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RESEARCH ARTICLE

REVISED Exposure assessment and risks associated with wearing silver nanoparticle-coated textiles

[version 2; peer review: 3 approved, 1 approved with reservations]

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Abstract

Background

Silver (Ag) nanoparticles (NPs) are used increasingly in consumer and healthcare fabrics due to their antimicrobial properties. Abrasive leaching experiments have shown that AgNPs can be released during textile wear and cause a dermal exposure. Derived-no-effect-limit value for AgNPs ranges from 0.01 to 0.0375 mg/kg-body-weight, and thus, low exposures levels can cause relevant risk.

Methods

In this study AgNP release from textiles by artificial sweat immersion and mechanical stress was investigated. A mass balance model was used to calculate dermal Ag exposure and potential intake via percutaneous absorption and inadvertent (peri-)oral intake during wear of face mask, suit with a full body exposure and gloves. Mass flow analysis was performed for up to 8-h wear time and by using Ag penetration rate constants reported for fresh-, cryopreserved- and glycerolized skin grafts.

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Results

Dermal intake risk characterization ratio (RCR) during 8-h wear time for glycerolized skin was up to 0.02 for face mask and 0.9 for full body wear in a worst-case condition. Wearing gloves for 1-h followed by single unintentional fingertip mouthing (contact area 11.5 cm^2) resulted in an RCR of 0.0002. RCR varied depending on the type of textile-product, exposure wear duration and skin type.

Conclusions

This study provides a comprehensive assessment of AgNPs release from textiles and their potential impact on human dermal exposure and was essential for understanding the safety implications for different exposure scenarios and mitigating potential risks.

Plain language summary
Silver (Ag) nanoparticles (NP) are widely used in textiles designed for general population and healthcare. However, there is ongoing research and concern about the potential health risks associated with the release of nanoparticles into the environment. Here, the risk was studied for face mask, gloves and a full body suit use scenarios and for different skin types and Ag NPs to identify realistic exposure scenarios that can lead to excessive risk. The major risk was associated with unintentional finger mouthing after wearing gloves containing AgNPs. However, the current release test settings are not designed for NP release assessment from textiles during realistic use scenarios. This study emphasizes following good hygiene practices when wearing Ag containing textiles to avoid intake via oral exposure.

Keywords
Nanoparticles, dermal exposure, mass balance, release, dermal intake, risk characterization ratio, conditions of use, REACH

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REVISED **Amendments from Version 1**

We have added more detailed description for the washing procedure. Details for analytical methods were added. Minor additions were included to the main text related to references, intake, environmental exposure and the model applicability for occupational exposure scenarios.

Any further responses from the reviewers can be found at the end of the article

List of abbreviations

ASINA	Anticipating Safety Issues at the Design Stage of NAno Product Development
Ag	Silver
NP	Nanoparticle
DNEL	Derived-No-Effect-Limit
RWC	Reasonable Worst-Case
RCR	Risk Characterization Ratio
AgCur	Silver nanoparticles, coupled with Curcumine
AgHEC6.4	Silver nanoparticles, embedded in cationic quaternized hydroxyethylcellulose matrix (DoE optimized molar ratio HEC/Ag:NaOH/Ag 6,4:1,4)
AgHEC	Silver nanoparticles, embedded in cationic quaternized hydroxyethylcellulose matrix (patented molar ratio HEC/Ag:NaOH/Ag 5,5:2,8)

Introduction

Silver (Ag) nanoparticles (NPs) are used in antimicrobial textiles, such as face masks and gloves, personal clothing, and wound dressings for their ability to inhibit the growth of microorganisms (Abazari *et al.*, 2023; Novi *et al.*, 2022; Salleh *et al.*, 2020). However, concerns have been raised regarding the potential intake via long term dermal exposure (Ferdous & Nemmar, 2019; Shah *et al.*, 2022; Yetisen *et al.*, 2016). In response to these concerns, regulatory actions have been taken in Europe where the European Commission (EC) has restricted the use of Ag NPs as a biocidal substance in fiber, leather, rubber and polymerized materials preservatives (EC, 2021). This regulatory measure reflects the need to mitigate potential risks associated with the widespread use of Ag NPs in consumer products.

