Sørli et al.:

An *In Vitro* Method for Predicting Inhalation Toxicity of Impregnation Spray Products

Supplementary Data

Supplementary methods

Mass spectrometric analysis protocol

TSPE-GCMS: 1 μl of raw product was injected into a stainless steel tube containing Tenax TA adsorbent. The tube was, after purging with He for 5.0 min, analyzed on a Perkin Elmer Turbo Matrix 350 thermal desorber (TD) coupled to a Bruker SCION TQ GC-MS system (Bruker Daltonics, Bremen, DE). Tube desorption was carried out at 275°C for 20 min and the low and high temperatures of the cryo trap were -20°C and 280°C, respectively. The GC column was a 30 m x 0.25 mm with 0.25 μm film thickness; VF-5MS (Agilent Technologies, Santa Clara, US). The oven program was as follows: 50°C for 4 min, ramp 1: 4°C/min to 120°C, ramp 2: 50°C/min to 250°C hold

Tab. S1: Number of mice used to assess the acute effects on respiration of the impregnation products

Product	No. of mice and type of *in vivo* tests	Total no. of mice	Power calculation[C] *n, Difference, SD, power*
"Footwear protector"	Screening test with 4 mice 2 concentrations of 8 mice/groups	20	8, 51, 20, **1.0**
"Wood impregnation"	Screening test with 4 mice 3 concentrations of 6, 8 and 8 mice/groups	26	6, 50, 20, **0.97**
"Car glass"	Screening test with 4 mice 8 mice exposed to maximal concentration possible to generate	12	
"Bath and tiles"	2 concentrations of 10 and 20 mice/group[A]	30	
"Textiles and leather"	Screening test with 4 mice 5 mice exposed to maximal concentration possible to generate	9	
"Special textile coating"	5 mice exposed to maximal concentration possible to generate[B] 6 mice exposed to maximal concentration possible to generate (repetition)	11	
"Textiles and leather concentrate"	mice exposed to maximal concentration possible to generate[B]	5	
"Non-absorbing floor materials" (POTS)	5 concentrations with 7, 9, 10 and 1 9 mice/group[A]	45	
"Rim sealer"	3 concentrations of 6 mice/group	18	6, 47, 5, **1.0**

[A] Data from Nørgaard et al., 2010

[B] Data from Nørgaard et al., 2014

[C] Power calculation performed by comparing mean Recovery period difference between highest and lowest concentration tested, using SD from highest concentration group and assuming $p<0.05$ for a 2-sided hypothesis. Calculations were only performed for already unpublished experiments, where the product caused adverse effects.

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for 2 min. Helium was used as carrier gas at an inlet pressure of 0.97 bar (1.5 ml/min). The mass spectrometer was operated in scan mode using electron or chemical ionization (methane at 1.4 bar inlet pressure). Valves, transfer lines and ion source were kept at 270°C. Collision induced dissociation (CID) was carried out using argon as collision gas and collision energies (CE) from 8-24 eV.

LTP-MS: 5 μl of raw product was applied to filter paper or Teflon and placed in front of the inlet of a Bruker micrOTOF-Q mass spectrometer. An in-house built LTP probe was used for ionization and measurements were carried out in both the positive and negative ion mode with a potential of 1.5-2 kV voltage applied to the MS inlet capillary. The flow rate and temperature of the dry gas were 2 l/min and 250°C, respectively. CID was carried out in a CE range of 2 to 30 eV. An ESI calibration solution containing fluoroalkyl phosphazines (Agilent tune mix) was used for external calibration resulting in mass errors for most assigned formulae of ± 5 mDa.

