Bioreactivity of combustion generated particulate matter (diesel and flame exhaust) in bacteria and in human immune cells

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Summary

There are still many ambiguities concerning the mechanisms of hazardous health effects of combustion generated nanoparticles. Furthermore, there is no general agreement on the most relevant parameter for quantifying the biological response to nanoparticle-exposure.

Particulate matter samples were generated by combustion of ethylene in a laboratory burner or by combustion of diesel in a generator. Combustion exhaust containing nanoparticles was collected in cold traps. The biological effect of these combustion condensates was investigated by using several experimental systems. The bacterial bioassay SWITCH (***S**almonella **W**eighting of **I**nduced **T**oxicity (Genotoxicity) and **C**ytotoxicity for Human Health*) gives rapid and reliable data on the cyto- and genotoxic potential of an applied agent (Baumstark-Khan C. et al. 2005. Journal of Environmental Science and Health. 40 (2) 245). Therefore it was used as a pre-screening test to estimate the dose-range of interest. For the detection of nuclear factor κB (NF-κB) activation, we developed the recombinant U-937-pNF-κB-EGFP/Neo cell line derived from U-937 human histiocytic lymphoma cell line.

The toxicity of combustion condensates, regardless of the fuel (diesel or ethylene), increases proportionally with total organic carbon (TOC) content in the sample. With the SWITCH test, we demonstrate that the same amount of TOC in diesel combustion condensates exerts the same toxicity, regardless of the motor operation mode during the sample generation. Prolonged storage of the diesel exhaust samples did not change their cyto- or genotoxicity up to 12 weeks storage time.

In the SWITCH test, ethylene combustion water samples showed geno- and cytotoxicity which was proportional to the TOC-content and the combustion stoichiometry (**C**arbon/**O**xygen ratio). One sample, obtained during fuel-rich combustion conditions (C/O=0.93), was selected for the further experiments with human cells. This sample exhibits in the U-937-pNF-κB-EGFP/Neo cell line a dose-dependent NF-κB activation. The same sample (C/O=0.93) dose-dependently impairs a cell cycle progression of U-937-pNF-κB-EGFP/Neo cells. After 24 h of treatment with 4.8 ppm of TOC, a G2-phase arrest occurs and small fraction of cells undergoes apoptosis, as observed by the appearance of a sub-G1 peak in the DNA-content analysis. The number of apoptotic cells in the population increases proportionally with the increased TOC-concentration of the sample. A two fold increase in TOC-concentration (from 4.8 ppm to 9.6 ppm) dramatically elevates (~5 times) the percent of early apoptotic cells in culture.

Our experiments suggest that the carbonaceous particulate matter, which comprises a significant fraction of the ambient particle mass, could play a pivotal role in diverse pathological effects. Furthermore, total organic carbon seems to be an appropriate parameter to evaluate the biological effect of atmospheric pollution due to combustion processes. Combustion condensates containing nanoparticles are cyto- and genotoxic in bacteria and induce NF-κB activation and apoptosis in the human monocytic cell line.

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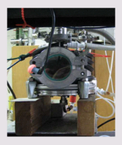
INTRODUCTION

There are still many ambiguities concerning the mechanisms of hazardous health effects of combustion generated nanoparticles. Furthermore, there is no general agreement on the most relevant parameter for quantifying their biological effect. Nanoparticles exist in combustion exhaust gases, and may significantly contribute to the environmental hazard due to their exquisite features (size <10 nm, water-solubility etc). Once inhaled, these small particles deposit in the respiratory tract, efficiently pass through the interstitium and enter the systemic circulation, where they can interact with human immune cells.

Sample preparation

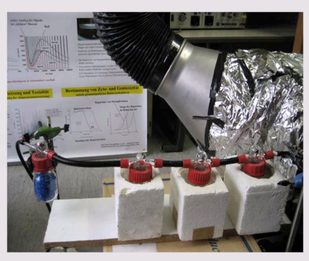
In the Institute of Combustion Technology (DLR, Stuttgart), ethylene combustion was performed under different stoichiometrical conditions (**C**arbon/**O**xygen ratio). Diesel exhaust samples were obtained from a generator under **no-load** and **load** motor operation mode conditions (4 kW). For each sample, 10 liter exhaust gas was collected under constant flow rate (0.4 l/min) and condensated in the cool traps.