Dermal exposure to NPs can occur via deposition from air to skin, intentional application of a product to skin or contact with articles containing NPs. NPs at the skin surface may be removed by volatilization, debridement, sweating, washing, and percutaneous absorption. Main pathways for dermal uptake are percutaneous absorption, direct absorption through damaged skin, or indirect uptake via inadvertent ingestion (Anderson & Meade, 2014; Gorman Ng *et al.*, 2016; Li *et al.*, 2021). Indirect exposure pathways are for example hand-to-object contact followed by finger mouthing or object-to-mouth contact for example when wearing a face mask. Dermal exposure mechanisms are well known and various mechanistic dermal exposure models are developed (e.g., Ernstoff *et al.*, 2016; Kvasnicka *et al.*, 2020; Li *et al.*, 2021).

The skin barrier is composed of the epidermis and dermis (Nafisi & Maibach, 2018). The epidermis is made of keratinocytes and non-keratinocytes primary cells that are stratified in layers as *stratum corneum* (the outermost layer) and viable skin of *stratum lucidum*, *stratum granulosum*, *stratum spinosum* and *stratum basale* (on top of dermis). Intake via percutaneous absorption is usually considered the fraction that penetrate into *stratum corneum* (Cleek & Bunge, 1993). From *stratum corneum*, the chemical can permeate to the viable skin and enter the systemic circulation that is considered as uptake. A simplified concept for dermal absorption across the *stratum corneum* is presented by van der Merwe *et al.* (2006). Chemical permeation is typically measured *in vitro* by using diffusion cell and skin samples with only *stratum corneum* and epidermis (ECHA, 2020; OECD, 2004).

Currently, there are no sophisticated mathematical models for predicting absorption of NPs through different layers of the skin (ECHA, 2020). This is because dermal absorption models are designed for neutral and low-molecular weight molecules whose dynamics are described with kinetic molecular theory which is not applicable for colloidal NP systems (Praetorius *et al.*, 2014). NP absorption becomes a complex phenomenon with diverse properties of NPs. The permeability is also effected by the properties of the vehicle and the structure and properties of skin along with their interactions (Alikhan *et al.*, 2009). Since the physical and chemical properties affecting dermal absorption of NPs are still undefined, permeability testing on a case-by-case basis is recommended (ECHA, 2020).

Empirical studies have indicated that dermal absorption through diffusion is minimal for compounds with molecular weights exceeding 500 g/mol (Buist *et al.*, 2017). Consequently, this phenomenon can be regarded as negligible for nanoparticles (NPs) with molar masses on the order of 10^6 g/mol and particle diameters around 10 nm. It has been shown that NPs can penetrate the skin via intra- or intercellular routes and transappendageal routes via hair follicles and sweat ducts (Larese Filon *et al.*, 2015). Particles that are not washed off generally remain in the upper layer of the epidermis, although there is evidence that some of the particles also reach the dermis. Potential risks associated with dermal exposure to insoluble NPs are (Brouwer *et al.*, 2016; Larese Filon *et al.*, 2016; Larese Filon *et al.*, 2015):

- ≤ 4 nm NPs can penetrate and permeate intact skin,
- 4 nm $< D_p \leq 20$ nm can potentially permeate intact and damaged skin,
- 20 nm $< D_p \leq 45$ nm can penetrate and permeate only damaged skin,
- > 45 nm NPs cannot penetrate nor permeate the skin.

Additionally, metallic NPs may dissolve and cause local effects or penetrate the skin in ionic form and cause systemic effects (Zanoni *et al*., 2021). Impurities present in NPs can cause both localized and systemic adverse effects. Also, NPs permeability may be better for non-rigid NPs.

