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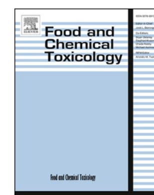

Risk assessment of consumer spray products using *in vitro* lung surfactant function inhibition, exposure modelling and chemical analysis

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ABSTRACT

Consumer spray products release aerosols that can potentially be inhaled and reach the deep parts of the lungs. A thin layer of liquid, containing a mixture of proteins and lipids known as lung surfactant, coats the alveoli. Inhibition of lung surfactant function can lead to acute loss of lung function. We focused on two groups of spray products; 8 cleaning and 13 impregnation products, and in the context of risk assessment, used an *in vitro* method for assessing inhibition of lung surfactant function. Original spray-cans were used to generate aerosols to measure aerodynamic particle size distribution. We recreated a real-life exposure scenario to estimate the alveolar deposited dose. Most impregnation products inhibited lung surfactant function at the lowest aerosolization rate, whereas only two cleaning products inhibited function at the highest rates. We used inhibitory dose and estimated alveolar deposition to calculate the margin of safety (MoS). The MoS for the inhibitory products was ≤1 for the impregnation products, while much larger for the cleaning products (>880). This risk assessment focused on the risk of lung surfactant function disruption and provides knowledge on an endpoint of lung toxicity that is not investigated by the currently available OECD test guidelines.

1. Introduction

The project included a pre-phase, where the market was analyzed both for impregnation- and cleaning products for overview of availability and use (Fig. 1). More than 200 products were identified. A subset of these products were then chosen for further study. These products underwent hazard assessment, by analyzing the products' chemical contents, estimating potential exposure during normal use and assessing effect on lung surfactant function *in vitro*. The data then fed into risk assessment, for derivation of a margin of safety (MoS) for use of the products.

Spray application of liquids is an efficient way to spread products evenly on large surface areas. This property is often utilized in the two product categories studied here: impregnation and cleaning products. However, spraying creates a cloud of aerosols in the breathing zone of the person using the product. This may pose a health risk, as small particles can be inhaled and penetrate deep into and damage the lungs. The human airways are efficient filters, and sequester a large proportion of the particulates in inhaled air, however, aerosols with an aerodynamic diameter between 0.005 and 0.5 μm primarily deposit in the alveoli ("Human respiratory tract model for radiological protection. A report of a Task Group of the International Commission on Radiological Protection," 1994). Many spray devices generate aerosols in this size range, therefore, using these products can potentially lead to aerosols depositing deep in the lungs - in the alveoli, where they can come into contact with the lung surfactant.

The alveoli are covered by a thin layer of liquid that contains lung surfactant. Lung surfactant is produced by the type II alveolar cells and consists of a complex mixture of 90% lipids (mostly phospholipids, with some neutral lipids -primarily cholesterol) and 10% surfactant proteins (Perez-Gil and Weaver, 2010; Zuo et al., 2008). It is released into the liquid covering the alveoli, and forms a film at the alveolar air-liquid interface that drastically reduces the surface tension and facilitates

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Abbreviations

6:2-FTMA	perfluorohexylethylmethacrylate
6:2-FTOH	perfluorohexylethyl alcohol
AOP	adverse outcome pathway
CDS	constrained drop surfactometer
DEHP	bis(2-ethylhexyl)phthalate
ELPI	Electrical Low Pressure Impactor
FF	far field
GC/MS	Gas Chromatography Mass Spectrometry
LC/MS	Liquid Chromatography Mass Spectrometry
MDF	medium density fiberboard
MoS	Margin of Safety
MSDS/SDS	material data safety sheet/safety data sheet
NAM	new approach methodology
NF	near field
NMR	Nuclear Magnetic Resonance
OECD	Organisation for Economic Co-operation and Development
OEL:	occupational exposure limit
PFAS	per- and polyfluoroalkyl substances
PFBA	perfluorobutanoic acid
PFCA	perfluorocarboxylic acid
PFHpA	perfluoroheptanoic acid
PFHxA	perfluorohexanoic acid
PFPeA	perfluoropentanoic acid
POD	point of departure
QCM	quartz crystal microbalance
SRER	size resolved emission rates

breathing. During expiration, the alveoli contract and reduce their surface area. To prevent alveolar collapse, lung surfactant reduces the surface tension at the air-liquid interface to near-zero values at the end of expiration. This is done by rearranging the surfactant components, so that the surface becomes enriched in molecules with higher surface activity (Keating et al., 2012; Possmayer et al., 2012). During inhalation, the alveoli are filled with air and their surface area increases. The surfactant film at the air-liquid interface is then quickly replenished with the less surface active components from the reservoirs, and the surface tension returns to equilibrium values. These continuous regulation mechanisms ensure effortless breathing.

The function of lung surfactant can be measured *in vitro* in the constrained drop surfactometer (CDS). In the CDS, the volume of a drop of lung surfactant is increased and decreased at a certain frequency and extent that correspond to the changes taking place in the lungs during breathing. The surface tension of the drop of lung surfactant can be measured as the drop is exposed to a test product or chemical. If the test product inhibits the function of the lung surfactant, the surface tension will no longer decrease sufficiently during compression. We have previously described how surfactant function inhibition leads to decreased lung function in an adverse outcome pathway (AOP) ((Da Silva, Vogel, et al., 2021) and https://aopwiki.org/aops/302). This AOP describes how inhibition of lung surfactant function can lead to alveolar collapse because of the resulting high surface tension at the end of expiration. Upon reopening of the collapsed alveoli, the alveolar-capillary membrane integrity can be damaged due to shear stress on the cells covering the alveoli, causing bleeding into the lungs. Alternatively the collapsed alveoli can remain closed resulting in reduced lung volume and hypoxemia. The combination of events initiated by inhibition of lung surfactant function can lead to decreased lung function (Da Silva, Vogel, et al., 2021).

Both impregnation and cleaning products may be applied as sprays; however, there is a vast difference in the respiratory toxicity and representation of symptoms after inhalation. Every year, impregnation products result in cases of acute illness where immediate medical attention is reported (Burkhart, Britt, Petrini, O'Donnell and Donovan, 1996; CDC, 1993; Daubert et al., 2009; Duch et al., 2014; Laliberte et al., 1995; Sørli et al., 2018; Vernez et al., 2006). By contrast, for cleaning products formulated as sprays, reports associated with acute symptoms are rare, although their continual use have been suspected to have long-term effects on the lungs (Hadrup et al., 2021); and professional cleaners have more self-reported respiratory symptoms or asthma than the general population (Clausen et al., 2020).

In this project, we aimed to further study the acute lung toxicity of consumer spray products using the non-animal method of lung surfactant inhibition *in vitro*. We analyzed 21 products. All were available on the Danish market; however, most have international distributors and are sold in many other countries as well. The analyzed products included 13 impregnation products for various surfaces, and 8 cleaning and disinfection products. We tested if the products could inhibit lung surfactant function *in vitro* in the CDS. To improve the understanding as to which ingredient chemicals caused the inhibition, we further tested eight different solvents that were present in many of the products. To simulate exposure to the products, we generated aerosols using the original spray device and used the data to estimate the lung deposited dose after use. These data were combined to estimate a MoS in the context of risk assessment of the products.

