Innovative Toxicology

TNO | Knowledge for businesss

Marcel van Vugt[1], Mariska Mulderij[1], Birol Usta[1], Gerrit Kadijk[2], Ingeborg Kooter[3], Cyrille Krul[1]*

1 TNO Quality of Life, P.O. Box 360, 3700 AJ Zeist, The Netherlands
2 TNO Science and Industry, Delft, The Netherlands
3 TNO Built Environment and Geosciences, Utrecht, The Netherlands

* P +31 30 694 41 67
F +31 30 694 49 89
Cyrille.Krul@tno.nl

Health effects of biofuel exhaust

Alternatives to fossil fuels receive a lot of attention. In particular, oil derived of specific crops forms a promising fuel. In order to warrant global expectance of such novel fuels, safety issues associated with combustion of these fuels needs to be assessed. Although only a few public reports exist, recently potential toxic effects associated with biofuels has been published. Here, we report the analysis of a comprehensive study, comparing the toxic effects of conventional diesel, biodiesel and blends thereof in various *in vitro* assays. Besides the toxicological evaluation also chemical analysis and the particle size of the emission was measured.

Material and methods

Emission extracts were obtained from European Transient Cycles (ETC) using a Euro3Truck engine. Seven different fuel compositions and once, a closed soot filter was used. Teflon-coated glass filters were extracted using EtOH:DCM (1:1). DCM was subsequently removed by vaporization and samples were adjusted using EtOH. Samples are described below.

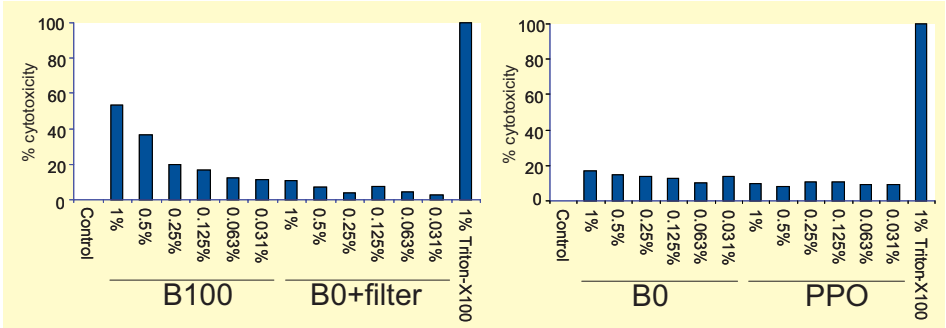
Code	Fuel	Particulate mass (mg)	Volume (m^3)	Concentration (mg/ml)
B0	Diesel[1]	3.3318	3.76	1.666
B5	5% Biodiesel[1]	3.0838	3.90	1.542
B10	10% Biodiesel[1]	3.0083	3.84	1.504
B20	20% Biodiesel[1]	2.4989	3.88	1.249
B100	Biodiesel[2]	1.6334	8.05	0.817
PPO	Pure plant oil[2]	2.8388	7.30	1.419
B0 + soot filter	[2]	0.3427	8.11	0.171
Clean air	-	0.115	4.11	0.058

1) exhaust collected from 3 ETC; 2) exhaust collected from 6 ETC

Results

Cytotoxicity

The Lactate dehydrogenase (LDH) assay was performed with dilution series of emission extracts. Highest concentration was 1% extract in culture medium. Mouse Raw264.7 macrophage cells were exposed to emission extracts for 24 hours. Only pure biodiesel induced a clear dose related cytotoxic response.

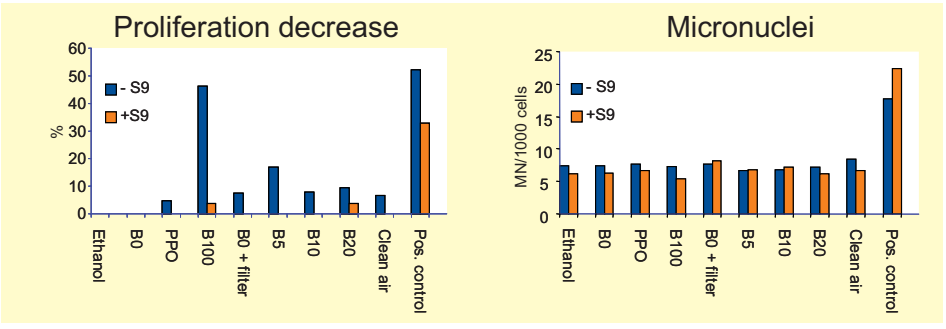


COMET analysis and HO-1 expression analysis

RAW264.7 cells were exposed for 24 hours to emission extracts that yielded a maximum of 20% cytotoxicity or lower. Cells and RNA were isolated. Expression levels of HO-1 were determined by RT-PCR, and DNA fragments were assessed by COMET assay. No significant increases were observed (data not shown).

