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Full length article

Evaluation of fiber and debris release from protective COVID-19 mask textiles and in vitro acute cytotoxicity effects

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Handling Editor: Adrian Covaci

Keywords:
Textile-based facemask
Debris release
Metal content
Acute lung cytotoxicity

\textbf{A B S T R A C T}

Since the start of the current COVID-19 pandemic, for the first time a significant fraction of the world’s population cover their respiratory system for an extended period with mostly medical facemasks and textile masks. This new situation raises questions about the extent of mask related debris (fibers and particles) being released and inhaled and possible adverse effects on human health. This study aimed to quantify the debris release from a textile-based facemask in comparison to a surgical mask and a reference cotton textile using both liquid and air extraction. Under liquid extractions, cotton-based textiles released up to 29 452 ± 1 996 fibers g\textsuperscript{-1} textile while synthetic textiles released up to 1 030 ± 115 fibers g\textsuperscript{-1} textile. However, when the masks were subjected to air-based extraction scenarios, only a fraction (0.1–1.1\%) of this fiber amount was released. Several metals including copper (up to 40.8 ± 0.9 µg g\textsuperscript{-1}) and iron (up to 7.0 ± 0.3 µg g\textsuperscript{-1}) were detected in acid dissolved textiles. Additionally, the acute in vitro toxicity of size-fractionated liquid extracts (below and above 0.4 µm) were assessed on human alveolar basal epithelial cells. The current study shows no acute cytotoxicity response for all the analyzed facemasks.

1. Introduction

In the COVID-19 pandemic, the “new normal” is characterized by non-pharmaceutical measures. Despite vaccines and further medication, non-pharmaceutical measures such as facemasks will remain an important tool to control COVID-19 and other diseases transmitted by droplets or aerosol infection. The collapse of the delivery chain of single use masks as well as concerns regarding their sustainability due to the enormous amounts of plastic waste initiated a boom and innovation in the ongoing pandemic. The usage of disposable facemasks generates millions of tons of plastic wastes to the environment in a short span of time [4–6]. Besides the sustainability aspects, reusable textile-based facemasks can be equipped with extra functionality such as antimicrobial/antiviral properties [7]. Although these masks offer promising characteristics in terms of functionality, consumer concerns on their safety raised along with the broad use of facemasks in general [8]. There is evidence that textiles release fibers, particles and debris during use and washing [9–11]. However, little is known about fiber and debris release from facemasks and cotton based textile masks during breathing in particular [7,8]. Small physical pollutants such as micron- and nano-sized particles [7,8], heavy metals (Pb, Cd and Sb) [8], and organic pollutants [8] were reported to be leached from masks and could potentially pose a threat to the environment and the public health. Therefore, the aim of this study was to investigate the type (fibers, particles) and amount of physical pollutants that are released during the
use of a cotton-based facemask, surgical mask and a reference cotton tissue. The release was assessed in a worst-case scenario (liquid extraction) as well as a more realistic air-based scenario using a model breathing head (Sheffield head). Additionally, we collected the released material and assessed its effects on human lung cells. To this end, the release of debris from facemasks was measured in air by a Sheffield head setup. To collect enough material for cytotoxicity study an alternative extraction procedure with purified water was applied prior to the execution of first in vitro acute cytotoxicity assessment. Therefore, the collected extracts were separated into a debris fraction containing fibers and particles $>0.4 \mu m$ and a soluble fraction containing water soluble compounds and particles below this size. The debris as well as the soluble fraction was applied on an A549 cell monolayer under an air-liquid interface cultivation to assure direct contact of the debris to the cell monolayer.

2. Materials and methods

2.1. Materials

Livinguard (LG) provided five textiles applied for the production of Pro Mask community facemasks, both uncoated (inner- and outer layer) and with an antiviral coating (inner- and outer layer; Table 1). For comparison, a standardized cotton textile (Swissastest, Ref 210) and two synthetic melt blown surgical masks manufactured by the companies Tengchuang Yiliao and Weian were included as controls in this study (Table 1, SI Table S3). Facemask and reference textiles were laser cut (tt-1300, Times technology, speed of 70%) to gain sample pieces with a defined textile surface area of 0.004 m² (Fig. 1 C, SI Figure S4). Laser cutting reduces the amount of fibers released from edges when compared to scissors [10]. To minimize burnt edges on textiles while cutting, the laser cutter power was adjusted for each textile separately (Table 1).

