wider application of new measurement technologies could thus be considered as an indirect but important quantitative environmental benefit. The environmental benefits associated with reduced air pollution, the economic benefits associated with reduced external costs associated with air pollution, the economic benefits associated with the adoption of less expensive measurement techniques, and the economic benefits associated with preferences of actions with lower cost-benefit ratios, cannot be reliably quantified at this time. Such quantification will be performed at a later date, when the contribution of the project actions will become more apparent.

For example, if the project results have contributed by a conservative estimate of 10% to the avoidance of development in Hradec Králové which would have produced approximately 10 tons of NOx and 0,4 tons of PM over the next 20 years, the benefits from only this action would have been 20 t NOx and 0,8 t PM.

The quantification of EU-wide actions, listed in the technical part of this report, will be more difficult and subject to much higher uncertainties.

For example, the independent measurements of real driving emissions were the trigger for the discovery and investigation of excess emissions of NOx from diesel passenger cars in the U.S., later known as "DieselGate". The external costs associated with the excess emissions of NOx have been valued by one study at 130 lives and 450 million USD annually in the U.S. (Barrett S.R.H., Speth R.L., Eastham S.D., Dedoussi I.C., Ashok A., Malina R., Keith D.W.: The impact of the Volkswagen emissions control defeat device on US public health. Environ. Res. Lett. 10 (2015) 114005.), and by another study of 45 thousands of disability-adjusted life years and 39 billion USD (Oldenkamp R., van Zelm R., Huijbregts M.A.J.: Valuing the human health damage caused by the fraud of Volkswagen. Environmental Pollution, 212, 2016, 121–127.)

It is likely that only a small fraction of the project on the benefits associated with such actions will be attributed to the project; however, the overall benefits are expected to be very high. Such benefits are therefore discussed in the next section as qualitative.

Long-term / qualitative environmental benefits

Article 47 of the EU Environment Action Programme claims that "The failure to fully implement existing policy is preventing the Union from achieving adequate air and water quality standards", and that "The Union will update targets in line with the latest science (…)". It is therefore expected that the scientific findings gathered during the project will make their way to decision making, involving the extent of support of advanced fuels and similar emissions reduction metrics, land-use planning, transportation planning, and other measures envisioned or purported to reduce emissions.

The project demonstration and dissemination activities have contributed to the advancement of real driving emissions (RDE) legislation set forth in the European Commission regulations no. 2016/427 (of 10 March 2016) and no. 2016/646 (of 20 April 2016).

It is expected that the project results may contribute, through demonstration of enabling technologies, to the following legislation:

- Expansion of RDE legislation to motorcycles and other L-category vehicles
- Expansion of RDE legislation to small non-road engines
- Expansion of RDE legislation to the measurement of ammonia (a secondary particle precursor)
- Expansion of RDE legislation to the measurement of methane and nitrous oxide (greenhouse gases)

Long-term / qualitative economic benefits

The demonstration of the low-cost miniature particle sensor serves as an enabling tool for the replacement or supplementation of the current opacity measurements during periodic vehicle inspections, with another method capable of assessing the functionality of a diesel particle filter.

It is also expected that the approaches and technologies, and primarily the toxicity assays demonstrated within the project, will be relatively directly and readily transferable and applicable to the assessment of emissions from home heating appliances and other distributed combustion sources, including the assessment under "real-world" performance.