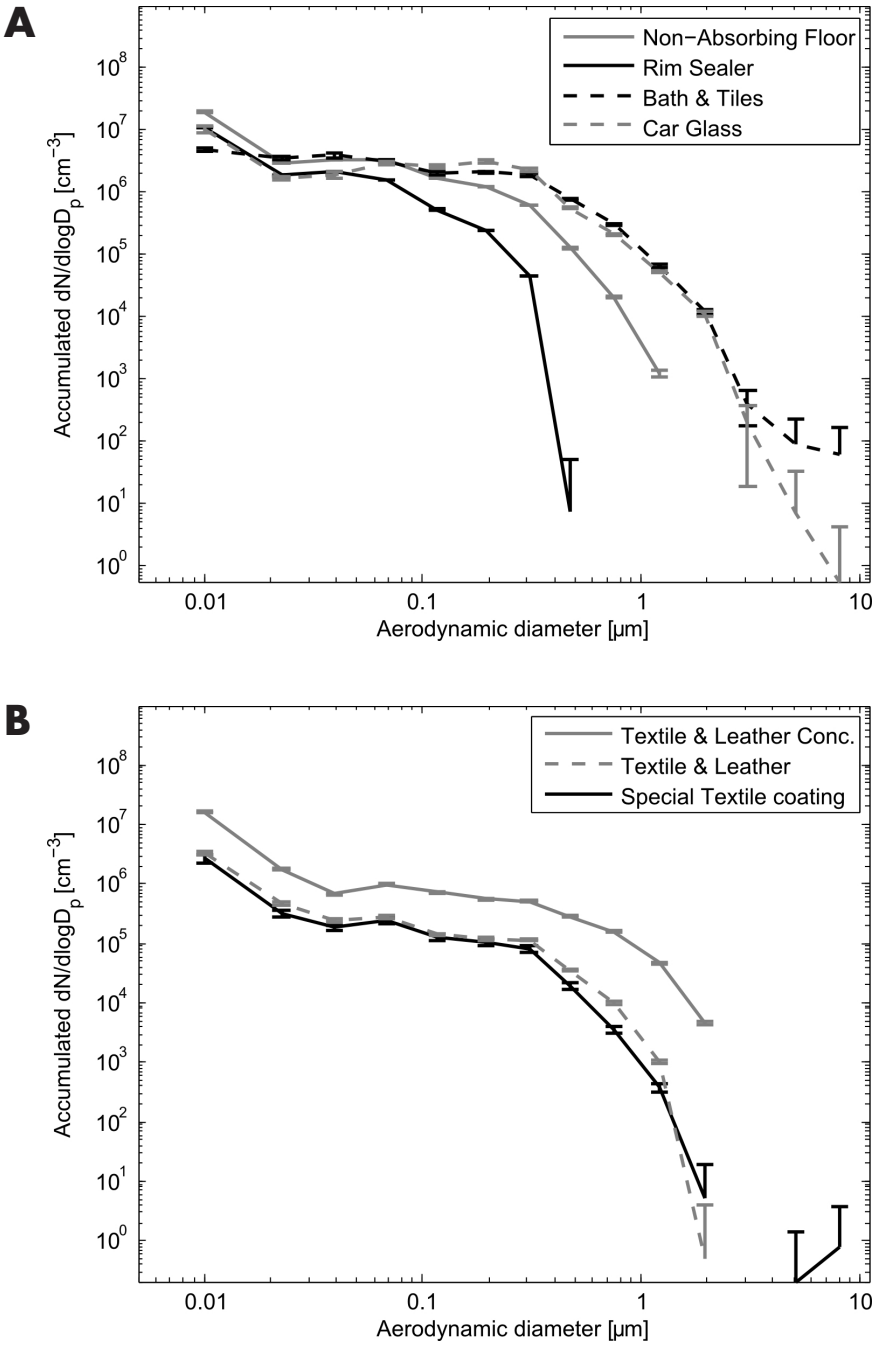
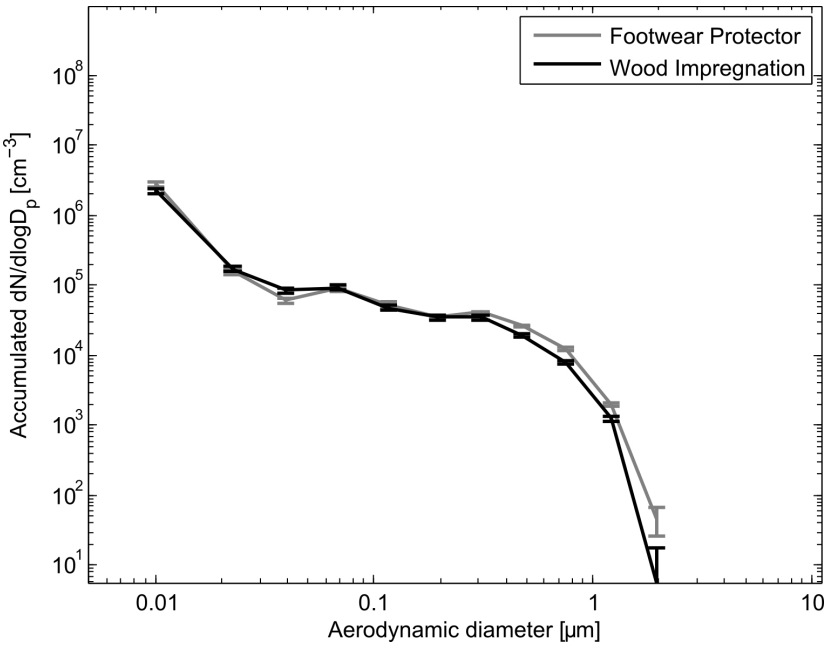


Fig. S1: Particle size distributions of aerosolized products as measured by ELPI

A-C show the particle size distribution of the aerosolized products as measured with ELPI inside of the mouse exposure chamber. All distributions have the main modes of particles within the respirable fraction and clear similarities in size and concentration can be seen for products of the same active compound. Differences in concentration level occur for different concentrations of active compound, with a higher concentration giving a higher particle number concentration.

C


Footwear Protector
Wood Impregnation
Accumulated dN/dlogD$_p$ [cm^{-3}]
10^8
10^7
10^6
10^5
10^4
10^3
10^2
10^1
0.01
0.1
1
10
Aerodynamic diameter [µm]