2.2. Air based debris extraction with Sheffield heads

The fibers, particle and debris release of the masks was assessed with two air based debris extraction setups applying Sheffield heads. A textile-based community facemask Pro Mask size L green (coated) consisting of two (inner-/outer layer) cotton based layers as well as a polypropylene based melt blown filter textile (middle layer) was analyzed and compared to a surgical mask (SM A) (Table 1). The collection and quantification of fibers $>12.0 \mu m$ was performed on a 12.0 µm Whatman™ Nuclepore filter membrane (Merck, Ref. 111116) with air. Therefore, a filter holder was designed allowing a constant airflow to pass through the filter membrane via the mounted filter holder (Fig. 1 A1). The filter holder is suitable to mount on a Sheffield head design according to DIN EN 149 (Fig. 1 A2). A respiratory ventilation air flow intensity of 14.2 L min⁻¹ (850 L h⁻¹) was applied to simulate a light breathing equivalent to activities like sitting and walking at a speed of 3.2 km h⁻¹ Johnson, 2016; Johnson et al., 1992. For each measurement a fresh mask, either SM A as control or Pro Mask size L green was fitted onto the Sheffield head with mounted filter holder and fresh filter membrane for fiber recovery. The ventilation airflow intensity was set to 14.2 L min⁻¹ for a release duration of 1 h (Fig. 1 B). During this measurement period the same mask was additionally taken off and on the Sheffield head 7 times to simulate mechanical stress on the mask during daily wearing. Each measurement was performed in triplicates (N = 3) and the released fiber amount and length distribution of fibers were quantitated with FiberApp software (version: 1.51) [14] and a micrometer calibration slide (Spectrographic Limited, 0.07–1.5 mm Circles), see supplementary information (SI) Table S1.

The particle release was quantified on a Sheffield head, which simulates natural inhalation and exhalation in a controlled environment with HEPA filtered clean dry air and automatically detects particles and aerosols in a size range of 0.3–10 µm with an Abakus® air particle counter (Klotz GmbH, Germany). A sinusoidal respiratory air flow of 30.0 L min⁻¹ (1’800 L h⁻¹) was applied to simulate a light to moderate breathing (Johnson, 2016; Johnson et al., 1992) during 8 h after fitting the masks on the Sheffield head and initiating the controlled environment. The particle measurement took place once per minute for 6 s (4800 data points per mask). The particle counts of all measurement points were summarized and normalized per gram mask (SI Table S2). The measurement allows a comparison of particle release between the different masks but no total particle release.

2.3. Liquid fiber and particle extraction procedure

The standardized microplastic fibers extraction procedure described by [10] was adapted for facemask and reference textiles with only minor modifications to allow a subsequent testing of extracts on cell cultures under sterile conditions. No prewash of textiles was performed as recommended by Livinguard for its community facemasks, since these liquid extracts aim to represent a worst-case scenario containing the total amount of extractable and therefore possibly inhalable fibers and particles. Prior to the fiber and particle extraction, the Gyrowash containers (James Heal, Ref. 718-902, Fig. 1 D) and 6 mm steel balls (James Heal, Ref. 718–164) were rinsed three times with ultrapure deionized water of 18.2 MΩ cm (Millipore AG, Switzerland) to remove contaminants. Then 150 mL ultrapure deionized water, 10 rinsed steel balls and three layers of textile were added to each Gyrowash container. For SM A, SM B, uncoated- and coated textile a single piece of outer-/middle- and inner layer was added to each Gyrowash container while RCT and filter textile contained 3 identical textile layers. The Gyrowash containers were inserted to the Gyrowash instrument (James Heal, model1615) where the extraction took place for 45 min at 25 °C (Fig. 1 D). Subsequently, Gyrowash vessels were opened to remove the textiles with forceps. While constantly stirring, different volumes (120 mL, 12 mL or

| Identifier | Description | Supplier | Layers | Structure and details | Surface density (g/m²) ± SD | Laser cutter power (%) |
|------------|-------------|----------|--------|-----------------------|-----------------------------|-------------------------|
| SM A       | Surgical mask A | Tengchuang Yiliao | 3      | Melt-blown PP mask, CE and FDA label, no mask std., middle efficiency | 75.5 ± 1.7 | 45 |
| SM B       | Surgical mask B | Welan     | 3      | Melt-blown PP mask, CE, Typ IIR (EN14683), BFE >98%, high efficiency | 83.6 ± 0.9 | 45 |
| RCT        | Reference cotton textile | Swissastest, Ref 210 | 1      | Cotten textile 180 g m² plain, bleached w/o optical brightener | 176 ± 1.3 | 55 |
| uncoated   | Pro Mask textile | LIVINGUARD | 3 (inner-/outer layer coated) | Sample, inner-/outer layer cotton textile with middle layer melt-blown PP textile | 282 ± 1.8 | 70 |
| coated     | Pro Mask textile | LIVINGUARD | 3 (inner-/outer layer antiviral coated) | Sample, inner-/outer layer cotton textile with middle layer melt-blown PP textile | 288 ± 2.4 | 70 |
| filter     | Pro Mask filter textile | LIVINGUARD | 1 (middle layer uncoated) | Melt-blown PP textile | 31.0 ± 1.0 | 50 |