combustion of ethylene in a low-pressure burner



combustion of diesel in a generator

Combustion exhaust containing nanoparticles was collected in cold traps



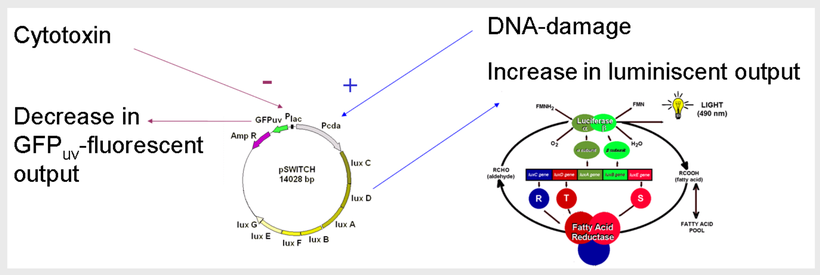
Combustion condensates before …

…and after thawing

Cellular models and investigated end points

BACTERIAL CELL MODEL:

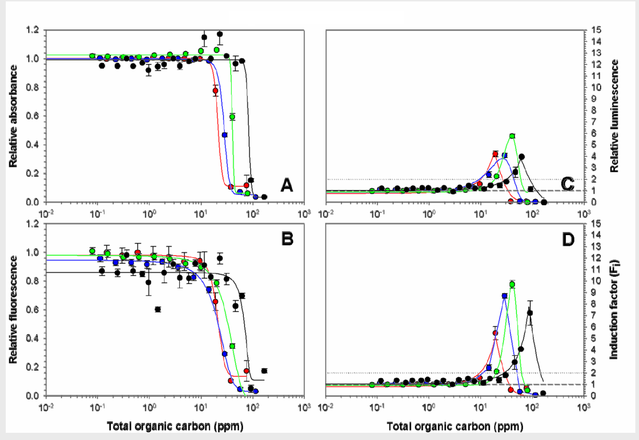
- Utilizes recombinant *Salmonella typhimurium TA1535 pSWITCH*
- SWITCH Test - bioassay on genotoxicity (DNA damage) and cytotoxicity (impairment of metabolism; cell death)
  - Based on 2 receptor-reporter systems: SOS-Lux and LAC-Fluoro
  - A two-fold increase in rel. luminescence is a conventional threshold for a genotoxicity. LD_{50} is a concentration by which a 50 % decrease in rel. absorbance (rel. fluorescence) occurs

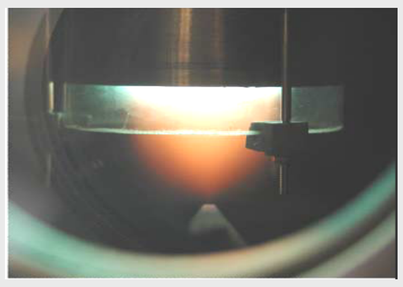


- Recombinant U-937-pNF-κB-EGFP/Neo cell line, derived from U-937 human histiocytic lymphoma cell line, was devised to investigate the activation of transcription factor NF-κB (role in a cell stress response)
- Annexin V-PE staining was used to study the induction of apoptosis (programmed cell death) in U-937 cells, after treatment with combustion water. Annexin V-PE binds selectively to the phosphatidylserine molecules of the outer membrane of the early apoptotic cells
- For the cell cycle progression analysis, a DNA-intercalating dye propidium iodide (PI) was used. Fluorescent signal of the bound PI is proportional to the DNA-content

RESULTS

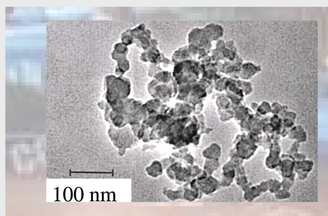
SWITCH test on ethylene combustion water



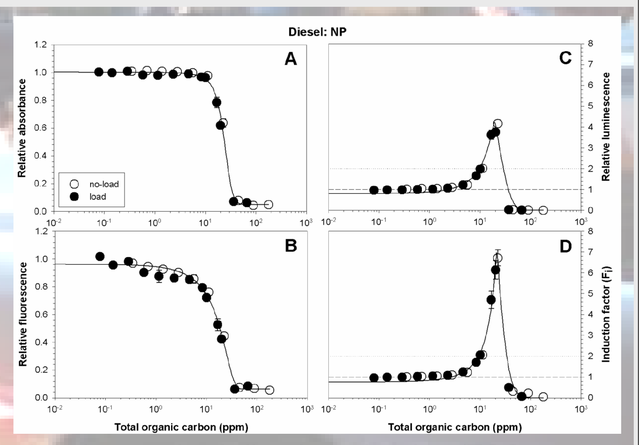


- On the graph, black (C/O=0.73), green (C/O=0.78), blue (C/O=0.83) and red colour (C/O=0.93) of the plots indicate different combustion conditions (C/O ratio)
- Both geno- and cytotoxicity of the samples are proportional to the total organic carbon (**TOC**) content and to the C/O ratio

SWITCH test on diesel combustion water



Primary chain aggregates from internal combustion emissions (*Xiong & Friedlander, 2001*).