2. Materials and methods

A review of the Danish market for impregnation products identified more than 100 products; and products for further analysis were chosen

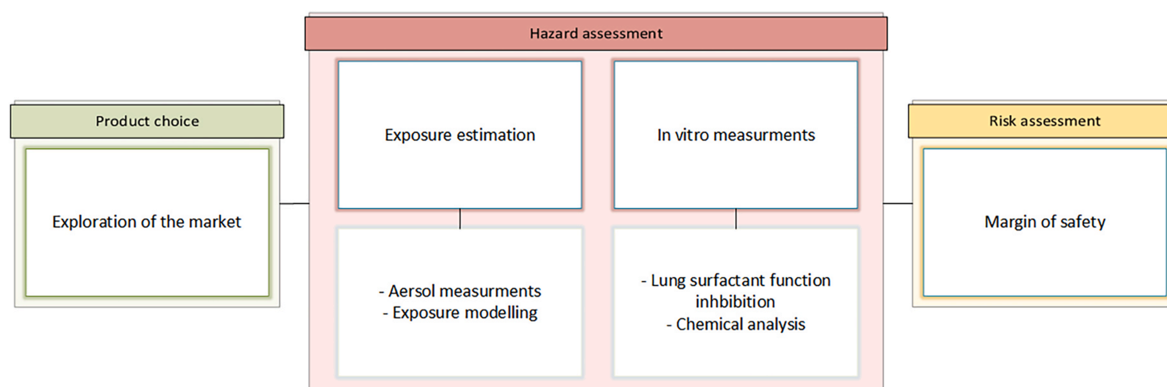

Fig. 1. Outline of the project.

following three criteria: 1) tonnage sold on the Danish market, 2) application method, and 3) involvement in human intoxication cases. The third point was included as we have previously been able to identify toxic products as we have received them directly form injured persons or health professionals (Duch et al., 2014; Sørli et al., 2018). Yet in the present study none of the products could be linked to such cases, thus, the products were chosen on tonnage and mode of application. As pressurized cans have frequently been associated with human cases of intoxication, these were prioritized for testing over other methods of application. Notably, two products initially included in the project damaged the polycarbonate of the exposure chamber and were not tested. We analyzed 13 impregnation products, most of which were packaged in pressurized cans (11 of 13) (Table 1). The last two impregnation products and all eight cleaning products were sold in trigger spray bottles, i.e. bottles where you have to push a lever to generate the spray. The cleaning products were chosen from a large database compiled after surveying the Danish market (Clausen et al., 2020). The number of products was narrowed down by choosing a subset based on how they were applied, tonnage sold on the Danish market, and chemicals with suspected involvement in airway disease (Hadrup et al., 2021). Several cleaning products were not included in the current project as they destroyed the polycarbonate, or did not produce adequate aerosols.

Table 1
Products and control solvents included in the project.

Abbreviation	Category	Function	Application
A	Impregnation	Multi-purpose	Pressurized can
B	Impregnation	Multi-purpose	Pressurized can
C	Impregnation	Multi-purpose	Pressurized can
D	Impregnation	Multi-purpose	Pressurized can
E	Impregnation	Multi-purpose	Pressurized can
F	Impregnation	Shoes	Pressurized can
G	Impregnation	Ski wax	Pressurized can
H	Impregnation	Outdoor equipment	Pressurized can
I	Impregnation	Outdoor equipment	Pressurized can
J	Impregnation	Outdoor equipment	Pressurized can
K	Impregnation	Leather furniture	Pressurized can
L	Impregnation	Indoor hard surfaces	Trigger spray
M*	Impregnation	Non-absorbing floor materials	Trigger spray
N	Cleaning	Glass and multipurpose cleaner	Trigger spray
O	Cleaning	Glass surfaces	Trigger spray
P	Cleaning	Glass surfaces	Trigger spray
Q	Cleaning	Mold removal	Trigger spray
R	Cleaning	Lime removal	Trigger spray
S	Cleaning	Disinfection	Trigger spray
T	Cleaning	Heavy-duty multi-purpose cleaner	Trigger spray
U	Cleaning	Glass and multi-purpose cleaner	Trigger spray
H_2O	Control solvent	Water	-
pH2	Control solvent	Water adjusted to pH 2	-
pH11	Control solvent	Water adjusted to pH 11	-
NaCl	Control solvent	0.9% NaCl	-
EtOH	Control solvent	96% ethanol	-
Glycerol	Control solvent	10% glycerol in water	-
HexDec	Control solvent	10:1 n-hexane: n-decane	-
DecHex	Control solvent	10:1 n-decane: n-hexane	-

* M has been studied extensively earlier, it contains fluorosilane in 2-propanol (Nørgaard et al., 2010). This product is now restricted for sale to private consumers according to Annex XVII, entry 73 of REACH (European Commision, 2019) due to content of "TDFA ((3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl) silanetriol and any of its mono-, di- or tri-O-(alkyl) derivatives) in an organic solvent". It has in earlier publications been named "NFP1" (nanofilm product 1), "POTS" (1H,1H,2H,2H-Perfluorooctyltriethoxysilane) and "non-absorbing floor materials") (Nørgaard et al., 2014; Nørgaard et al., 2010; Sørli et al., 2018).

All products were purchased online or in physical shops. In addition, eight solvent controls were analyzed (Table 1). To test for the effect of pH in the cleaning products, we tested water, and water adjusted to either pH 2 or 11. Salt, alcohol and glycerol are common in cleaning products and therefore the effect of 0.9% NaCl, 96% ethanol, and 10% glycerol in water was tested. Two mixtures of n-hexane and n-decane were used to represent common solvents used in the impregnation products.

2.1. Aerosol generation for the in vitro effect on lung surfactant function

All aerosols were generated by filling a syringe with the test liquid and then passing the liquid into an aerosol generator. Products in pressurized cans were first sprayed into a glass vial, otherwise the liquid was drawn directly into the syringe from the bottle. The product was led from the glass syringe by an infusion pump (Legato 100, Buch & Holm A/S, Denmark) into a Pitt no. 1 jet nebulizer (Wong and Alarie, 1982) via plastic tubing. In the jet nebulizer, the product was aerosolized by pressurized air. The aerosol was led from the nebulizer through glass tubing into the 1.9 L exposure chamber. The bottom of the chamber has hollow channels where air is sucked out of the chamber via holes. This air was passed through a HEPA filter, before exhausting into the atmosphere.