Polypropylene (PP), Bacterial Filtration Efficiency (BFE).
6 mL) of extract were filtered onto 0.4 µm Whatman™ Nuclepore filter membrane (Merck, Ref. 10417112) mounted on sterile analytical filter units (Thermo Scientific™, Ref. 130–4045) and connected to a vacuum pump (vacuubrand, model ME 2C NT) to assure a countable number of fibers as well as their length distribution were quantified. Analyzed fibers are indicated in blue (E2). The amount of particles as well as the size distribution of the particles present in the SDE ≤ 0.4 µm were analyzed with a NanoSight LM20 (Malvern) (F). Nitric acid digested mask textile samples for quantification of the total textile metal content fresh from the packaging without any pre-treatment by washing (G). A549 cells were seeded on the apical site of Thincert™ Tissue culture inserts in 12-well plates and treated under air-liquid interface cultivation conditions (H1). The sterile collected fibers were resuspended in 1 mL sterile ultrapure deionized water which equals the debris high fraction (DH, triplicates of SM A – left, RCT – middle and coated textile – right are shown) before further dilution to DM and DL took place (H2). 70 µL of fiber and particle extract (75.3%) suspended in 10-fold RPMI medium concentrate plus additives (24.7%) was added apically to the cell monolayer (DH coated condition is shown) (I1). Metabolic activity of viable cells (viable cells – brownish-red, non-viable – yellow color) was quantified colorimetrically at 490 nm absorbance using a standard in vitro viability MTS assay (I2). – color figure in print.

Fig. 1. Graphic summary of the applied methodology. Empa designed filter holder (A1) suitable to mount on a Sheffield head design according to DIN EN 149 (A2). A respiratory ventilation air flow intensity of 14.2 L min⁻¹ (850 L h⁻¹) was applied for 1 h for fiber recovery onto filter membranes (B). Laser cutting of textile sample pieces with a dimension of 4 × 10 cm (C). Liquid fiber extraction of textile sample pieces in Gyrowash containers inserted to the Gyrowash instrument (James Heal, model1615) for 45 min at 25 °C (D). The light-white fibers on dark-black background pictures (E1) were analyzed with FiberApp software 1.51 and the amount of fibers as well as their length distribution were quantified. Analyzed fibers are indicated in blue (E2). The amount of particles as well as the size distribution of the particles present in the SDE ≤ 0.4 µm were analyzed with a NanoSight LM20 (Malvern) (F). Nitric acid digested mask textile samples for quantification of the total textile metal content fresh from the packaging without any pre-treatment by washing (G). A549 cells were seeded on the apical site of Thincert™ Tissue culture inserts in 12-well plates and treated under air-liquid interface cultivation conditions (H1). The sterile collected fibers were resuspended in 1 mL sterile ultrapure deionized water which equals the debris high fraction (DH, triplicates of SM A – left, RCT – middle and coated textile – right are shown) before further dilution to DM and DL took place (H2). 70 µL of fiber and particle extract (75.3%) suspended in 10-fold RPMI medium concentrate plus additives (24.7%) was added apically to the cell monolayer (DH coated condition is shown) (I1). Metabolic activity of viable cells (viable cells – brownish-red, non-viable – yellow color) was quantified colorimetrically at 490 nm absorbance using a standard in vitro viability MTS assay (I2). – color figure in print.
subsequently analyzed with FiberApp software (version: 1.51) for the amount and length distribution of fibers present (analyzed fibers marked blue; Fig. 1 E2, SI Table S5)\(^4\). The fiber and particle extraction was performed for each textile combination in independent triplicates (N = 3) and water extractions without textile were performed to assess the amount of debris present during sample preparation. To gain fiber and particle extracts \(\geq 0.4 \mu m\) and the soluble fraction including debris with a size of \(\leq 0.4 \mu m\) (named soluble & debris extract, SDE) for the subsequent application on cell cultures, the procedure was performed under sterile conditions. The filtration was performed in a laminar flow bench with sterilized Whatman™ Nuclepore filter membranes. 120 mL sterile extract solution was filtered in order to collect the fiber and particle fraction \(\geq 0.4 \mu m\) on the membrane. The filters were transferred to 15 mL reaction tubes (Greiner) for resuspending the fiber and particle fraction from the filter membrane in 1 mL sterile ultrapure deionized water, which equals the debris high (DH) concentration (Fig. 1 H2). With different volumes of sterile water, the debris high (DH) extracts were diluted for further testing. Debris medium extract (DM) was established by resuspension of 120 mL extract solution in 5 mL water, whereas the debris low extract (DL) was resuspended in 25 mL of sterile water. The filtered solution of DH extract was transferred to 250 mL bottles (Nalgene) and served undiluted as SDE \(\leq 0.4 \mu m\).