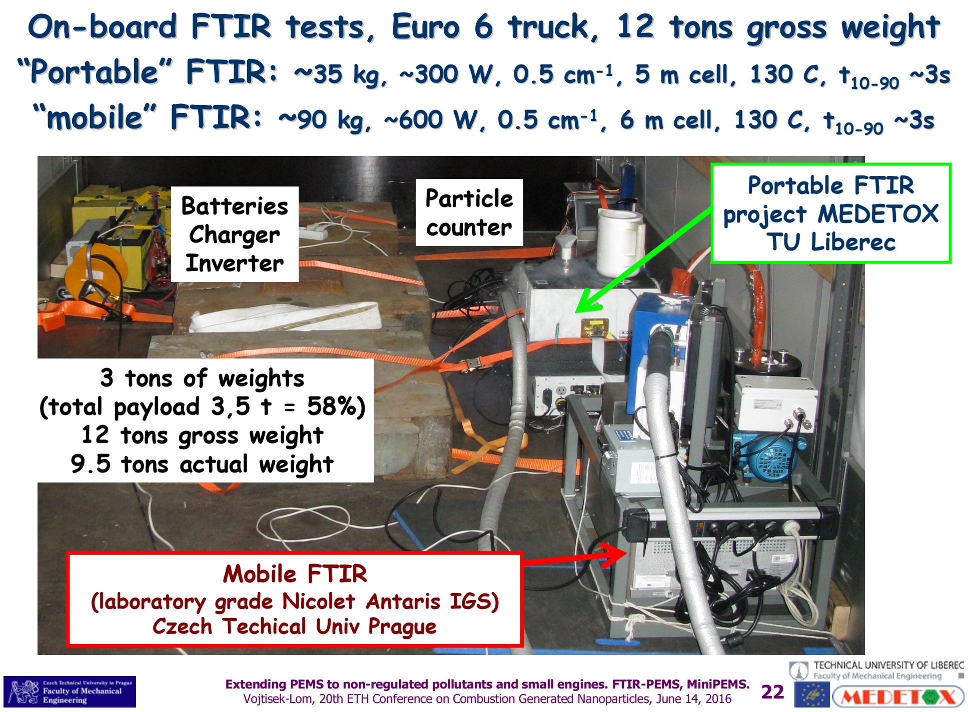
Long-term / qualitative social benefits

On the national level, the project findings were disseminated in multiple articles, radio and TV documentaries and news reports, during a lecture and several shorter hearings in front of the Committee for Sustainable Transport of the Czech government, public lectures at the Committee of the Environment of the Czech Academy of Sciences, lectures at automotive technician trade shows, and many lectures for academia, legislators and the general public.

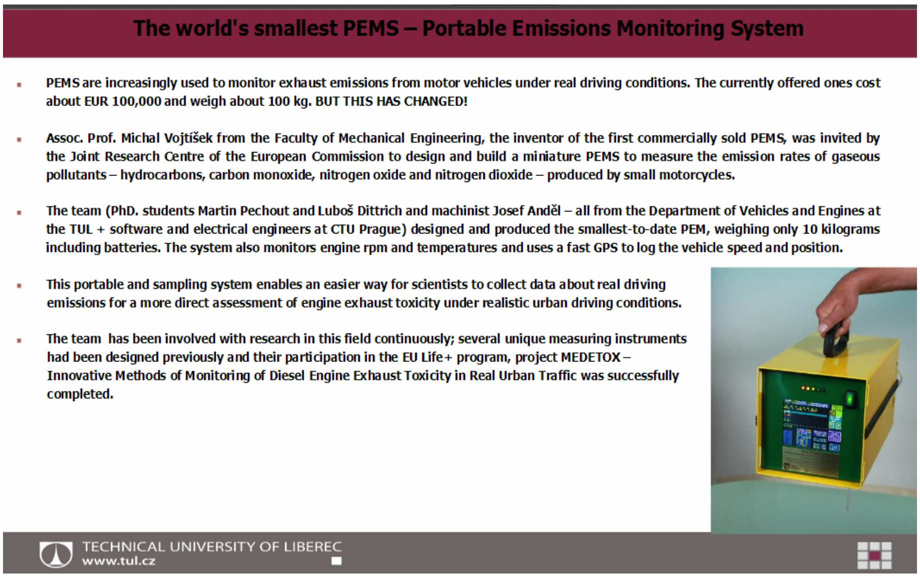
Ambient air measurements have sparked an interest from local authorities and citizen groups to assess air quality on a local (microscale) basis. Project findings and background information gathered during the project have been used in citizen actions against unwise land-use choices that would have likely resulted in the deterioration of air quality, including a construction of a large shopping centre which would have brought large amounts of traffic into a historical city centre. In the long term, the project results should contribute to improvement of living environments and to a reduction of adverse health effect from city traffic.

### 5.4.2. Replicability and Technology Transfer, Continuation of the project actions

The approach was taken up by other research groups. For example, a monitoring system based on the project prototype was built by the Czech Technical University in Prague and used for commercial evaluation of advanced motor fuels (one larger project consisting of about 50 days of vehicle testing time carried out in 2016), and various emissions abatement approaches (i.e., effects of traffic flow regulation). The photo below shows MEDETOX and CTU monitoring systems during comparison testing on a Euro 6 truck.