2.2. Lung surfactant function measurements and determination of inhibitory dose

A droplet of lung surfactant, 10 μL of 2.5 mg/mL Curosurf (Chiesi, Parma, Italy), in a buffer containing 0.9% NaCl, 1.5 mM $CaCl_2$, and 2.5 mM HEPES, adjusted to pH 7.0, was placed on a hollow pedestal. Curosurf is made from solvent extracted minced porcine lung tissue and contains ~99% w/w phospholipids and 1% w/w hydrophobic surfactant-associated proteins (SP-B and SP-C) (Zhang et al., 2011). Curosurf only contains the solvent extracted constituents of lung surfactant, and lacks some components found in natural lung surfactant, however it has the advantage that it is commercially available. The pedestal was connected to a motorized syringe pump that adds and removes liquid from the droplet at a defined volume and frequency. The droplet was cycled with a change of surface area of 26.5 ± 4.6% and at 3-s cycles to simulate breathing lungs. During the experiment, a camera took five pictures per second of the backlit drop. The ADSA (axisymmetric drop shape analysis) software (Yu et al., 2016) was used to analyze the pictures to calculate the surface tension of the droplet. The pressurized air in the nebulizer and the exposure chamber were heated, and the temperature inside the exposure chamber was monitored using the TinyTag Plus 2 data logger (TGP-4017, Gemini Data Loggers Ltd, United Kingdom). We aimed at heating the chamber to 37 °C, however the setup was sensitive to the outside and room temperature, and the mean temperature in the experiments was 32.2 ± 4.6 °C. The temperature at each day of extermination was stable. A quartz crystal microbalance (QCM, Vitrocell, Waldkirch, Germany) was positioned close to the cycling lung surfactant droplet. The QCM measurements were used to estimate the dose of each product that inhibited lung surfactant function. Lung surfactant function was defined as being inhibited if at

least 3 consecutive minima in surface tension were larger than 10 mN/m. The time of inhibition i.e., the first minimum above 10 mN/m, was combined with data from the QCM to estimate the inhibitory dose. At the time of inhibition the deposited mass on the QCM was recorded and converted to the mass deposited on the lung surfactant droplet by multiplying with the average surface area of the drop throughout the experiments (0.18 cm^2).

2.3. Impregnation product exposure

The impregnation products were tested at the lowest infusion rate that resulted in a reproducible deposition on the QCM, this was 0.1 mL/min. Below this rate no deposition could be measured on the QCM. Notably, some impregnation products caused instant inhibition of lung surfactant function at this QCM rate and the inhibitory dose was likely lower than what could be measured in the experimental setup. The experiments followed the setup: 40 s of baseline was followed by 5 min exposure. After each exposure, air was passed through the chamber to flush out the aerosol of the product. The experiments were repeated 3–7 times. If there was no inhibition at the infusion rate of 0.1 mL/min, the experiment was carried out with a higher infusion rate, until inhibition was observed or until the maximum infusion rate that could be aerosolized was reached. Two of the impregnation products did not inhibit lung surfactant function at low infusion rate and were tested at maximum infusion rate (0.5 or 1 mL/min; F and L, respectively). The solvent controls HexDec and DecHex were tested at 0.1 mL/min infusion rate as described above.

2.4. Cleaning product exposure

The cleaning products were all tested at an infusion rate of 0.5 and 1 mL/min, the products could not be tested at higher infusion rates since the nebulizer could not keep up with aerosolizing the liquid. As we expected the cleaning products to be less inhibitory than the impregnation products, we chose a start infusion rate of 0.5 mL/min. At both infusion rates, the experiment followed the setup: 30 s of baseline was followed by 5 min exposure and 5 min of no exposure. The experiments were repeated 5 times (at infusion rate 0.5 mL/min) or 3–5 times at infusion rate 1 mL/min. The solvent controls of deionized water, 0.9% NaCl and 96% ethanol were tested at an infusion rate of 1 mL/min, whereas water adjusted to pH 2 by adding HCl, water adjusted to pH 11 by adding NaOH, and 10% glycerol in water were tested at an infusion rate of 0.5 mL/min.

2.5. Exposure estimation and modelling

2.5.1. Aerosol measurements for products in small-scale exposure chamber

The release of aerosol particles during the use of impregnation and cleaning products from the original aerosol device was investigated to assess the potential for exposure in humans. Aerosol number concentrations and particle size distributions of particles between 6 nm and 10 μm were measured using an Electrical Low Pressure Impactor (ELPI, HR-ELPI+, Dekati, Finland) in 14 channels with a 1 s time resolution. A small-scale chamber 0.8 m × 0.96 m x 0.71 m (h x w x d) with a total volume of 0.55 m^3 was used (Fig. 2). Two 9-V computer fans were placed in the chamber to ensure efficient mixing of air and particles during the experiments. The fans contributed with a respirable particle mass concentration of 0.0051 mg/m^3, and this was subtracted from the spray activity concentrations along with chamber background concentrations. An external pump provided additional flow out of the chamber with the replacement air entering through an inlet attached to a HEPA filter. The total air flow out of the chamber by the pump and ELPI was 38.5 L/min, resulting in an air exchange rate of 4.2 h^{-1}.

Prior to spraying, unused pressurized cans and trigger sprays were shaken thoroughly for 30 s. The spray activity was conducted by spraying continuously for 30 s inside the chamber for the pressurized cans or by a series of pumps on the lever for the trigger sprays at a frequency of 60 pumps in 30 s. The spray was directed towards a thin piece of medium density fiberboard (MDF) set up at the end of the wall to simulate spraying a surface as impact of particles on a surface removes particles from aerosol dispersion. The distance from the spray point to the MDF was 50–60 cm.

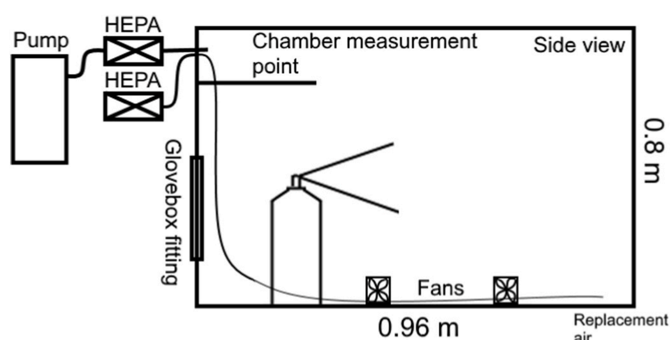

Fig. 2. Conceptual drawing of the small-scale chamber experimental setup.

The resulting aerosol chamber concentrations were used to calculate the size resolved emission rates (SRER) calculated according to Ott et al. (Ott et al., 2006) under the assumption of a fully mixed system (Eq. (1)), as well as respirable mass emission rates.

$$ER_i = \frac{V_{tot} \cdot (C_{m,i} - C_{0,i}) \cdot \exp(-\gamma_i \cdot (t_m - t_0))}{t_m - t_0}$$

Equation (1): Where, V_{tot} is the total volume of the chamber, $C_{m,i}$ is the maximum concentration of the ith bin, t_m is the time at C_m, C_0 is the respirable concentration at the start of the experiment, and t_0 is the time at the start of the experiment. The loss rate, γ, was calculated for each experiment by fitting a first order exponential decay function to the concentration in each size bin. For conversion into respirable mass emission rates the size bins of the ELPI with a geometric mean D50 diameter of ≤4.4 μm were used. For the conversion from number concentration to mass concentration, spherical particles with a density of 1 g/cm^3 were assumed. The midpoint of each bin was used for this conversion.