### 2.4. Particle characterization

To assess the amount of particles and the size distribution of the particles present in the SDE \(\leq 0.4 \mu m\), the extracts were analyzed with a NanoSight LM20 (Malvern) (Fig. 1 F). Three separate extractions (N = 3) of each textile combination were tested. For high precision of the readings, >20 particles per frame are required. Therefore, higher concentrated SDE \(\leq 0.4 \mu m\) were diluted 10 times with ultrapure deionized water. Only the RCT, uncoated- and coated textile extracts were diluted while all other SDE were already in a suitable range containing \(< 20 \) particles per frame. The instrument settings were as follows: Frames Processed: 2700 to 2700; Frames per Second: 30.00; Calibration: 166 nm/pixel; Blur: Auto; Detection Threshold: 10 Multi; Min Track Length: Auto; Min Expected Size: Auto; Temperature: 22.00 °C; Viscosity: 0.95 cP. The accuracy of the instrument was tested with a certified 100 nm polystyrene latex particle standard (NanoSphere™, Thermo Scientific) which yielded a mode diameter of 101 nm (D50 = 106 nm; SD = 31%) when diluted 100’000 times in purified water (19.8 particles per frame) [15].

### 2.5. Quantification of total and water-extractable metals in mask textiles via ICP-MS

For quantification of the total textile metal content a known amount (between 0.4 and 0.8 g) of untreated mask textile, was collected using a cutter on evenly distributed positions on the textile in order to account for area specific variability. For each textile type, three separate sample preparations were conducted (n = 3). Subsequently, the samples were subjected to a 2-step digestion procedure to avoid excessive reactions or foaming (Fig. 1 G). To this end, the collected textile pieces were placed in 25 mL quartz digestion tubes and 8 mL concentrated ultrapure nitric acid (HNO\(_3\), 69%, Normatomo®, WVR chemicals) were added. The first step of the textile digestion was then performed in a nitrogen gas pressure (_N\(_2\)_™) with a certified 100 nm polystyrene latex particle standard (NanoSphere™, Thermo Scientific) which yielded a mode diameter of 101 nm (C; Viscosity: 0.95 cP. The accuracy of the instrument was tested with a certified multi-element standard containing the elements of interest (CCS-6, Inorganic ventures) was digested and analyzed using the same procedure as for the textile samples (SI Table S7). Recoveries were calculated for all quantified single metal elements, which included Cr, Cu, Fe, Mn, Ni and Pb. An overall recovery average of 93.4% (mean value of single metal element recoveries, SI Table S7) was found with lowest recovery for Ni (44.5%) and highest recovery for Fe (120.0%). Metal contents of aqueous textile extracts (0.75 g L\(^{-1}\) linear alkylbenzene sulfonic acid dissolved in ultrapure deionized water with pH 9.2 according to [9] performed in 50 mL plastic tubes on a Polymax 1040 shaker (Heidelberg, Germany) for 60 min. were determined directly without further dilution.

### 2.6. Cell culture

Alveolar human lung epithelial cells (A549, ATCC no. CCL-185, Lot: 60150896) were grown in Roswell Park Memorial Institute (RPMI)-1640 medium (Sigma Aldrich, R0883-500ML) supplemented with 10% fetal bovine serum (FBS) (Sigma-Aldrich, F9665), 1% L-glutamine (Sigma-Aldrich, G7513) and 1% penicillin-streptomycin solution (Sigma-Aldrich, P4458) under standard cell growth conditions (37 °C, 5% CO\(_2\) in an incubator (Thermo Electron Corporation, Hera cell 240). Cells with a passage number ranging from P13 – P15 were trypsinized, counted and resuspended after centrifugation for the treatment at air-liquid interface cultivation conditions (Fig. 1 H1).