Estevan *et al*. (2022) estimated a risk related to wear of a face mask containing Ag NPs by the general population considering both daily use for 2 h/day and in workplace use for 8 h/day. The dermal exposure was calculated for the face mask with a contact surface area of 555 cm^2 face mask containing 113 µg-Ag/cm^2. It was assumed that Ag NPs in the textile are immediately released to skin surface. Dermal flux was set to correspond Ag NP penetration in a glycerolized human skin (3.8 ng/cm^2/h) that is similar to necrotic skin (Bianco *et al*., 2014). Under these worst-case conditions described above, the risk ratio was ≤0.014 and the risk was adequately controlled. The approach is applicable for face mask safety assessment, but a more detailed approach is needed in a generic exposure scenario with longer wear times and larger contact surfaces by wearing other clothing containing Ag NPs.

In this study, a more realistic approach considering Ag release rates from textile to skin is considered rather than assuming instantaneous release and intake time trends. We demonstrate how Ag NP leaching from textile affects the Ag NP dermal exposure and uptake, while also assessing the role of unintentional oral exposure and the impact of hand/body washing in reducing exposure. The assessment was performed for antimicrobial textiles developed in Anticipating Safety Issues at the Design Stage of NAno Product Development (ASINA) for face mask wear and a full body exposure that is considered as the reasonable worst-case (RWC) exposure assessment. The simulations are performed for RWC for i) general population wearing face mask and ii) worst-case occupational exposure scenarios during wear of face mask and a full-body suit containing Ag NPs that are in contact with body with dermal exposure surface area of 20 000 cm^2 (2 m^2). Overall, by considering realistic exposure scenarios and incorporating factors such as leaching rates, unintentional exposure pathways, and the effectiveness of washing practices, the study aims to provide valuable insights into the potential risks associated with the use of Ag NP-containing textiles.

2. Methods

2.1 Dermal exposure model

Here non-volatile and inert NPs absorption to the skin via direct contact, and removal from the skin by wear and washing, and finger-to-mouth transfer mechanisms are considered. By considering these absorption, removal, and transfer mechanisms, the study aims to provide an understanding of fate and potential exposure pathways. The change in NP mass m (mg) on the skin surface over the exposure duration t (h) can be described with:

$$\frac{dm(t)}{dt} = S_p(t) - \left(k_{sc} + k_o + k_{ww}\right) \times m(t) \quad (1)$$

Where $S_p(t)$ (mg/min) is the NP application rate from a product p and rate constants k_{sc} (1/min), k_o (1/min) and k_{ww} (1/min) respectively account for the NP losses from the skin surface by transfer to *stratum corneum* (*sc*), inadvertent oral exposure (*o*) and wear and washing (*ww*). All rate constants are considered as net rates and the transport from receiving receptor back to the donor is insignificant.

2.1.1 Emission source $S_p(t)$. The emissions from the product to skin can be described by using the product initial NP mass m_p (mg) and NP transfer rate from the product to the skin as:

$$S_p(t) = k_p \cdot m_p(t) \cdot (1 - e^{-k_p t}), \quad (2)$$

Where the k_p (1/min) is the first order loss rate constant and the product initial mass concentration $m_p(t{=}0) = m_{p,0}$ can be calculated from the amount of product in contact with skin AP (g), the product NP concentration C (mg/g) and the fraction of NPs available for dermal exposure f_a (-), which is set to 1 corresponding to a situation where all NPs are available for dermal exposure.

Emission source for dermal exposure can be categorized according to the emission pattern as an instant-, continuous-, or varying emitter. For instant emission, all product is instantaneously in contact with skin (e.g., applying lotion), i.e., k_p is very high and $S(0) \approx m_0$. For a continuous emitter, the transfer rate is small compared to the contact period and the mass loss from product is small, *i.e.,* $m(t) \approx m_0$. Otherwise, the NP mass in the product decreases according to the emissions when assuming that there is no other loss mechanism than transfer to skin.

Indirect dermal exposure describes dermal exposure when skin is in contact with contaminated surfaces. This is described with an instant application source where the mass transfer is described with surface contamination m_s (mg/cm^2), dermal contact area A_d (cm^2) and transfer efficiency TF (-) estimating the fraction transferred from the contaminated surface to the skin.

