

Toxicity of the Airborne Brake Wear Debris

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ABSTRACT

Particulate air pollution from road traffic currently represents significant environmental and health issue. Attention is also paid to the “non-exhaust pollution sources,” which includes brake wear debris. During each brake application, the airborne and nonairborne particles are emitted into the environment due to wear. High temperatures and pressures on the friction surfaces initiate chemical and morphological changes of the initial components of brake pads and rotating counterparts. Understanding of impact of matter released from brakes on health is vital. Numerous studies clearly demonstrated that particulate matter caused potential adverse effects related to cytotoxicity, oxidative stress, stimulation of proinflammatory factors, and mutagenicity on the cellular level. This paper compiles our main results in the field of genotoxicity, immunotoxicity, and aquatic toxicity of airborne brake wear particles. The brake wear particles were generated using an automotive brake dynamometer. *In vitro* human peripheral blood cell model was used for the genotoxicity and immunotoxicity. Assessment of aquatic toxicity was performed on the green algae *Raphidocelis subcapitata*. Obtained results point to potency of toxicity related to the generated airborne brake wear debris.

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INTRODUCTION

Each year, about 400,000 premature deaths are attributed to the air pollution in the Europe only and the road traffic emissions contribute significantly to this burden [1]. The fine particles play role in adverse health effects and, so far, mainly exhaust emissions and wear of tires were considered to be the major source of the finest particulate matter (PM). However, several recent studies indicated that exhaust and non-exhaust emissions sources contribute almost equally to generation of the finest airborne PM [2, 3, 4]. Non-exhaust emissions are mostly connected with the friction processes which are associated with wear of automotive hardware including brakes, tires, and road surfaces with horizontal traffic marks. The brake wear represents one of the major non-exhaust sources and the brake wear released amounts increased drastically with the recent development of

transportation needs. Although several reviews addressed this important topic recently [5-6], there is no standardized procedure dealing with environmental and health impact testing related to the brake wear debris (BWD) and additional research is required.

This contribution represents our latest results from studies focusing on toxicity of the airborne brake wear debris. Genotoxicity and immunotoxicity assessments were performed using airborne BWD (BWD-1), collected after dynamometer test from a model brake pad formulation rubbed against grey cast iron rotor. Genotoxicity assays indicate on the potential of generated wear debris to modify human DNA, and its damage then usually leads to a series of health challenges. Immunotoxicity tests reflect on the capacity of wear debris to suppress the function of the human immune system, which also has a considerably negative impact on health.

The second part of this paper focused on the assessment of aquatic toxicity. The aquatic toxicity data indicate on the potential of brake wear debris to contaminate water used by humans and other living organisms, including plants. The toxic materials in water could then be further circulated within the food chain. In contrast to the “BWD-1” debris generated from one model sample type, the “BWD-2,” used in aquatic toxicity study, was collected after dynamometer testing performed on ten different commercial brake pad materials rubbed always against grey cast iron. In both experiments, the collected BWD was characterized by Raman microspectroscopy.

EXPERIMENTAL

Friction Testing and Brake Wear Debris Generation

Automotive full scale brake dynamometer model M2800 (Link Engineering) and the AK Master test procedure (SAE J 2522 - Dynamometer Global Brake Effectiveness) were used for generating the airborne BWD. The airborne fraction was collected from filters (KS 85 Klima - Service, a.s., Czech Republic) standardly located in the brake dynamometer exhaust system and by sweeping of the surface of the air flow pipes.

While the collected BWD-1 particles are from one model brake pad only, the BWD-2 contains particles from ten different commercial low metallic brake pads. One could argue that the BWD-2 mixture may probably better represents the wear debris released from the currently available low-metallic pads used in Europe. In contrast to the model pad, the exact formulations of the commercial brake pads are not available.

Model Friction Composite for Automotive Brake Pads

The model brake pads were manufactured at the Southern Illinois University laboratories and they represent a composite with components typical for the majority of brake pads currently used worldwide. Formulation of the model low-metallic polymer matrix composite brake material is presented in Table 1. Although, these pads “passed the BEEP procedure” [7], their formulation is not intended for commercial manufacturing.

Table 1. Formulation of the model brake pad.

Constituent	Weight [%]
Phenolic Resin	24
Coke	15
Steel Fiber	10
Barite	9
Synthetic Graphite	5
Vermiculite	5
Synthetic Graphitic Carbon	5
Nitrile Rubber	5
Copper	4
Iron Powder	3
Tin	3
Magnesium Oxide	3
Stibnite	3
Zircon	2
Twaron	2
Molybdenite	1
Aluminium Oxide	1

Assessment of Immunotoxicity

Phagocytic Activity of Granulocytes and Monocytes and Respiratory Burst of Phagocytes

In the performed *in vitro* experiments with human blood cells, a commonly used range of concentrations were applied. The highest concentration of BWD-1: 75 µg/cm² corresponds to 106 µg/ml, which represents the highest concentration generally proposed for *in vitro* experiments.