### 2.7. Treatment at air-liquid interface cultivation conditions

In 12-well plates (Greiner Bio-One, 6651080) containing Thincert™ Tissue culture inserts (Greiner Bio-One, 665630, Density: 0.6 pore \(\times 10^6\) cm\(^{-2}\), Pore size: 3 μm, Culture surface: 113.1 mm\(^2\) \(0.5 \times 10^8\) A549 cells were added apically in 1 mL complete RPMI medium into the inserts (Fig. 1 H1). On the basolateral side, 1.5 mL RPMI medium was added. For cell growth and the formation of a cell monolayer on the apical side of the inserts, the 12-well plates were kept under standard cell growth conditions for 4 days. After 4 days, the A549 cell monolayer was transferred from submerged conditions to air-liquid interface (ALI) conditions by removal of the medium on the apical side and replacement of the used 1.5 mL RPMI medium with 1.0 mL fresh RPMI medium on the basolateral side. The A549 cell monolayer was maintained for 24 h ± 1 h at ALI conditions before exposure. For ALI exposure, the RPMI medium on the basolateral side was replaced with 1 mL fresh RPMI medium containing 70 μL of fiber and particle extract (75.3%) \(\geq 0.4 \mu m\) (DH, DM, DL) or \(\leq 0.4 \mu m\) SDE suspended in 10-fold RPMI medium concentrate (24.7% medium fraction plus additives, Sigma Aldrich, R1145) was added apically to the cell monolayer (Fig. 1 I1). A final fiber concentration of 0.3 ± 0.1 (Blank – ext. w/o textile), 7 ± 2 (SM A), 1841 ± 53 (RCT), 1400 ± 95 (uncoated), 1018 ± 30 (coated) and 151 ± 2 (filter) present within these 70 μL debris-medium-solutions high (DH). Cells were exposed to two additional debris concentrations DM and DL. The undiluted SDE were diluted only by addition of the medium fraction plus additives (75.3% final particle concentration) before cell culture exposure. The exposure time of 48 h ± 1 h took place under standard cell growth conditions (Fig. 1 H1).
and 5 parts phenolred free RPMI medium) was added apically. After 30–40 min at standard cultivation conditions, 3 times 100 μL supernatant (triplicates) was pipetted into 96-well plates to determine the absorbance at 490 nm using a plate reader (Berthold Technologies, Germany). (Fig. 1 D). Each acute toxicity assay contained negative controls (NC, n = 3) by which only RPMI medium (70 μL) was added to the A549 cell monolayer apically and absorbance readings were set to 100% viability for comparison of the other absorbance values (positive control and textile extracts, SI Table S8). The sensitivity of the assay was assessed with positive controls containing cadmium sulfate (CS) at three different concentrations each in triplicates (1 mM; 10 mM; 50 mM) in RPMI medium. The acute toxicity assay was repeated 3 times with different A549 cell passages (P13–P15, biological replicates, n = 3) and 3 independent washing extracts (N = 3), see SI Table S8.

3. Results and discussion

3.1. Fiber and particle release from masks and mask textiles

Personal protective facemasks have become part of our daily lives since the COVID-19 pandemic. The vast majority of these facemasks are assessed for splash resistance, particle filtration efficiency and air permeability prior to market launch. The fiber and particle release of mask textiles and potential adverse health effects have been little studied to this date. In our experiments, fibers were released from all synthetic (SM A, SM B, filter) and cotton-based (RCT, uncoated, coated) masks and mask textiles. To ensure comparability the results were stated per gram of textile. Fig. 2 summarizes the total release data of all the tested scenarios. For the estimation of the fibers and particles released during daily use of the medical facemasks and textile masks, the air-based Sheffield head extraction was performed in two scenarios, light to moderate breathing rates (14.2 & 30 L min⁻¹). In the first scenario, a Sheffield head based on DIN EN 149 [16] was used to investigate the release of larger fibers and particles (≥0.4 μm) collected on filter membranes, which are released during 8 mechanical exposures by donning and doffing the mask at constant airflow.

In this scenario, SM A released 6 ± 1 fibers per gram mask with a median size of 310 μm and a size distribution of (195–650 μm, 25th and 75th percentile) (Fig. 2). The Pro Mask size L green released 25 ± 7 fibers per gram mask with a median size of 660 μm and a size distribution of (413–1089 μm, 25th and 75th percentile) compared to 6 ± 4 fibers per filter with a median size of 256 μm and a size distribution of (201–768 μm, 25th and 75th percentile) collected from the environment without mask. Mechanical stress on the mask caused by repeated donning and doffing of the mask increases the amount of fibers (SM A: 1.5x; coated: 12.5x) compared to masks treated without additional mechanical exposure (SI Table S1).