2.1.2 Assessment of the rate constants k_{sc}, k_o, and k_{ww}. The rate constants can be estimated individually by measuring initial NP mass in the product and the NP mass in the receiving compartment. When $S(t) = 0$ and x is the only significant removal process, the rate constant for process x can be calculated as:

$$k_x = -\frac{1}{t} \ln\left(1 - \frac{m_x(t)}{m(t=0)}\right) \quad (3)$$

Where m (mg) and m_x (mg) are the donor and acceptor (receiving compartment) masses, respectively.

2.1.3 Mass transfer via stratum corneum k_{ss} (intake). Ag NP penetration varies from $0.27{\times}10^{-6}$ to $34{\times}10^{-6}$ depending on the skin type (Table 1). In this evaluation a precautionary assessment and penetration rate constants measured by Bianco *et al*. (2014) were used.

Table 1. Reported median penetration fluxes for Ag NPs under different conditions.

Study	Skin type	Exposure media	AgNP flux penetration, [ng/cm²/h]	Lag time, [h]	Rate constant, k_{sc}, [1/h]
(Bianco *et al.*, 2014)	Fresh human skin graft	Ag NPs (19±5 nm) in synthetic sweat using a Franz diffusion cell apparatus for 24 h. The donor solution surface load was 113 µg-Ag/cm².	0.2	8.2	1.8×10^{-6}
	Cryopreserved human skin graft. Skin viability is reduced by 36% compared to fresh skin		0.3	10.9	2.7×10^{-6}
	Glycerolized human skin graft. Comparable to necrotic skin from a morphological and structural point of view		3.8	6.3	34×10^{-6}
(Larese Filon *et al.*, 2009)	Intact skin graft.	Ag NPs (25±7.1 nm) in synthetic sweat using a Franz diffusion cell apparatus for 24 h. The donor solution surface load was 70 µg-Ag/cm².	0.02	<1	0.27×10^{-6}
	Damaged skin graft abraded according to the Bronaugh and Stewart (1985) protocol		0.10	<1	1.4×10^{-6}

2.1.4 Mass transfer via inadvertent oral exposure k_o. Two mechanisms are responsible for mouthing mediated ingestion:

1) Surface-to-hand contact followed by hand-to-mouth contact, and

2) direct mouthing of objects and ingestion of contaminants (Li *et al.*, 2021).

Relevant parameters for inadvertent oral exposure are surface loading (mg/cm²), transfer efficiency (-), mouthing frequency (1/h) and contact surface area (cm²). The transfer efficiency of NPs in hand-to-mouth contact is scarcely studied. Surface-to-hand studies showed that zinc oxide NPs transfer efficiency is *ca.* 30 times higher than for micron sized zinc oxide particles from metal and wood surfaces (Brouwer *et al.*, 2016). For powders, the hand-to-mouth transfer efficiency can be nearly complete (Ng *et al.*, 2012). The default surface area of both hands varies from 840 to 900 cm² for males (OEHHA, 2008; te Biesebeek *et al.*, 2014). Fingers mouthing frequency is studied in occupational environments (Gorman Ng *et al.*, 2016). Consumers finger mounting is studied only for children and infants: Surface area of fingers contacting accidentally mouth during fish tackle handling was estimated to be 19 cm² (three 3 fingertips and that each fingertip is 30% of the finger) and the contact frequency was assumed to be 9 times per hour (OEHHA (2008). For adults, the average surface area for three fingertips per hand is 11.5 cm² (range: 9.3–14.4 cm²; $n = 12$) (Sahmel *et al.*, 2015).

2.1.5 Inadvertent oral ingestion, intake, and uptake. Intake via inadvertent ingestion from hand to mouth contact can be estimated from the dermal load, contaminant transfer efficiency from hand to oral or perioral region and number of contacts (Ng *et al.*, 2012). In ingestion, the intake is assumed to be complete, *i.e.* $F_{ret} = 1$, and the uptake can be estimated when the absorption of the substance from gastrointestinal tract is known. This model is applicable for solids, powders, pastes, gels and liquids including products with nanomaterials (SCCS, 2019). The retention factor is usually poorly known, that is the main limiting factor considering the model reliability. NP absorption across intestine, *i.e.* uptake, can be calculated by using simplified mechanistic models when NPs permeability is derived from Caco-2 or Ussing chamber experiments (Lundquist & Artursson, 2016; Ölander *et al.*, 2016).

