Supporting Information

Brake wear (nano)particle characterization and toxicity on airway epithelial cells *in vitro*

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Supporting Information Figure S1 – Size distribution from BWP found on commercial transport vehicles



Figure S1: Distribution of particle detected by optical microscopy obtained from a motor vehicle testing center operating on light duty vehicles. The fitting curve is given by a log-normal function.

Supporting Information Figure S2 – Characterization of brake wear powder



Figure S2: Characterization of brake wear powder originating from an automotive brake dynamometer

a) Brake wear powder sample obtained from an automotive brake dynamometer for pad and drum testing. BWP composition was characterized using thermogravimetry analysis (b) and X-ray fluorescence for elemental analysis (d). Note that for the sample coming from the motor vehicle testing center, the iron and copper proportions were 29% and 17% respectively, whereas the values here are 43 and 34 %. Moreover, this brake pad formulation contained Sb and Mo, most probably in form of sulfides (since content of S is 3.9%). Content of Ba (0.2%) suggests that Ba was not used as a filler. To evaluate the size and morphology of the wear particles optical (c) and scanning electron (e) microscopy were performed. The insets in c) are $24 \times 40 \ \mu m^2$ fields.

Supporting Information Figure S3 – Size distribution from BWP collected from an automotive brake dynamometer



Figure S3: Distribution of particle detected by optical microscopy obtained from an automotive brake dynamometer for pad and drum testing. The fitting curve is given by a log-normal function.

Supporting Information Figure S4 – Light scattering data for BWP dispersed and sonicated with proteins



Figure S4: a) Scattering intensity and b) hydrodynamic diameter measured by light scattering of BWP particles dispersed in a 1 g L⁻¹ BSA solution as a function of the sonication delivered sonication energy *DSE*. In the configuration used, a one-minute sonication translates into a total input energy of 30 J mL⁻¹.

Supporting Information Figure S5 – Dynamic light scattering in cell culture medium (EMEM)



Figure S5: Intensity distributions for the BWP nano-sized fraction in DI-water (a) and in cell culture medium (EMEM) at 10 min (b) and 24 h (c). The maxima at 24 h are at 170 nm, 320 nm and 1000 nm.

Supporting Information Figure S6 – Complementary TEM images and EDX spectra from brake wear nanoparticles



gure S6: a-f) EDX spectra from different brake wear nanoparticles showing that elemental composition underlines the presence of only one type of atoms per particle, indicating that the particles are generated from different parts of the braking component. Examples of particles containing iron (a), silicon (b), zirconium (c), tin (d), bismuth (e) and titanium (f) are provided.

Supporting Information Figure S7 - Reactive oxygen species production in Calu-3 cells



Figure S7: Reactive oxygen species production was detected by DCFH probe fluorescence in Calu-3 cells exposed for 4 h to BWP from 1 to 100 μ g cm⁻². H₂O₂ and TBHQ were used as positive controls

Supporting Information Figure S8 – IL-6 and TNF α release by Calu-3 cells exposed to BWP or its nanosize fraction



Figure S8: IL-8 and TNF α cytokine release by Calu-3 cells exposed for 24 h to BWP and its nanosize fraction at different concentrations. Cytokine measured by ELISA was used to characterize the pro-inflammatory response after a 24 h exposure. Statistical analysis was performed using One-way ANOVA and Dunett's post-test to compare particles effects with no exposure. The symbols (#) and (°) refer to the BWP and to its nanosize fraction respectively. Two-way ANOVA complemented by Holm-Sidak post-test was performed to compare the BWP size effects (*).

Supporting Information Figure S9 - Cell viability of Calu-3 cells exposed either to particles or the soluble fraction



Figure S9: Cell viability of Calu-3 cells exposed for 24h to different concentrations of BWP, their nanosize fraction and the corresponding soluble fraction. Mitochondrial activity reflecting cell viability was assessed using WST-1 assay. The soluble fraction was the supernatant recovered after BWP sonication, filtration and centrifugation.

Supporting Information Figure S10 – Application of the in vitro sedimentation, diffusion and dosimetry model to the brake wear nano- and microparticles



Figure S10A: Table - List of input parameters injected in the in vitro sedimentation, diffusion and dosimetry model reported by DeLoid and coworkers (DeLoid G.M. et al. Nat. Protoc. 2017, 12, 355-371) for 1.5 µm brake wear particles. The size distribution refers to the data from Fig. S1. **a**) The fraction of deposited particles as a function of the time. It was found that after 45 min of incubation the dose delivered to the cells saturates at 79% of the total administered dose. **b**) Bottom concentration as a function of the time. The initial concentration in the dispersion was set at 0.1 g L⁻¹ (corresponding to 1 mg cm⁻³, see Table). **c and d**) Time evolution of the concentration as a function of the cell culture well. The kinetics shows that the sedimentation occurs rapidly, the concentration profiles stabilizing after 45 min. Note that in d) the abscissa has been expended from 2.99 to 3 mm to emphasize that most particles are at the bottom of the well.



Figure S10B: Table - List of input parameters injected in the in vitro sedimentation, diffusion and dosimetry model reported by DeLoid and coworkers (DeLoid G.M. et al. Nat. Protoc. 2017, 12, 355-371) for the brake wear nano-sized fraction. The size distribution refers to the light scattering data in Fig. S5c. **a)** The fraction of deposited particles as a function of the time shows a saturation around 7% of the total administered dose after 24 h. **b)** Bottom concentration as a function of the time. The initial concentration in the dispersion was set at 0.1 g L⁻¹ (corresponding to 1 mg cm⁻³). **c and d)** Time evolution of the concentration as a function of the position in the different colored curves correspond to different snapshots of gradient concentration.

Supporting Information Figure S11 – Rescaling toxicity assays with the effective dose



Figure S11: a) Cell viability of BWP particles corrected from the soluble fraction. b) Mitochondrial activity of brake wear nano- and macroparticles expressed as a function of the effective dose. c) Reactive oxygen species production detected by DCFH probe oxidation after 4 h exposure *versus* effective concentration for BWP and its nano-sized fraction. d) Same as in c) for the IL-6 cytokine release measured by ELISA after a 24 h exposure.