One hundred and fifty µl of human heparinized whole blood (diluted 1:1 in RPMI 1640 medium containing 10% FCS) was dispensed into wells of a 96 well microtiter culture plate under sterile conditions. BWD-1 were added in a volume of 25 µl and exposed with cells for 48h. After incubation, 10 µl of hydroethidine solution (Sigma) was added to 30 µl of human heparinized whole blood and incubated for 15 min at 37°C. Three microliters of fluorescein-labelled *Staphylococcus aureus* bacteria (1.4x10⁶ per test) (Molecular Probes) were added to the “test” tubes. Tubes were incubated for 15 min at 37°C. Samples were put on ice and 700 µl of cold lysis solution was added. To the “control” tubes, the *S. aureus* were added after the lysis solution. Samples were analyzed by flow cytometry within 30 min.

Proliferative Activity of Lymphocytes

One hundred and fifty µl of human heparinized whole blood diluted 1:15 in RPMI 1640 (Sigma-Aldrich) medium containing 10% foetal calf serum (FCS, PAA) was dispensed in triplicate wells of a 96 well microtiter culture plate under sterile conditions. Mitogens (Sigma-Aldrich): concanavalin A (Con A) (25 µg/ml), phytohemagglutinin (PHA) (25 µg/ml), pokeweed mitogen (PWM) (2.5 µg/ml), and antigen CD3 (3 µg/ml) (Beckman Coulter) and BWD (0.12 and 75µg/ml) were added in a volume of 25 µl in different exposure intervals (4, 24, 48, and 72h). The plates were incubated at 37°C and 5% CO₂ for 48h; then wells were pulsed with 1 µCi ³H-thymidine (Moravek) diluted in 20 µl medium at 37°C for additional 24 h. After incubation, cell cultures were harvested onto glass filter paper. Filters were placed into scintillation fluid (Perkin Elmer). Radioactivity was measured using a Beta Scintillation counter Microbeta 2 (Perkin Elmer). Calculations: Counts per minute (cpm)/per culture were measured in triplicate for each variable.

Levels of Tumor Necrosis Factor Alpha (TNF-α) in Cell Culture Supernatants

Unstimulated and/or PHA and PWM mitogen stimulated cell cultures were prepared as described above. After 48 hours of exposure to BWD, cell culture supernatants were frozen (-80°C). Levels of cytokine were measured using ELISA kit according to the manufacturer instructions.

Assessment of Genotoxicity

NP Dispersion Protocol

Stock solutions of BWD-1 were prepared fresh each time. Concentrations were selected according to the FP7 NanoTest project [8]. To prepare 1 ml of stock solution, 1 ml of 10% foetal bovine serum (FBS) in PBS was added to 5 mg of BWD NPs in a glass

centrifuge tube. The dispersion was sonicated with a HD 3100 SONOPULS sonicator by BANDELIN electronic (Berlin, Germany) for 15 min at 100 Watt (cycle: 100%). The dispersion was cooled during sonication with an ice/water bath in order to prevent heating of the dispersion. The stock solution was equal to the highest concentration ($75 \mu\text{g}/\text{cm}^2$). Serial dilutions in the dispersion medium were made to obtain the full range (3, 15, and $75 \mu\text{g}/\text{cm}^2$ equivalent to 5.4, 27, and $135 \mu\text{g}/\text{ml}$) of BWD-1 NPs suspensions. BWD-1 NP solutions were added to cultures immediately in a volume of $100 \mu\text{l}$.

Cytokinesis-Block Micronucleus test

Peripheral blood samples from a single donor (non-smoking female, aged 28) with no recent exposures to genotoxicants or ionizing radiation were used. Ethical approval was obtained before the study. Blood was collected by venipuncture from fasted subjects, and aliquoted in heparinised tubes. Cultures were set up in duplicate in two independent experiments by adding 0.5 ml of whole blood to 4.5 ml of RPMI medium with L-glutamine and NaHCO_3 (Sigma) supplemented with 10% or 2% FBS (Sigma), phytohaemagglutinin (Remel, $0.18 \text{ mg}/\text{ml}$) and antibiotics (penicillin and streptomycin, Gibco) in 9 cm^2 flasks. 24 h after seeding (G1/S exposure), cultures were treated with different concentrations of BWD-1 (3, 15, and $75 \mu\text{g}/\text{cm}^2$) 20 h prior to cytochalasin B addition (Sigma, $6 \mu\text{g}/\text{ml}$) and the cultures were incubated for additional 28 hour (48 h treatment total).