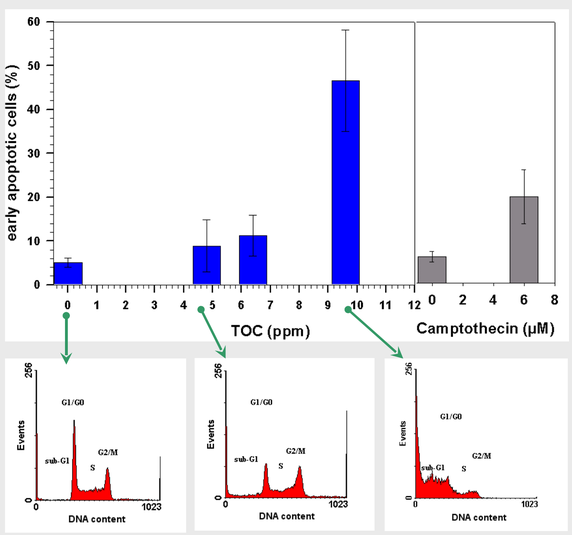
- Higher emission of organic carbon was observed during no-load diesel combustion ($TOC_{no\text{-}load}$ = 1040 ppm; $TOC_{4kW\ load}$ = 257 ppm)
- The same amount of TOC in diesel combustion condensates exerts the same toxicity, regardless of the motor operation mode during the sample generation (s. graph above)
- Prolonged storage of the diesel exhaust samples did not change their cyto- or genotoxicity up to 12 weeks storage time

Induction of apoptosis in monocytes

- A sample obtained during fuel-rich ethylene combustion (C/O=0.93), showed the biggest toxic potential in the SWITCH test, and therefore was used in experiments with mammalian U-937 cells. This sample was tested for the induction of apoptosis and cell cycle impairment after 24h of incubation
- A two fold increase in TOC-concentration (from 4.8 ppm to 9.6 ppm) leads to an impairment in cell cycle progression and dramatically elevates (~5 times) the percent of early apoptotic cells in culture

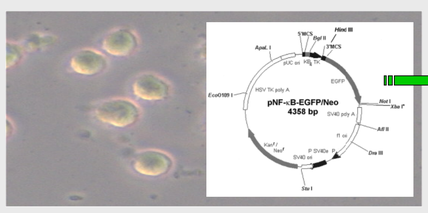
Annexin V-PE staining

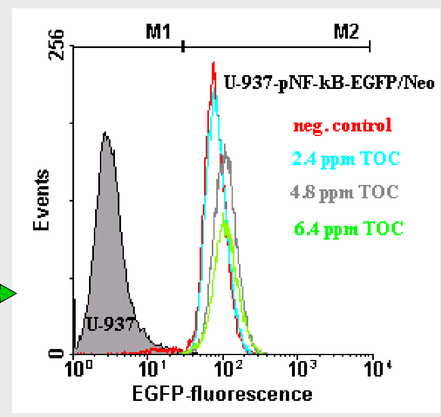
Propidium iodide staining



NF-κB-activation in U-937-pNF-κB-EGFP/Neo

Binding of the endogenous NF-κB to the κB enhancer elements on the plasmid, triggers the expression of downstream positioned reporter gene for green fluorescent protein (EGFP). A 24h incubation with ethylene combustion water (C/O=0.93) activates NF-κB in the U-937-pNF-κB-EGFP/Neo cell line in a dose-dependent manner





CONCLUSION

- Our experiments suggest that the carbonaceous particulate matter could play a pivotal role in diverse pathological effects. Total organic carbon seems to be an appropriate parameter to evaluate the biological effect of atmospheric pollution due to combustion processes.
- Combustion condensates containing organic carbon are cyto- and genotoxic in bacteria and induce NF-κB activation and apoptosis in the human monocytes

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