In the second scenario, the release of possible inhalable particles (0.3–10 μm) was investigated on a Sheffield head simulating both inhalation and exhalation over a longer period of 8 h in a particle-free environment. In this scenario, an increased number of particles was released from all masks (3.2–6.9% of the total particle sum) during the first 3 min compared to the blank measurement without textile (1.1% of the total particle sum). In the case of the SM A, a sum (480 measurement timepoints) of 242 ± 132 particles with a median size of 300 nm (300–567 nm, 10th and 90th percentile) were released and in the case of the Pro Mask size L green 63 ± 25 particles with a median size of 300 nm (300–433 nm, 10th and 90th percentile) per gram mask (Fig. 2). Within the blank air measurements, an average of 6477 ± 5482 particles with a median size of 300 nm (300–400 nm, 10th and 90th percentile) were counted. Therefore, particles in the range of 300–567 nm were released in very low quantities (242 ± 132 for SM A and 63 ± 25 for coated textile per g) from masks. Based on the results, both masks capture more particles in the range of 0.3–10 μm from the ambient air than the masks

![Fig. 2. Fiber-/ particle release and length distribution of air-based and liquid extracts from masks and mask textiles. (A&B) Debris ≥ 0.4 μm. Fiber-/ particle counts per gram textile are shown as bars and relate to the y-axis (left) while the length distribution of the released fibers is shown as blue circles and relates to the second y-axis (right). (A) The air-based Sheffield head extraction of debris ≥ 0.4 μm was performed for 1 h and quantified with FiberApp. (B) The liquid extraction of debris ≥ 0.4 μm was quantified with FiberApp. (C&D) Debris ≤ 0.4 μm. Fiber-/ particle counts and lengths distributions are shown as described in (A). (C) Sheffield head extraction of debris 0.3–10 μm was performed for 8 h and quantified with an Abakus® air particle counter. (D) The liquid extraction of debris ≤ 0.4 μm was quantified with NanoSight.](image-url)
emit themselves. Additionally, it can be assumed that a proportion of these particles originated from the environment before the chamber was purged exclusively with particle-free air. The connecting hose from Sheffield head to the detector was rinsed with water as a test to exclude electrostatic deposition of the particles and fibers. The complete Sheffield head debris release results are shown in the SI Table S2.

Due to the low fiber and particle concentration released during the air-based Sheffield head extraction and the non-sterile sampling procedure, an additional liquid extraction of the mask textiles with GyrOwash was performed under sterile conditions. This worst-case scenario is intended to show the maximum concentration of extractable fibers and particles from mask textiles exceeding the amount of presumably inhalable released debris during daily wearing of protective masks. However, under the two air-based Sheffield head extraction scenarios, only a fraction (SM A: 1.1%; coated: 0.1%) of the fiber amount was released compared to the water-based extraction procedure.

Mainly fibers and large particles ≥ 70 μm present in this extract were filtered, photographed, subsequently counted with FiberApp software (version: 1.51) [14] and displayed in a size distribution chart (SI Table S5). Additionally, the particles present in the range of 50–400 nm in the soluble water extract were characterized with a NanoSight LM20. Within the blank sample 7 ± 3 fibers (>70 μm) with a median fiber length of 297 μm with a 75th percentile at 540 μm and a 25th percentile at 135 μm were counted within 120 mL of extract originating from the environment (Fig. 2). In the soluble blank water extract, 2.8 ± 3.4 × 10^7 particles with a median size of 294 nm and 80% of the particles ranging between 274 and 360 nm in size were counted. The coated textile consisting of one cotton based inner-/ and outer layer as well as a polypropylene based melt blown filter textile layer released on average 20 949 fibers ± 622 (≥70 μm) g^-1 textile and a median fiber length of 416 μm (234–650 μm, 25th and 75th percentile) was found (Fig. 2). The particle count g^-1 of coated textile yielded 1.4 ± 0.3 × 10^11 particles g^-1 textile with a median size of 135 nm and an 80% particle range between 100 and 273 nm. In regard of fiber and particle count and size distribution all tested cotton-based textiles yielded similar release results. The uncoated textile released 29 452 ± 1 996 fibers (>70 μm) g^-1 textile; 390 μm (189–702 μm, 25th and 75th percentile) median fiber length; 7.5 ± 1.7 × 10^10 particles g^-1 textile; 147 nm (87–210 nm 80% particle range) median size whereas the RCT released 20 728 ± 591 fibers (>70 μm) g^-1 textile; 540 μm (270–905 μm, 25th and 75th percentile) median fiber length; 1.1 ± 0.4 × 10^11 particles g^-1 textile, 125 nm (96–261 nm 80% particle range) median size via liquid extraction (Fig. 2). In comparison with the cotton-based textiles, synthetic melt blown mask fabrics from Welan and Livinguard released a lower amount of fibers with a smaller median fiber size as well as a slightly decreased particle amount with a particle size distribution similar to cotton. The SM A released 519 ± 153 fibers (>70 μm) g^-1 textile; 216 μm (108–378 μm, 25th and 75th percentile) median fiber length; 3.5 ± 1.5 × 10^10 particles g^-1 textile, 98 nm (81–166 nm 80% particle range) median size the SM B released 1 030 ± 115 fibers (>70 μm) g^-1 textile; 81 μm (78–225 μm, 25th and 75th percentile) median fiber length; particles g^-1 textile and median size was not determined and the filter textile released (9 638 ± 108 fibers (>70 μm) g^-1 textile; 130 μm (78–338 μm, 25th and 75th percentile) median fiber length; 1.5 ± 0.8 × 10^10 particles g^-1 textile, 132 nm (96–210 nm 80% particle range) median size via liquid extraction (Fig. 2).