Another example is the building of a miniature portable on-board monitoring system for the Joint Research Centre of the European Commission (shown below)




Acellular tests of DNA adducts and oxidative damage may be used in the future for high throughput analyses of toxic effects induced by engine emissions.

Toxicity assays demonstrated within the project have, along with different types of laboratory toxicity assays carried by several other groups, contributed to the increase in cooperation between engineering and toxicology groups in the field of a more direct assessment of the effects of new fuels and technologies on health risks.

All three project partners will stay engaged in further research in the concerned field, and in the dissemination of project results.

### 5.4.3. Best practices pioneered

Some of the best practices demonstrated by the project and already in the process of adaptation by other groups include:

- the use of an on-board FTIR to measure real-world emissions of greenhouse gases methane and nitrous oxide, and to measure the emissions of reactive nitrogen species (ammonia, nitrogen monoxide and dioxide);
- the use of a rotating disc diluter and particle counters or charge-based detectors to measure real-world particle number emissions;
- the use of a charge based device (in this case, an ionization chamber) as a low-cost, simple device to assess the functionality of a diesel particle filter;
- the use of acellular tests of DNA adducts and oxidative damage for high throughput analyses of the toxic effects induced by engine emissions.

### 5.4.4. Contribution of EU funding

The mentioned activities would have been carried out to some extent anyway by this or other groups. The EU funding has:

- enabled a focused joint effort by engine and toxicity assessment groups which would have taken place to an extremely limited extent or not at all;

- fostered, to a great extent, interdisciplinary networking with other national and EU groups;
- accelerated the development of innovative portable systems for particulate matter emissions measurement, and for the measurement of many unregulated gaseous pollutants;
- increased national media and citizen awareness;

Thanks to the project existence and to the EU funding of the project, the project team, along with the Czech Technical University, were able to act as independent research laboratories during the "excess emissions" investigation (known as "DieselGate"), resulting in a 30-minute documentary aired by the BBC, and shorter documentaries by German TV WRD and by Czech Television.

### 5.4.5. Long term indicators of project success

The following quantitative indicators are proposed to monitor the long-term success of the project:

- Number of entities (laboratories, groups) adopting at least one method/technology/approach demonstrated within the project
- Number of cases of adoption of project methods in a field other than engine emissions
- Number of citations of project outputs in scientific and technical literature
- Number of citations of project outputs in governmental reports and policy documents
- Number of citations of project outputs and other references to the project in citizen actions, documents, media, and on the World Wide Web
- Number and, if available, total budget of subsequent studies or work relevant to the project theme, and using or building on, demonstrated technologies and approaches, by any of the consortium members and their collaborators

The main anticipated impact of the project, contribution towards a wide use of real-world emissions measurement and a wide use of toxicity assays, resulting in an improvement in decision making about emissions abatement and in improved air quality, cannot be readily quantified, as there are other drivers for such actions.

It is also anticipated that there will be many, at the current time, unforeseen effects.

It is proposed that for a minimum of the following seven years, by the end of years 2017-2023, the project team will draft its own evaluation of the project outcomes and value. and post it on the project website.

# 6. Comments on the financial report

The standard statement of expenditure is included in Section 8

## 6.1. Summary of Costs Incurred

The overview of the total expenses by cost category is summarized in **Table 1:**

| PROJECT COSTS INCURRED | | | | |
|---|---|---|---|---|
| | Cost category | Budget according to the grant agreement* | Costs incurred within the project duration | %** |
| 1. | Personnel | 579 660 | 620 137.50 | 107.0 |
| 2. | Travel | 58 620 | 77 030.36 | 131.4 |
| 3. | External assistance | 94 600 | 65 112.62 | 68.8 |
| 4. | Durables: total non-depreciated cost | | | |
| | *- Infrastructure sub-tot.* | 0 | 0 | |
| | *- Equipment sub-tot.* | 0 | 0 | |
| | *- Prototypes sub-tot.* | 149 500 | 138 458.60 | 92.6 |
| 5. | Consumables | 182 900 | 208 193.01 | 113.8 |
| 6. | Other costs | 78 200 | 52 833.41 | 67.6 |
| 7. | Overheads | **80 044** | **78 320.99** | **97.8** |
| | **TOTAL** | **1 223 524** | **1 237 922.95** | **101.2** |