2.5.2. Modelling of exposure

The exposure model used in this work was based on a previously published population balance model (Jensen et al., 2018). The modelled scenario with contextual parameters was based on a described exposure case and used to assess room concentrations resulting from the use of each product. The computational domain was defined as a two-box, near field/far field (NF/FF) setup. The calculated SRER from each product was used as source input in the exposure model.

The modelled exposure scenario consisted of a 36 m^3 room with a defined NF volume of 1.2 m × 1.2 m x 1.2 m (h x w x l) centered around the source at all times. An air exchange rate of 1.5 h^{-1}, corresponding to multiple open windows (Howard-Reed et al., 2002) was used based on the description of the exposure case. Spray time was defined to be 30 min. The total modelled exposure duration was 120 min, this represents a person performing the spraying activity in the room and remaining inside the room for another 90 min after the spraying activity has ended.

The average particle number concentrations (particles/cm^3) in the NF, as well as the deposited alveolar dose were calculated from the exposure scenario according to eq (2).

$$DD_{alv} = \sum_i D_{f,alv,i} \cdot t \cdot C_i \cdot Inh_r$$

Equation (2): Where DD_{alv} is the deposited dose in the alveolar region, $D_{f,alv,i}$ is the alveolar deposition fractions in size bin i as calculated according to (Hinds, 1999), Inh_r is the inhalation rate, here we used 12 L/min corresponding to light exercise, C_i is the average aerosol

concentration in size bin i, and t the exposure duration defined in the scenario.

2.6. Comparison of results from in vitro lung surfactant measurements with the estimated deposited alveolar dose during use

To enable comparison of the alveolar deposited dose estimated in the exposure scenario to the inhibitory dose found *in vitro*, both were converted to mg product per mg lung surfactant. This conversion relies on several assumptions. Firstly, for the *in vitro* inhibition of lung surfactant function: The dose deposited on the QCM is measured as ng/cm^2. However, the volume of the drop is changed constantly to simulate breathing. This results in a varying surface area. The average area (0.18 cm^2) during the experiments was used to relate the deposited dose on the QCM to the deposited dose on the lung surfactant drop. E.g. if at inhibition of the lung surfactant function, the QCM read 200 ng/cm^2, this was converted to the deposited dose on the lung surfactant droplet by multiplying with 0.18 cm^2, i.e. 36 ng in this case. To further convert the deposited dose on the surface of the drop into amount per mass of lung surfactant, it was divided by 0.025 mg, as each tested drop is 10 μL with a lung surfactant concentration of 2.5 mg/mL. In the previous example this would result in 0.000036 mg divided by 0.025 mg lung surfactant, i.e. an inhibitory dose of 0.0014 mg product per mg lung surfactant. Secondly, the alveolar deposited dose during use that was estimated in the exposure scenario comes out in mg of product deposited in the alveoli. To convert this into mg product per mg lung surfactant, we first estimated the amount of lung surfactant in the lungs by assuming that the alveolar region is covered by a total of 20 mL liquid with a concentration of 50 mg lung surfactant/mL (Sørli et al., 2016). We further assumed that the product distributed evenly in the lung surfactant. Thus the estimated deposited dose was divided by 1000 mg lung surfactant, to obtain values of mg product per mg lung surfactant.

2.7. Estimation of MoS

"Margin of safety" is often used in pharmacology to assess the margin between the effective dose and the toxic dose. The larger the margin, the safer the drug. The concept has also been used in "Next-generation risk assessment", where the point of departure (POD) for various biological effects were determined using new approach methodologies (NAMs) (Baltazar et al., 2020). The *in vitro* PODs were plotted with the estimated *in vivo* exposure to calculate a MoS. Using this approach, MoS is a tool that can be used to make a safety decision by integrating *in vitro* data and modelled or measured exposure. If the *in vitro* POD is lower or equal to the estimated exposure level the MoS will be one or less than one, and there is likely an adverse effect *in vivo*. On the other hand, if the POD is higher than the exposure estimation, it is less likely that there is an adverse effect *in vivo*. The larger the MoS the less likely the adverse effect *in vivo*.

In the present work we used the level of exposure that inhibited lung surfactant function as POD. To estimate the MoS we divided the *in vitro* inhibitory dose with the estimated alveolar deposited dose, both converted to mg product/mg lung surfactant as described above (Fig. 3).

2.8. Chemical composition of products

2.8.1. Chemical analysis of impregnation products

The impregnation products studied in this project constitutes a subset of the 110 identified products described earlier (Danish EPA *in prep*). NMR-spectroscopy (Nuclear Magnetic Resonance) together with Gas and Liquid Chromatography in combination with Mass Spectrometry (GC/MS and LC/MS) were employed to determine the composition of the impregnation sprays (Tables 3 and 4). Densities were determined by weighing a measured volume of the product, immediately after release from the aerosol container. An unknown amount of propellants (mainly C3-C4 hydrocarbons) was lost to evaporation, therefore the apparent densities are indicative only and may deviate from the densities of the product in the container (Table 5).

2.8.2. Chemical composition of cleaning products

The eight spray cleaning products included in this project constitute a subset of the database of 101 products identified and described previously (Clausen et al., 2020). All products were found in the Danish Product Registry. The registry is a confidential database of substances and materials for professional use in Denmark, maintained by the Danish Working Environment Authority with volumes updated every second year. The chemical composition of the cleaning products is reported in the database, and the composition is summarized in Table 6.

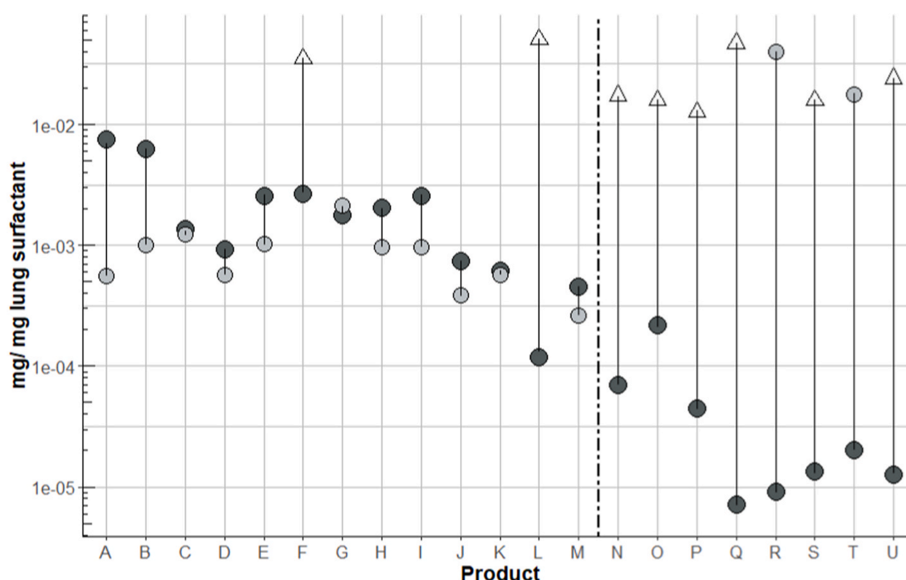

Fig. 3. Margin of safety plotted as the distance between the estimated exposure, and the inhibitory (or highest tested dose) *in vitro*. Dark grey circles: the deposited alveolar dose estimated by the exposure model; light grey circles: the dose that inhibited lung surfactant function *in vitro;* arrowheads: highest tested dose of non-inhibitory products; black lines: margin of safety.