To summarize, during water-based extraction, cotton-based textiles (uncoated: 29 452 ± 1 996 fibers g^-1 textile; coated: 20 941 ± 622 fibers g^-1 textile) show a higher fiber release compared to synthetic textiles (SM A: 519 ± 153 fibers g^-1 textile; SM B: 1 030 ± 115 fibers g^-1 textile). The amounts of fibers found for cotton fabrics are in a comparable range as described in the literature (3’500–4’500 fibers g^-1 textile [17]; 5’000–7’000 fibers g^-1 textile [18]; 9’000–14’000 fibers g^-1 textile [19]). Fiber release can be influenced by parameters such as mechanical stress during the washing process, reduced surface tension due to the use of detergent and temperature [17,18]. In terms of particle release ≤ 0.4 μm, comparable amounts were obtained for both cotton-based and synthetic textiles with liquid extraction. In a recent study, micro and nano scale polymeric fibers (size range: 25–2’500 μm) and particles (size range: 0.36–500 μm) were found in aqueous leachable extracts from all tested disposable plastic facemasks [8]. Furthermore, [20] reported a significant release of nanoplastic particles (<1000 nm) during washing and abrasion from polyester based synthetic textiles. Although the fiber quantity is increased during water-based extraction compared to air-based extraction, the quality of the released fibers in terms of median length distribution was similar, independent of the extraction method (air-based extraction: SM A: 310 μm; coated: 660 μm, water-based extraction: SM A: 216 μm; coated: 416 μm). Thus, the use of the water-based fiber extracts in the cytotoxicity assessment is likely to represent the type of fibers that might be inhaled.

### 3.2. Metal content of mask textiles

Both the mask textiles and aqueous textile extracts (wash-outs) obtained from these mask textiles were additionally tested for their metal content due to reports of possible metal particle contamination on protective masks [8] (Fig. 3). First, we determined the total metal content of fresh masks (metal mass per g textile dry mass) without any pre-treatment by washing. Different metals were detected in a low ppm concentration range in acid dissolved textiles. The sum metal content of calibrated elements (Cr, Co, Cu, Fe, Pb, Mn, Ni, Ag, Zn) was 43 ± 2 μg g^-1 for SM A, 8 ± 0.4 μg g^-1 for RCT and 16 ± 3 μg g^-1 for coated textile. This analysis includes metals accumulated from the environment by the cotton plant, metals from additives such as the paint applied to the masks, as well as metals from other sources in a particulate or non-particulate form [21]. Iron was dominant in cotton-based textiles. The Fe content of 7 ± 0.3 μg g^-1 for the RCT and the 7 ± 0.8 μg g^-1 for the coated textile is likely to be caused by biologically bound metals since the natural content of untreated cotton material was reported to be 20–90 μg g^-1 [22,23]. However the detected copper content of 41 ± 1 μg g^-1 for SM A and the 6 ± 0.3 μg g^-1 for the coated textile potentially originates from the blue- respectively green, black and white coloration of the mask layers [8,21]. Lead, a known toxic metal, was only found in sub ppm concentrations in all analyzed textiles with the highest value of 0.07 ± 0.01 μg g^-1 for the coated textile. It is known that a broader range and higher amounts of metallic elements is found in dyed textiles
In addition, the water and detergent-based wash extracts were examined to assess, if the different metals are mobilizable from the fabric either by dissolution or dispersion (SI Figure S6). A sum metal content of $0.07 \pm 0.09 \mu g^{-1}$ respectively $0.2\%$ compared to the total textile content (acid dissolved textile) for the SM A, $0.8 \pm 0.3 \mu g^{-1}$ (10% of total textile content) for RCT and $0.7 \pm 0.2 \mu g^{-1}$ (4.4% of total textile content) for coated textile was detected $g^{-1}$ of each textile within three independent ($N = 3$) textile washing extracts (Fig. 3, blue bars overlay represents the metal content of water-based wash extracts). A graphical illustration of the percentage of each metal element leached from the analyzed textiles in comparison to the mask total metal content is included within the SI Figure S6.