The Commission contribution remains at 50% of €1,223,524 as approved in the Grant agreement.
*) If the Commission has officially approved a budget modification indicate the breakdown of the revised budget. Otherwise this should be the budget in the original grant agreement.
**) Calculate the percentages by budget lines: e.g. the % of the budgeted personnel costs that were actually incurred

SC decided that since 2014, most of the sample preparation for toxicity assays as well as chemical analysis of samples will be done in the laboratories of IEM. This required some transfers of budgets between categories as reported in the Progress Report (8.7. 2015) – increase of Personal Costs and Consumables and decrease of External Assistance and Other Costs. These transfers were within the flexibility limits of +/- 10% or +/- 30,000 € as required by General Provisions. These changes are also accompanied by an increase of the total incurred costs by 14,399 €. The EC contribution remains at 50% of 1,223,524 € as approved in the Grant agreement, and all additional expenses are covered by the own resources of the beneficiaries.

**The changes are described in detail below:**

Justification of all transfers between budget categories as shown in Table 1:

1. SC decided that since 2014 most of the sample preparation for toxicity assays as well as chemical analysis of samples will be done in the laboratories of IEM instead of the external company. This will require some transfers of the budget between categories – increase of Personal Costs (+43,538 € / +7%) and Consumables (+ 25,293 € / +14%) and decrease of External Assistance (- 29,488 € / -31%) and Other Costs (-25,367 € / -32%).
2. As suggested in the Mid-term Report (20.11. 2013), due to an increase of travelling connected with very high dissemination activities (see Section 5.2.1.), 20,790 € was transferred from the category Other Costs to the category Travel. As demonstrated in Outputs and Indicators, the results of the project were reported in 45 (instead of 12 originally planned) international conferences and in 22 (instead of 15 originally planned). This enormous increase was due to outstanding interest of experts, stakeholders and the general public in the results of the project, particularly after opening the "dieselgate" affair.
3. The total price of the prototype was 138,459 € instead of the expected 149,500 €, the difference of 11,041 € was transferred to Consumables and to Personnel to cover expenses connected with the maintenance of the prototype.

**Justification of the significant increase of daily rates for some project experts as required by Commission letter [Ref. Ares (2016)2734889 from 13.6. 2016]**

The daily rates costs of some employees of the coordinating beneficiary IEM significantly exceeded the original planned rates. This is partly caused by higher mean salaries of the IEM staff compared to the project proposal. The mean salary of IEM employees is very unstable and depends mostly on the actual number of national and international grants and related personal costs. Therefore, it is impossible to predict it for the next several years. For example, MEDETOX coordinator, Jan Topinka was almost permanently involved in the leading position of 6 projects and thus he received a corresponding salary. The system of calculation of daily rates according to the Common provision is based on the relevant part (percentage of working time) of brutto salary received from all the projects. The same applies (although to a lesser extent) for other IEM employees (Mr. Radim Sram, Ms. Jana Schmuczerova, Mr. Rossner, Ms. Novakova). As suggested in the Mid-term Report, **the discrepancy was compensated by a lower total number of hours dedicated to the project without the effect on the project goals and the quality of project results.** Particularly in the last year of the project, the extent of the experimental work was limited and some savings of time dedicated to the project were possible.

**Budget transfer between IEM and TUL**
Some money transfers were agreed between partners as specified in Amendment No. 1 to the contract between IEM and TUL. This change was reported and approved in the previous report (see Progress Report, 8.7. 2015, Annex 7.5.6.). One of the consequences of the budget transfer between IEM and TUL was that two components of the prototype were purchased by IEM. According to the internal rules of IEM, no tender is necessary for these 2 components, since they were under the price limit for public tender.
Public tender documentation on FTIR (component of prototype) is attached (Annex 8.9.)

## 6.2. Accounting system

All project expenses were registered in appropriate Financial tables downloaded from the LIFE toolkit.