2.1.6 Mass transfer via wear and washing k_{ww}. NPs loss from skin by wear and washing is scarcely studied. Hand washing frequency in general population is *ca.* 8 times per day according to SDA (2005). NP removal efficacies by wear or washing are not available. Here, wear is not considered, and washing is simulated by using exposure time. These removal pathways can be applied when information on rate constants is available and if there is need for more detailed exposure assessment.

2.2 ASINA Ag NP textiles

Polystyrene textile (Klopman International, Vektron 8200; weight 145 g/cm²) were coated with Ag NPs by using a spray coating system as described by Del Secco *et al.* (2022). The coating suspensions consisted of Ag (Sigma Aldrich, Milan, Italy) capped with hydroxyethylcellulose (Univar Solutions SpA, Milan, Italy) (AgHEC), hydroxyethylcellulose with changed molar ratio (AgHEC6.4) or adding curcumin (AgCur) as capping agent instead of HEC, all dispersed in water at concentrations of 0.1% w/w. The AgHEC, AgHEC6.4 and AgCur aqueous nano suspensions were produced by CNR- ISSMC (Faenza, Italy) using a patented production process (patent no. WO2016125070A1). Three different Ag NPs were applied to textiles by using the spray coating technique (Table 2). The Ag concentration in each textile was measured by using inductively coupled plasma mass spectrometry according to ISO 11885:2007 (elemental concentration in different media).

Table 2. Textile coating parameters and Ag load in textile. Ag concentration on coating solution is 0.1 wt% for all samples. Plasma neutralization during spray coating is shown with (P).

Sample code	Embedding material	NM concentration on solution [wt%]	Embedding concentration on solution [wt%]	Flow rate [mL/min]	R2R speed [m/min]	Ag concentration [ng Ag/cm²]
AgCurA	Curcumin	0.1	0.06	60	6	849 ± 31
AgCurB	Curcumin	0.1	0.06	80	6	1444 ± 203
AgCurC	Curcumin	0.1	0.06	60	4	1530 ± 151
AgCurC(P)	Curcumin	0.1	0.06	60	4	1147 ± 165
AgCurD	Curcumin	0.1	0.06	80	4	1957 ± 22
AgCurE	Curcumin	0.1	0.06	60	2	2258 ± 14
AgHEC6.4A	HEC6.4	0.1	1.07	60	6	1149 ± 144
AgHEC6.4B	HEC6.4	0.1	1.07	80	6	1222 ± 49
AgHEC6.4C	HEC6.4	0.1	1.07	60	4	1538 ± 41
AgHEC6.4C(P)	HEC6.4	0.1	1.07	60	4	1529 ± 82
AgHEC6.4D	HEC6.4	0.1	1.07	80	4	1861 ± 96
AgHEC6.4E	HEC6.4	0.1	1.07	60	2	2651 ± 399
AgHECC	HEC	0.1	0.92	60	4	1311 ± 671
AgHECC(P)	HEC	0.1	0.92	60	4	1406 ± 318
AgHECD	HEC	0.1	0.92	80	4	1574 ± 302

2.3 Textile washing

The washing simulation was based on the "ISO 105-C06 (A1S) for colour fastness to domestic and commercial laundering (2010)". The tests were performed with a lab washing machine (Linitest Plus, Atlas Electrical Device); motor speed 40 ± 1 rpm, steel vessels (75 ± 5 mm diameter, 125 ± 10 mm height, 550 ± 50 mL) and a washing solution of 4 ± 0.01 g L^{-1} ECE Colour Fastness Test Detergent 77 in deionized water (pH 10) and ten steel ball (Ø = 0.6 cm) to simulate the mechanical energy applied by friction to the textiles. Five pieces of each textile type were cut with an area of 40 cm^2 per piece and stacked in the vessel. A volume of 150 mL of washing solution was added to the vessel, together with the steel balls and the textiles. In order to do a conditioning of the washing solution inside the vessels at the same temperature as the washing machine, preheat of the washing solution at 40°C was doneunder stirring at 300 rpm for 30 min.