After the incubation period, cells were hypotonically treated in 0.075 M KCl (room temperature) and fixed twice with fixative (methanol/glacial acetic acid, 3:1). The fixed cells were immediately dropped onto microscope slides; air dried and stained with 3% Giemsa-solution for 10 minutes. All experiments included treatment of cells with Mitomycin C (MMC, Sigma-Aldrich, $0.05 \mu\text{g}/\text{ml}$) as the positive control and the dispersion medium as the vehicle control.

The frequencies of micronuclei (MN) were determined in 4000 binucleated cells (BNCs) and 2000 mononucleated (MNCs) from two independent cultures. The proportion of mono-, bi- or multinucleated (MTCs) cells was determined in a total 1000 cells and the cytokinesis-block proliferation index (CBPI) was calculated as follows [9]: $\text{no. of MNCs} + 2 \times \text{no. of BNCs} + 3 \times \text{no. of multinucleate cells} / \text{total no. of cells}$.

Statistical Analysis

SPSS 16.0 software was used for statistical analysis. Normality was tested by Shapiro-Wilcoxon's test. To test for significant differences between groups the Independent samples T-test (or Paired-Samples T-test) for normally distributed data, and the Mann-Whitney U-test (or Wilcoxon's test) for non-normally distributed data were used. Differences between three groups were tested by One way analysis of variance (ANOVA) and by Bonferroni's test if equal variances were assumed or by Tamhane's test if equal variances were not assumed. Kruskal-Wallis's test was used for non-normally distributed data. The data were expressed as mean values with standard error of mean (means $\pm$ SEM). Differences at $p < 0.05$ were considered to be statistically significant.

Assessment of Aquatic Toxicity

Aquatic toxicity assessment was evaluated by using green algae *Raphidocelis subcapitata*, which is commonly used as a bioindicator species to assess the levels of nutrients or toxic substances in freshwater environment. The leachates were prepared in accordance with European technical standard EN 12457-2 [10]. BWD-2 was leached in deionized water in continuous rotation container for 24 h. After mixing for 24 h the mixture filtered through a nitrocellulose membrane filter (Pragopor, Pragochema s.r.o.) with an average pore size of $0.45 \mu\text{m}$.

In this study the toxicity assessment was performed in accordance with the OECD 201 methodology, i.e. monitoring the growth inhibition of organisms (*Raphidocelis subcapitata*) after 72-h exposure to the tested material [11]. For the evaluation of the parameter EC_{50} the following concentration series: 100, 50, 30, 10, and $5 \text{ g}/\text{l}$ were used. The highest concentration was chosen to compile solid to liquid ration 1:10 according to the European standard EN 12457-2 [10].

Data obtained for exposed samples were compared with the control samples and are given as percentage of algae growth inhibition or stimulation. To determine the acute aquatic toxicity of the aqueous leachates of the studied samples it was necessary to adjust pH value to be within the physiological range of 8.1 ± 0.2 for the given species. The concentration series and control samples were inoculated with the same volume of the algae suspension to achieve the cell concentration of $10,000 \text{ cells per cm}^3$. Thus the prepared test samples were cultivated ensuring constant temperature and light conditions for 72 h. Algal cell density was measured by using Olympus CX31 light microscope and Bürker counting chamber.

Raman Microspectroscopy

Both collected airborne BWD were characterized by Smart Raman Microscopy System XploRA™ (HORIBA Jobin Yvon, France). Raman spectra were acquired with 532 nm (20 - 25 mW) excitation laser source, and 1200 grooves/mm grating in the range $100 - 4000 \text{ cm}^{-1}$. The intensity of the laser beam was changed based on the sample signal from 1 to 10% of initial laser beam. As the Raman system is coupled with a microscope, measurements were done with a 50x objective of the optical microscope Olympus BX41/51. Detection of Raman radiation is carried out by 1,024 pixel $1''$ CCD chip, which is high sensitive and air-cooled. Spectra were corrected in the software LabSpec and compared with coupled Raman spectral library. Raman microspectroscopy was chosen due to possibility of measuring individual regions of interest, which is necessary for multicomponent heterogeneous samples.

RESULTS AND DISCUSSION

The mechanism of phagocytosis represents a part of the innate immune response, essential for the protection of the organism. Phagocytosis is a major mechanism used to remove pathogens and cell debris from tissues. The failure of this activity results in recurrent and chronic bacterial and fungal infections. Phagocytic cells also play important role in removal of BWD from the blood. Neutrophil granulocytes and monocytes are both "professional" phagocytes.