In summary, the water and detergent-based wash extracts indicate that only a limited amount of metals is released from the textiles, which originate either from water-soluble metallic compounds or from water-insoluble metal particles. Copper was detected in elevated concentrations in dyed textiles in accordance with previous studies [8,23]. However, the suspected copper-containing dye was immobilized in the fibers [25,26] and therefore could only be removed with water to a very small extent (<1.2%).

### 3.3. Acute in vitro cytotoxicity of mask textile debris

An in-depth characterization of the released fibers and particles was not foreseen during this study. However, the mask extract debris collected under sterile condition were applied in different concentrations to an in vitro acute alveolar human epithelial cells (A549) toxicity assay to assess their potential acute toxicity. The fractions tested included both fairly large fibers $> \mu m$ (inhaleable but not reaching the deeper airways of the lungs) and smaller $\leq \mu m$ potentially respirable particles [27]. During the acute toxicity assay $2 \times 10^5$ (coated textile) and $0.8 \times 10^5$ (SM A) times more fibers where applied even within the lowest DL concentration compared to the more realistic Sheffield head scenario (fibers exposed on a defined insert area compared to mask fiber release by air to the lung surface area). The performed T-test analysis ($p$-value $\leq 0.01$) revealed no significant acute toxicity (equivalent to a decrease in viability compared to the negative control - NC) for any analyzed mask textile debris (D; DH, DM, DL) or extract (SDE) in the tested concentration range (Fig. 4 SI Table S8). The lowest viability (90.7%) in a textile debris sample was reported for coated DM concentration whereas the highest viability (104.5%) yielded from the filter SDE. Cell viability deviations of $\pm 10\%$ (90–110% viability) are likely caused by biological cell assay variance rather than a mild acute cytotoxicity of the sample and are therefore not considered to be toxic. The sensitivity of the assay was proven by three different concentrations of cadmium sulfate (CS or CdSO$_4$) as positive controls, which resulted in a significantly decreased cell viability of $83.1 \pm 6.2\%$ (1 mM CS, **$p \leq 0.01$), $4.2 \pm 1.5\%$ (10 mM CS, $p \leq 0.001$) and $-0.9 \pm 0.7\%$ (50 mM CS, ***$p \leq 0.001$) (Fig. 4). The in vitro acute cytotoxicity assessment does not allow prediction of possible long-term exposure effects. Further research should focus on the in depth chemical characterization of inhalable and respirable particles ($\leq 5 \mu m$) and the long-term toxicity assessment of these mask debris on in vitro and in vivo lung exposure models. In a review by [28] it was concluded that the current studies are inconclusive in regard of toxicity of cellulose nanofibers and cellulose nanocrystals in both in vitro and in vivo lung exposure models. Furthermore, a correlation between acute and long-term (repeated) dose is not compulsory since for instance oxidized graphene derivatives (10–800 nm) show an acute mouse in vivo peak toxicity on day 2 and clearance effects resulted in no- to mild-toxicity after repeated dosage at the endpoint of 3 months [29].

In conclusion, this research quantified for the first time the fiber and particle debris release of textile masks. During a normal wearing habit scenario, only a limited quantity of fibers and particle debris was released. Natural cotton based textile released slightly more debris in comparison with synthetic mask textiles. Although high mask debris doses show no acute in vitro cytotoxicity to human lung cells, further research is needed on long-term toxicity, co-exposition and under asthmatic conditions.

Ethics approval and consent to participate.
Not applicable as no human participants or research material requiring ethics approval were involved in the study.
Consent for publication.
Not applicable since no human participants were involved.
Availability of data and materials.
Data supporting this study are provided in this article, the supporting information, the source data file or upon reasonable request to the corresponding authors. Textile fabrics can be provided on request by Livinguard AG.

**Competing interests.** Philipp Meier reports financial support and equipment, drugs, or supplies were provided by Livinguard AG.
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.

Acknowledgments

We thank Jörg Gschwend and Stefan Gfeller from Empa for designing and construction of the customized Sheffield head filter membrane holder for the fiber analysis.

The textile mask manufacturer Livinguard AG founded this research.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2022.107364.

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