Table 2
The lowest infusion rate that caused inhibition, or the highest infusion rate tested in *in vitro* experiments assessing lung surfactant function.

Number	Category	Infusion rate, mL/min	Inhibition
A	Impregnation	0.1	Yes
B	Impregnation	0.1	Yes
C	Impregnation	0.1	Yes
D	Impregnation	0.1	Yes
E	Impregnation	0.1	Yes
F	Impregnation	0.5	No
G	Impregnation	0.1	Yes
H	Impregnation	0.1	Yes
I	Impregnation	0.1	Yes
J	Impregnation	0.1	Yes
K	Impregnation	0.1	Yes
L	Impregnation	1	No
M	Impregnation	0.1	Yes
N	Cleaning	1	No
O	Cleaning	1	No
P	Cleaning	1	No
Q	Cleaning	1	No
R	Cleaning	1	Yes
S	Cleaning	1	No
T	Cleaning	1	Yes
U	Cleaning	1	No
H_2O	Control	1	No
pH2	Control	0.5	No
pH11	Control	0.5	No
NaCl	Control	1	No
EtOH	Control	1	No
Glycerol	Control	0.5	No
HexDec	Control	0.1	Yes
DecHex	Control	0.1	No

2.8.3. pH

The pH of the cleaning products was measured using a pH meter (PHM220 Lab pH meter from MeterLab, Radiometer, Copenhagen) (Table 6). The pH of the impregnation products was not measured as they were primarily hydrocarbon based.

3. Results

If the test substance inhibited lung surfactant function, the inhibitory dose, i.e. the deposited mass on the QCM at the time where at least 3 consecutive minimum surface tension values were >10 mN/m was determined. For the impregnation products, we started out at the lowest possible infusion rate (0.1 mL/min) and continued at higher rates if there was no inhibition. The two control solvents were tested at 0.1 mL/min (Table 2). All but two impregnation products (F and L) inhibited lung surfactant function at an infusion rate of 0.1 mL/min. One of the two control solvents (HexDec) also inhibited at 0.1 mL/min. Product F was tested at a higher infusion rate of 0.5 mL/min, and product L at a rate of 1 mL/min, but none of them inhibited lung surfactant function (Table 2). The control solvents (HexDec and DecHex) evaporated before they could be registered on the QCM, thus the inhibitory dose for HexDec could not be determined.

For the cleaning products, we tested at two pre-determined infusion rates (0.5 and 1 mL/min). The deposition on the QCM was between 300 and 1700 ng/cm^2 for the non-inhibiting cleaning products at the highest infusion rate. For the two products inhibiting lung surfactant function, the QCM deposition was 2900 and 11800 ng/cm^2 (T and R, respectively). Six control solutions with common solvents were also tested; water, 0.9% NaCl and 96% ethanol (at infusion rate 1 mL/min) and 10% glycerol, and water adjusted to pH 2, or adjusted to pH 11 (at infusion rate 0.5 mL/min). The control solutions, water, water adjusted to pH 2, pH 11 and ethanol, did not register on the QCM, whereas the salt in the 0.9% NaCl solution and the 10% glycerol deposited on the QCM (the final deposition was approximately 200 and 5200 ng/cm^2, respectively).

Table 4
Yield of fluoro-compounds (in weight%) from product I (Danish EPA *in prep*).

PFBA	PFPeA	PFHxA	PFHpA	Sum of PFCAs	6:2 FTOH
0.027%	0.049%	0.092%	0.010%	0.178%	0.23%

PFBA: perfluorobutanoic acid, PFPeA: perfluoropentanoic acid, PFHxA: perfluorohexanoic acid, PFHpA: perfluoroheptanoic acid, PFCAs: perfluorocarboxylic acids, 6:2 FTOH: perfluorohexylethyl alcohol.

Table 5
The relative volatility of the products was evaluated by the weight ratio of the non-volatile residue or the volatile part to initial content after 12 h standing in an open test tube.

	Original sample, g	Non-volatile residue, g	Volatile part, g	Ratio residue/ volatile	Ratio volatile/ residue
A	0.55	<0.01	0.54	<0.02	>54
B	0.43	0.02	0.41	0.05	20.50
C	0.7	0.22	0.48	0.46	2.18
D	0.77	0.74	0.03	24.67	0.04
E	0.57	0.13	0.44	0.30	3.38
F	0.71	0.20	0.51	0.39	2.55
G	0.79	0.44	0.35	1.26	0.80
H	0.61	0.48	0.13	3.69	0.27
I	0.55	0.07	0.48	0.15	6.86
J	0.71	0.38	0.33	1.15	0.87
K	0.75	0.61	0.14	4.36	0.23

n-Decane was a major component of the "heavy" hydrocarbon fraction by GC-MS, therefore it was selected as a control solvent for the non-volatile residue of the impregnation sprays. n-Hexane was selected as control solvent for the volatile part, though the exact composition of the volatile hydrocarbon part was not investigated in detail.

Table 3
The density and chemical composition of the impregnation products measured by NMR (Danish EPA *in prep*).

		Weight % w/w							
Product	Density g/cm^3	Hydrocarbons	Methyl siloxanes	Isopropanol	Butyl acetate	Ethanol	Dihexyl ether	Isopropylacetate	DEHP
A	0.65	96	2.5	n.d.	n.d.	n.d.	n.d.	n.d.	1.0
B	0.66	93	5.0	n.d.	n.d.	n.d.	n.d.	n.d.	2.1
C	0.74	41	2.1	47	10	n.d.	n.d.	n.d.	0.3
D	0.77	95	3.8	n.d.	n.d.	n.d.	n.d.	n.d.	0.9
E	0.73	36	1.3	52	11	n.d.	n.d.	n.d.	0.3
F	0.73	43	1.4	46	9	n.d.	n.d.	n.d.	0.3
G	0.79	n.d.	n.d.	n.d.	n.d.	84	16	n.d.	n.d.
H	0.72	95	4.6	n.d.	n.d.	n.d.	n.d.	n.d.	0.3
I	0.68	88	5.1	n.d.	n.d.	n.d.	n.d.	6	1.0
J	0.76	95	4.1	n.d.	n.d.	n.d.	n.d.	n.d.	0.8
K	0.77	96	4.0	n.d.	n.d.	n.d.	n.d.	n.d.	0.3
L	1.01	Trace	Trace	n.d.	n.d.	trace	n.d.	n.d.	n.d.

DEHP: bis(2-ethylhexyl)phthalate, n.d.: not detected.