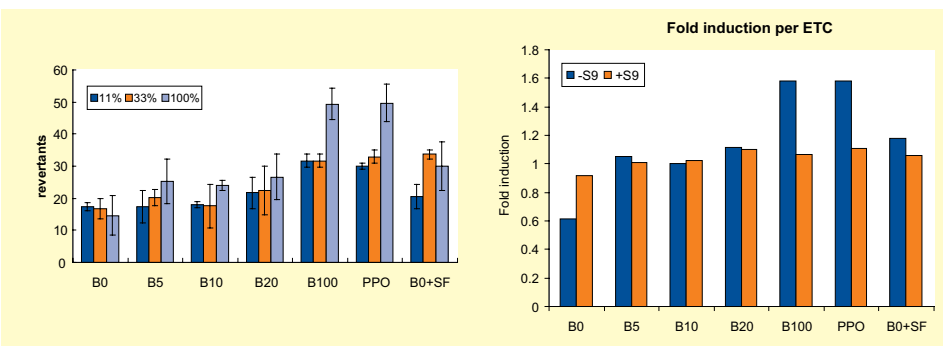
Micronucleus assay

The micronucleus assay was performed to assess irreversible chromosome damage in Raw264.7 macrophages. Cells were incubated with 1% (final concentration) of emission extracts, or positive controls mitomycin C (-S9) or cyclophosphamide (+S9). Cells were exposed for 4 hours, and subsequently cultured for 20 hours in the presence of cytochalasin B. Proliferation indices (left panel) and micronucleated binucleates per 1000 cells (right panel) are indicated.



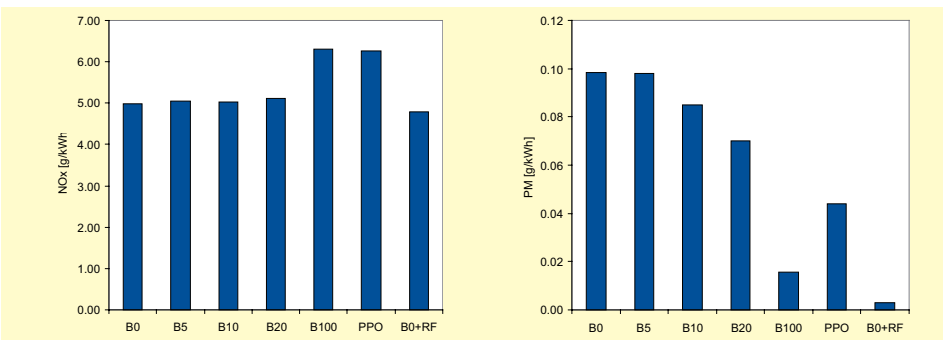
Ames test

Salmonella strains TA98 and YG1024 were exposed to three dilutions of emission extracts. Exposure was conducted in triplicate in the absence and presence of S9 metabolic activation. Absolute numbers of revertants and fold induction compared to per ETC are indicated. Only results of YG1024 are displayed. TA98 showed similar, but less pronounced results.



Emission characterization

Chemical analysis of the emission revealed an increase in nitrogen-oxides (NO_x) and a concurrent decrease in carbonmonoxide (CO) when biofuel was used. Particle size was measured by using the Electrical Low Presser Impactor (ELPI) and biofuel resulted in significant lower levels of PM_{10}. No differences were observed in the total distribution of particulate size exhausted.



Conclusions

- Biofuel resulted in lower amounts of emission per m^3 exhaust.
- However extracts of emission of biofuels increased mutagenicity, decreased proliferation and enhanced cytotoxicity (per unit mass and per ETC). This might be induced by increase NO_x emission.
- Although effects induced by increased NO_x emmission are measured *in vitro*, these observations warrant further investigation of the chemical compounds that might induce the toxic effects, to prevent concern in the future usage of biofuels.
- Current legislation based on mass of PM_{10} emission might not adequately reflect health effects of environmental particulates.

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