METHODS

1. SAMPLING

a) Engine
   Type of engine: diesel Zetor 1505, turbocharged, 4.16 liter, 90kW
   Fuels: on-road diesel (EN 590), 100% biodiesel (FAME)
   Operating modes:
   - engine coupled to dynamometer and operated at steady-state conditions selected to represent different phases of engine operation during the transit traffic congestion:
     - 100 rpm above idle, 2% load (870 rpm, 10 Nm) - corresponds to low-speed "creep"
     - intermediate rpm, 30% load (1500 rpm, 150 Nm) - corresponds to "highway cruise"
     - intermediate rpm, 100% load (1500 rpm, 500 Nm) - corresponds to hill climb / acceleration
   To collect the sample of emission particles in sufficient quantities for various toxicity tests, laboratory operations in each operating mode ran in different time intervals.

b) Sampling equipment
   atmospheric high-volume samplers (EcoTech 3000, 8\"x10\" filters)
   filter: PPTE filter (TX402H120W, Pall)

c) EOM extraction
   1. Extraction by dichloromethane
   2. Evaporation under a stream of nitrogen with 1,2-propanediol as a keeper
   3. Re-dissolution in dimethylsulfoxide (DMSO)

2. TOXICITY TESTS

1) Acellular assays (calf thymus DNA L1 rat liver microsomal 59 fraction)
   - incubation at 37°C for 24 hours
   - DNA adducts (by 32P-postlabelling)
   - 8-oxo-dG

2) Cellular assays (model of human lung epithelial cells - A 549 cells)
   a) Cytotoxicity – test WST-1 cell proliferation test (Roche)
      - doses of 1 and 10 dm³ of undiluted emissions were tested
      - incubation for 24 hours
   b) Genotoxicity – DNA adducts (by 32P-postlabelling)
      - micronucleus test
   c) Oxidative damage - oxidative damage of proteins: carbonyl groups
      - oxidative damage of lipids: 8-isoprostanene

RESULTS

1) ACCELLULAR ASSAYS (ct DNA)
   concentration 10 dm³ emissions/ml
   DNA adducts

   The results suggest that highest genotoxicity is induced by operating mode 1500/500 (deposit burn-off), particularly for diesel. For biodiesel genotoxicity substantially lower.

   No significant DNA oxidative damage was observed for all operating conditions for both diesel and biodiesel.

2) CELLULAR ASSAYS (HUMAN LUNG CELLS A549)
   a) Cytotoxicity

   Significant cytotoxicity was observed for higher dose of 10 dm³ of the undiluted emissions.
   All toxicity tests were performed at the subtoxic dose of 1 dm³.

   b) Genotoxicity
      concentration 1 dm³ emissions/ml culture medium

   Similar to the acellular test, highest genotoxicity (DNA adducts) was detected for engine operating mode 1500/500 (deposit burn-off). Higher DNA adducts were induced by diesel compared to biodiesel.
   No significant genotoxicity was observed by micronucleus test.

   c) Oxidative damage
      concentration 1 dm³ emissions/ml culture medium

   No significant induction of the oxidative damage of proteins and lipids in A549 was observed at any operating mode for diesel and biodiesel.

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