Table 6
The chemical composition of the spray cleaning products as reported in the Danish Product Registry, in percentages of the whole product. Different single chemicals have been grouped in overarching categories. The pH for each product is also included.

	Water	Perfume	Color	Alcohol	Tenside	Base	Acid	Glycol and glycol ethers	Disinfectant	Preservative	Salts	Chelator	pH
N	95	x	x	4.95	0.09								7.9
O	89			10.00	0.03			0.60					8.9
P	99.8	x		0.00	0.13		0.01	0.07		0.02			3.6
Q	98	x			0.47	0.4					0.9		9.7
R	92	x			1.50		4.7						2.5
S	98				0.61		0.11	0.56	0.28				2.0
T	84		x	1.80	3.26	1.0		8.00			0.1	1.60	11.6
U	96	x	x	0.10	3.51		0.01	0.07		0.02			5.9

X indicates the presence of perfume and/or color added to the product.

None of the six control solvents inhibited lung surfactant function.

For products that inhibited lung surfactant function, the inhibitory dose was calculated as described (Fig. 3). If the product did not inhibit lung surfactant function, they were plotted as an arrowhead to indicate that the inhibitory dose was higher than the tested dose (Fig. 3). The estimated alveolar deposited dose from the exposure scenario was plotted in the same figure. The emission rate during spraying of the products strongly influences how much of the material is estimated to deposit in the alveoli. Thus most of the impregnation products (to the left of the dotted line in Fig. 3) have a higher deposited dose than the cleaning products. The vertical lines connecting the estimated exposure during use and the inhibitory dose gives a graphical representation of the MoS. For the cleaning products (to the right of the dotted line in Fig. 3), inhibition was only observed for to products; for the rest, the highest tested dose was plotted as arrowheads. Note that increasing the infusion rate only increased the deposited dose for about half of the cleaning products, reflecting that the aerosolization device could not aerosolize all of the product as the infusion rate increased (this was confirmed by observation of un-aerosolized product collecting in the pit).

3.1. Chemical analysis - impregnation products

The main components in the majority of the impregnation products were saturated hydrocarbons, often declared as "hydrogenated naphtha" in the product specification. However certain products contained some oxygenated solvents, such as alcohols, ethers, or esters. All but two products contained mixtures of methyl siloxane derivatives. Their content was estimated to be in the range of 1.3–5.1% by weight. Such siloxanes are common components in many household or personal-care products, and most of the products had siloxanes declared in the specifications. The plasticizer bis(2-ethylhexyl)phthalate (DEHP) was found in all but two products at levels of 0.3–2.1% by weight. This was not declared in any of the specifications. DEHP has an occupational exposure limit (OEL) in Denmark of 3 mg/m^3, this level will be reached by dispersing 0.14–1 g of the studied products per cubic meter of air.

Traces of PFAS (per- and polyfluoroalkyl substances) precursors perfluorohexanoic acid (PFHxA) and perfluorohexylethylmethacrylate (6:2-FTMA) were detected only in product I. Based on the structure of these trace contaminants, the presence of a FTMA-based polymer was suspected. Oxidation (Houtz and Sedlak, 2012) and hydrolysis (Nikiforov, 2021) of the sample allowed detection and quantification of transformation products – perfluorocarboxylic acids (PFCAs) C4-C7 and perfluorohexylethyl alcohol (6:2 FTOH).

3.1.1. Chemical content – cleaning products

The eight cleaning products investigated in this project are a subset of 101 spray cleaning products identified in the Danish Product Registry (previously described in (Clausen et al., 2020). The content has been collected as volume percentages of different chemical subgroups in Table 6.

3.1.2. Emission rate measurement and alveolar dose estimated in exposure scenarios

The calculated SRER, used as source input in the exposure scenarios, are shown in the Supplementary Information Table S1. We found the particle number emission rates ranged between 5.06×10^6 and 8.42×10^{10} particles/s, and respirable mass emission rates between 0.002 and 3.35 mg/s (Table 7). The products contained in pressurized cans generally generated higher emission rates than the trigger sprays. Emission rates were in general lower for trigger sprays compared with the pressurized cans due to the lower nozzle pressure for the trigger sprays. One exception to this was product M which was packaged in a trigger spray bottle and released aerosols at a rate of 3.16×10^{10} particles/s. Furthermore, several of the cleaning products produced a foam rather than an aerosol fog, which reduced the number of particles remaining airborne after application.

4. Discussion

The investigated impregnation products are more toxic than cleaning products.

We have used "the margin of safety" to evaluate of the relative risk of using either impregnation or cleaning spray products, i.e. the larger the margin, the safer the product. The MoS was generally much lower for the investigated impregnation compared to cleaning products. Thus we

Table 7
The particle number emission rate, average total number concentration, respirable mass emission rate and calculated deposited dose in the alveolar region based on the modelled concentrations for impregnation and cleaning products.

	Particle number emission rate	Average total number concentration, NF	Respirable mass emission rate	Deposited dose in the alveolar region
	particles/s	particles/cm^3	mg/s	mg
A	8.42×10^{10}	6.27×10^5	3.35	7.49
B	4.93×10^{10}	4.41×10^5	2.40	6.32
C	1.78×10^{10}	$2.44E \times 10^5$	0.71	1.35
D	4.04×10^9	7.53×10^4	0.39	0.92
E	2.82×10^{10}	3.22×10^5	0.90	2.58
F	3.52×10^{10}	3.23×10^5	1.04	2.67
G	1.20×10^9	2.25×10^4	0.82	1.78
H	5.05×10^{10}	4.57×10^5	0.95	2.06
I	4.50×10^{10}	3.40×10^5	1.25	2.58
J	2.47×10^9	5.45×10^4	0.28	0.74
K	1.32×10^9	3.17×10^4	0.32	0.62
L	2.30×10^9	3.70×10^4	0.05	0.12
M	3.16×10^{10}	4.86×10^5	0.154	0.45
N	5.37×10^7	2.17×10^3	0.042	0.07
O	5.93×10^8	1.63×10^4	0.079	0.22
P	5.06×10^6	9.06×10^2	0.025	0.04
Q	7.70×10^7	2.70×10^3	0.002	0.01
R	2.29×10^7	1.28×10^3	0.003	0.01
S	6.73×10^7	3.10×10^3	0.005	0.01
T	4.67×10^7	1.10×10^3	0.004	0.02
U	6.15×10^7	2.51×10^3	0.005	0.01

NF: near field.

conclude that the investigated impregnation products were generally associated with higher risk of illness than the cleaning products.

To estimate how much of the product would end up in the person's lung during application, we used an exposure scenario recreated from a case of real-life intoxication. In this case the person developed respiratory symptoms after using an impregnation product for 30 min and stayed in the room 90 min thereafter. As most impregnation products come with the warning "use in a well ventilated area" the person had opened all the windows in the room. It is reasonable to assume that an application time of 30 min is realistic, e.g. when impregnating a new piece of furniture, or when cleaning. However, for some of the products, both the application time and time spent in the room after application could be shorter, and we consider this scenario a worst case.