The accounting system of project beneficiaries was reported as Annexes 7.5.4. and 7.5.5. of the Progress Report (08/07/2015). A special internal number identifying project costs was given by each beneficiary to monitor the project costs in the analytical accounting system: IEM: 470-244 (EU contribution), 471-244 (Contribution of ME to coordinating beneficiary IEM), 404-244 (co-financing); TUL: 1765 (EU contribution), 1473 (co-financing), ME: 09723.
We used recommended standard time-sheets to record the time spent on the project by individual workers. These time-sheets were repeatedly checked and approved by the Commission. The timesheets were completed electronically and signed by the worker and by the project coordinator (an example of a filled time-sheet is in Annex 8.5.).
All orders of materials and external services and corresponding invoices were stamped by project number and signed by the project coordinator. According to the rules of IEM and TUL, prototype components are regarded as materials or consumables but not investments.
The time registration system of IEM relies on a special book where the time of arrival and departure from the institute is filled in. The same applies for TUL with the difference that the time is registered by the secretary of the department. ME has electronic control of working time at the main entrance.
All orders of materials and external services and corresponding invoices were stamped by project number and signed by the project coordinator. See Annex 8.6. as an example of the order and the corresponding invoice.

## 6.3. Partnership arrangements

The rules of the financial transactions between the coordinating beneficiary and the associated beneficiaries are solved by official Partnership agreements attached in Annexes XX and XX. In agreement with Common Provisions, the relevant proportion of the EU contribution was transferred by the coordinating beneficiary to the associated beneficiaries within 30 days from receiving the EU contribution. Moreover, on the basis of special agreement (Annex 8.8.), ME contributed to IEM by the amount of € 154,200. This budget was proportionally distributed between IEM and TUL. On the basis of Amendment No. 1 to the Partner agreement between IEM and TUL, the original distribution of the budget between IEM and TUL was modified as specified in the amendment (Progress Report, 08/07/2015, Annex 7.5.6.). IEM entered the information in the financial tables for expenses of IEM and ME (on the basis of documents provided by ME). The data of TUL were entered directly by TUL and periodically checked by the IEM financial officer.

## 6.4. Auditor's report/declaration

The project audit was performed by a selected auditor: Ing. Pavel Antoš, auditor (official registration number 1416, Certificate reported as Annex 7.5.7 in the Progress Report, 8.7. 2015), office: AUDITORSKÁ A DAŇOVÁ KANCELÁŘ, s.r.o., Vodičkova 41, 110 00 Praha 1, Czech Republic (registration number: 181). The auditor's report is included as Annex 8.4. with the financial report, and it follows the format of the standard audit report form available on the LIFE website.

## 6.5. Summary of costs per action

As reported in the Mid-term Report in the Financial Review (20.11. 2013), most of the expenses (particularly consumables, external assistance and personal costs) relate to various

Actions, and the spending is reported according to the individual categories without reporting spending within individual Actions.

# 7. Annexes

## 7.1. Administrative annexes

## 7.2. Technical annexes

7.2.1. Key words
7.2.2. Abbreviations
7.2.3. Summary monitoring protocol 1
7.2.4. Summary monitoring protocol 2
7.2.5. Summary monitoring protocol final

## 7.3. Dissemination annexes

7.3.1. Layman's report
7.3.2. After-LIFE Communication plan
7.3.3. Other dissemination annexes
7.3.3.1. Paper in impacted journal: Stolcpartova et al. Atmosphere (2015)
7.3.3.2. Paper in impacted journal: Vojtisek-Lom et al. Atmospheric Environment (2015)
7.3.3.3. Paper in impacted journal: Libalova et al. International Journal of Molecular Sciences (2016)
7.3.3.4. Submitted manuscript in impacted journal: Vojtisek-Lom et al. Fuel (2016)
7.3.4. Dissemination activities and publications
7.3.5. MEDETOX Monograph

## 7.4. Outputs and Indicators

# 8. Financial report and annexes

8.1. Standard Payment Request and Beneficiary's Certificate
8.2. Consolidated Cost Statement for the Project
8.3. Financial Statements of the Individual Beneficiaries (IEM, TUL, ME)
8.4. Auditor's report
8.5. Example of time-sheet and related payslip
8.6. Example of order and related invoice
8.7. Copy of sample of TUL vehicle log and vehicle mileage accounting
8.8. Agreement on cofinancing between IEM and ME
8.9. Tender for FTIR (prototype) documentation