The estimated lung deposition was higher for the impregnation products than for the cleaning products. The MoS is determined by 1) the estimated deposited dose, and 2) the dose at which inhibition of lung surfactant function is observed. Hence, the mode of application is an important determinant in the observed differences in the MoS between the product groups. Most of the impregnation products came in pressurized cans, and these produced many small particles, resulting in a high predicted deposition in the respiratory parts of the lungs. The cleaning products came in trigger spray bottles, and these release larger and much fewer particles during application. A trend of moving away from pressurized cans and towards trigger and foam sprays for cleaning products to reduce exposure has been observed in the Danish cleaning industry in recent years (BFA, 2018). Indeed, in some cases the nozzles on the cleaning products are designed so that instead of a spray, the product forms foam when extruded, resulting in very few particles detected in the air, and a very low estimated alveolar deposited dose in the exposure scenario. The MoS is also affected by the dose at which the product inhibited lung surfactant function. Most of the impregnation products inhibited lung surfactant function at a low dose (resulting in a low MoS, most $\leq$1), whereas a dose that inhibited the lung surfactant function could not be established for most of the cleaning products (resulting in a large MoS). When the products were tested for inhibitory potential of lung surfactant function, the application method was bypassed as all products were aerosolized in the same way.

We used Curosurf as a lung surfactant model for the study of surfactant function analysis. Curosurf contains the solvent extracted fraction of minced porcine lungs, and as a result has less of the hydrophobic proteins SP-B and –C, and does not contain the hydrophilic protein SP-A, and it has an altered lipid composition (Bernhard et al., 2000). Curosurf does not function as well as native lung surfactant when measuring surface tension lowering ability *in vitro* (Bernhard et al., 2000), however it is easily obtainable for use in research, as compared to native lung surfactant that requires access to lungs from freshly slaughtered animals and expertise in isolating the surfactant. In addition, we have previously compared native lung surfactant and Curosurf in the CDS and found the results to be comparable (Da Silva, Hickey, Ellis, Hougaard and Sørli, 2021). The ease of access of commercially available lung surfactant preparations outweighs the slightly reduced functionality, when it comes to testing for inhibition of function *in vitro*.

The difference in toxicity between impregnation and cleaning sprays is probably also linked to different physical-chemical properties of the ingredients, dictated by their function. Impregnation products are intended to make surfaces dirt and water-repellent. Hence, it is desirable that the solvent evaporates fast after application, allowing the film forming substances to adhere to the treated surface. This typically results in products with volatile solvents contained in pressurized cans for fast and even application. In contrast, cleaning products aim to remove dirt and lime scale, and increase surface wettability for easy cleaning. As most cleaning products are used in combination with water, the active substances are commonly dissolved in water or water soluble solvents. Rather than the solvent evaporating and leaving a treated surface, it is washed away together with the rest of the product.

If toxic and harmful chemicals could be identified during product formulation by application of a safe-by-design approach, this could help producers to develop safer products. A prerequisite for this approach is that we are able to associate specific chemicals to the toxic potency of the products. Nonetheless, in a market analysis of impregnation products available on the Danish market performed in 2020 (Danish EPA *in prep*), the chemical composition were only readily available in the SDS/MSDS for approximately 20% of the surveyed impregnation products. This limits the impact of our research both upstream and downstream. We are unable to inform upstream producers which chemicals or chemical mixtures to avoid. Downstream consumers or workers are unable to avoid potentially toxic chemicals based on the listed ingredients.

Additionally, film forming ingredients in impregnation products may differ in toxicity, depending on the solvent they are dissolved in (Nørgaard et al., 2014). This is likely the cause of several described outbreaks of impregnation spray toxicity, where products that had been on the market for a long time had some solvents replaced by others, and suddenly became the cause of many intoxication cases (Burkhart et al., 1996; CDC, 1993; de Groot et al., 2004; Hubbs et al., 1997; Laliberte et al., 1995). If this is the case also for other active ingredients, it is complex to pinpoint specific chemicals involved in the effect; and combining different chemicals may result in synergy (Hadrup, 2014; Hadrup et al., 2016; Kortenkamp, 2007; Olmstead and LeBlanc, 2005). This makes the testing of the complete formula in products even more important. It emphasizes the need for efficient *in vitro* methods allowing for testing of a variety of chemical combinations in a safe-by-design approach, prior to deciding on the specific ingredient combinations in marketed products.

Several groups of substances have been suspected as the cause of impregnation product inhalation toxicity. One suspected culprit is PFAS - commonly used to create water- and dirt-repelling properties. The fluorine content of impregnation products found on the Danish market has been tested several times. In 2008, 14 of the 16 tested impregnation products contained fluorine (Feilberg, 2008). In 2017, 5 of the 11 impregnation products analyzed contained fluorine (Tænk Kemi, 2017). These earlier analyses found a much larger proportion of fluorine containing products than the present survey. In the current project the chemical composition of 13 products were determined and only one contained fluorine. Interestingly, a product with the identical trade name as the one that contained fluorine in the 2017 report, was bought and tested in the present project, but without detection of fluorine, indicating that fluorine had been removed from the ingredient list. Even with the relative small sample size of these three projects, there seems to be a general trend towards moving away from fluorine in impregnation products. This trend is likely aided by EU regulation prohibiting the sale of TDFA (a fluorine containing compound) in organic solvents to consumers (European Commision, 2019).

The literature on other substances with acute pulmonary effects in impregnation products or cleaning agents is sparse pointing to chemicals such as "hydrocarbons" and "resins". This is likely because most case studies are described retrospectively and the exact product and composition cannot be retrieved.

The effect of solvents on the inhibition of lung surfactant function *in vitro*.

In addition to investigating the products, we tested product solvents (Table 2). The impregnation products were analyzed for volatility, and found to be more or less volatile (Table 5). We used a 10:1 mixture of n-hexane: n-decane (HexDec) as solvent control for the highly volatile products and a 10:1 n-decane: n-hexane (DecHex) as control for the less volatile products. The HexDec control inhibited the lung surfactant function at an infusion rate of 0.1 mL/min. As the mixture is highly volatile, the QCM could not detect its deposition, and an inhibitory dose could therefore not be determined. The DecHex solution did not inhibit the lung surfactant function under the same test conditions. As for the HexDec solution the QCM could not detect the chemicals. The most volatile products were A and B. These inhibited the lung surfactant

function, and the effect cannot be ruled out to be caused by the solvent, as the HexDec solution also inhibited function. However, the less volatile products represented by D, I, J and K inhibited the lung surfactant function to a similar degree as A and B, whereas the less volatile DecHex did not inhibit function. This indicates that it is less likely that the inhibition is driven by the solvent, or that the toxicity only partly can be ascribed to the solvent.

Alkaline and acidic ingredients determine the pH of a product. Extremes of pH can cause acute inhalation toxicity albeit through other mechanisms than surfactant inhibition, e.g. corrosive effects on the cells lining the airways. The pH in the lung lining fluid is 7 in healthy lungs (Hobi et al., 2014), and when lung surfactant function is measured *in vitro* the optimal pH ranges between 4 and 7 depending on the method used (Amirkhanian and Merritt, 1995; Camacho et al., 1996). The lung surfactant function was only affected by cleaning products R and T, both have extreme pHs of 2.5 and 11.6, respectively. These products were only toxic when large amounts were aerosolized, measured as high deposition on the QCM. We tested if the inhibition was due to pH extremes by adjusting the pH of water to 2 or 11. We did not see effect on the lung surfactant function, however, we could not reproduce the same high aerosolization rates for water as for products R and T. This is likely due to the physical-chemical properties of the cleaning liquids that eases aerosolization. We could therefore not rule out that the inhibitory function of products R and T was due to extreme pH. We also tested water, 0.9% NaCl, 96% ethanol and 10% glycerol as examples of solvents in cleaning products (Table 6). None of these inhibited lung surfactant function *in vitro*, making it likely that it was the effect of other ingredients in the two cleaning products that inhibited lung surfactant function.

4.1. Implications of our findings for risk assessment

In this project we cannot pinpoint single toxic ingredients, but we can draw conclusions based on product groups and make recommendation on this background. One recommendation is that impregnation products need to be strictly regulated. Certain products ought to be labelled as toxic (or even banned). This is reflected by the low MoS for these products, as most were below one. This indicates that the predicted alveolar deposition was higher than the dose at which *in vitro* toxicity was observed (Fig. 3). However, as noted previously, the MoS is based on an exposure scenario that may not be applicable to all products types, thus for risk assessment of individual products the exposure scenario may need to be adjusted. As the exposure estimates reflects that the aerosols released by the impregnation products consists of very small particles, an alternative recommendation is to ban marketing of products in pressurized cans.

In contrast, the cleaning products, which are used in larger quantities, seem less acutely toxic as measured by the dose needed to inhibit lung surfactant function. This may reflect that the chemicals used in cleaning products are less toxic to the lung surfactant than those in the impregnation products. On the other hand, it could reflect that harsh cleaning agents have historically been sorted out from spray products at an earlier time point. The adverse effects of cleaning products are mainly related to longer term and/or repeated exposure, e.g. data points towards a potential for asthma induction (Hadrup et al., 2021). This indicates that other endpoints, in addition to acute effects, have to be taken into account in the complete risk assessment spray products.

4.2. Assumptions and limitations of the approaches

We used the *in vitro* lung surfactant function assay to test for the potential acute toxicity of the products. We have previously demonstrated strong correlation between this *in vitro* technique and *in vivo* acute toxicity, both in humans that have accidently inhaled products, and in mouse inhalation studies (Duch et al., 2014; Larsen et al., 2020; Larsen et al., 2014; Nørgaard et al., 2014; Scheepers et al., 2017; Sørli et al., 2018; Sørli et al., 2018). Inhibition of lung surfactant function *in vitro* correlates with development of acute respiratory symptoms such as couching, tightness in the chest, difficulty breathing and flu-like symptoms in accidently exposed humans, and a sudden rapid irreversible reduction in tidal volume in exposed mice. Inhibition of function *in vitro* also correlates to described clinical signs of respiratory toxicity in rats after exposure to chemicals tested according to OECD test guidelines for acute inhalation toxicity (Da Silva, Hickey, et al., 2021).

The approach we have used to estimate the MoS comes with several limitations and pitfalls. These are related to the estimation of exposure, identification of the inhibitory dose *in vitro*, and correlating *in vitro* to *in vivo* findings: 1) The *in vitro* system has a finite amount of lung surfactant, whereas in the lungs the lung surfactant is a dynamic system of catabolism and release. 2) The device used to measure exposure in the *in vitro* system, the QCM, measures what deposits and stay deposited, and this is used to estimate what lands on the drop of lung surfactant. For volatile compounds, such as the impregnation products, this measure is likely an underestimation as a large proportion of the product evaporates during the experiment. 3) The correlation between findings *in vitro* and *in vivo* are based on many assumptions, where the values are not known, e.g., the lung surfactant concentration in the alveoli have a wide range of reported values, likely due to the difficulty of measuring it exactly. 4) The modelling of exposure scenarios are also based on several estimates. 5) The exposure scenario was composed to mimic a worst case use of the products and for an extended duration. It is representative of reported cases of acute airway toxicity, but is probably not representative of intended uses of the products. The intended use of impregnation spray for footwear may likely not last for 30 min. On the other hand, when using the spray cleaning products the time of application and amount used could be higher than used in the exposure scenario. The duration of use, i.e. the duration of activation of the active source, is the main property affecting the resulting indoor concentrations (Jensen et al., 2018; Ribalta et al., 2021). 6) The inter-zonal airflow between NF and FF has also been shown to affect the concentrations in the NF when assessing exposure (Jensen et al., 2018; Ribalta et al., 2021). Here we assume an inter-zonal air exchange rate similar to the general room ventilation rate. The room concentrations, however, depend on mixing of air inside the room. 7) For determination of the deposited alveolar dose we assumed deposition fractions calculated during light exercise and with a 12 L/min inhalation rate to yield precautionary and conservative estimates. 8) Some channels in the ELPI came out with negative emission rates due to low chamber concentrations compared with background concentrations (Table S1). These emission rates were omitted from calculations carried out in the modelling. 9) Last but not least, the MoS calculated in the current work only pertains to one endpoint, and a complete risk assessment would have to include other endpoints too.

5. Conclusion

The impregnation products strongly influenced the lung surfactant *in vitro* by inhibiting its function. Although we were not able to pinpoint specific substances responsible for the effect, it is unlikely that the effect was caused by common solvents in the products. We propose impregnation products should be firmly regulated. For cleaning products, acute lung toxicity owing to inhibition of lung surfactant function seems less likely. Moreover, it is highly relevant to have an efficient *in vitro* technique to screen toxicity of a high number of products. This would also reduce the need for animal experiments.

This adverse outcome is only one of several potential outcomes after inhaling aerosols. In the future, results from lung surfactant function inhibition can be combined with other *in vitro* approaches to replace animal experiments in risk assessment of chemicals. Already now, surfactant function inhibition can be used to prioritize substances in safe-by-design of less toxic products as well as prioritize already in-use substances for further toxicological investigation.

CRediT authorship contribution statement

J.B. Sørli: Conceptualization, Methodology, Investigation, Formal analysis, Writing – original draft, Writing – review & editing, Funding acquisition. **S. Sengupta:** Investigation, Writing – original draft. **A.C.Ø. Jensen:** Conceptualization, Software, Investigation, Formal analysis, Writing – original draft. **V. Nikiforov:** Conceptualization, Investigation, Formal analysis, Writing – original draft. **P.A. Clausen:** Formal analysis, Writing – review & editing, Funding acquisition. **K.S. Hougaard:** Resources, Writing – review & editing, Funding acquisition. **Sara Højrriis:** Writing – review & editing. **M. Frederiksen:** Resources, Formal analysis, Writing – review & editing, Funding acquisition. **N. Hadrup:** Resources, Writing – review & editing, Writing – original draft, All authors read and approved the final draft of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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