After the washing process, the washing water was collected, and the textiles were rinsed one by one dipping them five times in 10 mL of deionized water. Once this step was finished, the water was renewed to avoid accumulation of silver released in water. Finally, the textiles were placed on a paper, covered, and dried overnight at room temperature.

The washing water was filtered at different pore size (20 µm pore size, 0.45 µm pore size and 10 kDa, sequentially) and stored for electron microscopy characterization and silver quantification by using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). For the ICP-MS, 0.5 g of liquid sample is taken to perform an acid digestion with 5 ml of concentrated ultrapure nitric acid (HNO_3 70%) in an analytical microwave system at 250°C. Then, the obtained digestion residue is properly diluted to analyze the elements of interest by ICPMS. The quantification is performed by interpolation in a calibration curve prepared with commercial standards of the elements of interest. Electron microscopy images were treated by ImageJ (Rasband, 2018)."

2.4 Abrasion tests

The simulation of a textile touching the skin was based on an adaptation of BS EN ISO 105-X12:2016 - Tests for color fastness Colour fastness to rubbing. The test was performed using a crock-meter (302-P, JBA). The equipment applies low energy wear on a 10 cm surface by a cylindric tip (ø = 16 mm) covered with cotton tissue. Before starting the experiment, calibration of the tip pressure was done by altering the weight until no pressure is applied on surface. Then, a 9 N weight was applied to the tip. Pieces of textile were cut at an approximate area of 40 cm^2 and stuck by double face tape into the dedicated spot for rubbing. A cotton napkin was dipped in a simulated sweat solution at pH 6.5 (0.5 wt% sodium chloride, 0.1 wt% lactic acid, 0.1 wt% urea) and rinsed for 1 min before covering the crock-meter tip. A total of 10 rubbing cycles

(1 second/cycle) were performed by triplicates to unwashed textiles. The cotton napkin was replaced every 10 cycles. Characterization was performed to the cotton tissue (NM release receiving compartment) using ICP-MS.

Characterization was performed to the rubbered textiles (remaining NM concentration) and to the cotton tissue (NM release receiving compartment) using ICP-MS and SEM techniques. For the ICP-MS analysis (limit of detection of 5 mg NM/kg textile), all sample were weighed to perform an acid digestion with 3 mL of concentrated ultrapure nitric acid (HNO_3 70%) plus 1 mL of concentrated ultrapure hydrofluoric acid (HF 48%) in an analytical microwave system at 250°C. Then, the obtained digestion residue is properly diluted in order to analyse the element of interest (Ag) by ICPMS. The quantification is performed by interpolation in a calibration curve prepared with commercial standards of the elements of interest.

2.5 Risk characterization

Ag penetrated to *stratum corneum* is assumed to enter blood circulation immediately. Intake is normalized using 60 kg body weight (bw) according to the U.S. EPA (2011) recommendation. Estimated systemic derived-no-effect-limits (DNELs) ranged between 0.01 and 0.0375 mg/kg-bw/day for general population and between 0.02 and 0.075 mg/kg-bw/day for occupational settings (Estevan *et al.*, 2022). The risk characterization ratios (RCRs) were calculated by using bw-normalized daily intake divided with the lowest DNEL for general population (0.01 mg/kg-bw/day).

3. Results and discussion

3.1 Model comparison

Dermal model was compared with the dermal exposure model by von Goetz *et al.* (2013) They measured Ag releases for commercial Ag NP coated textiles in 30 min artificial sweat immersion and mechanical stress test based on modified ISO method 105-C06 for "color fastness to domestic and commercial laundering". The release fractions and release constants calculated here (Table S1, *External data*; Koivisto *et al.*, 2024) were:

a) T-shirt, 83% polyester and 17% wool textile containing Ag 183 mg/kg: Release was 6.8% for acidic sweat and 5.0% for alkaline sweat corresponding to average release constant of 0.0017 1/min.

b) Trousers, 93% polyamide and 7% elastane containing Ag 41 mg/kg: Release was 13.2% for acidic sweat and 14.0% for alkaline sweat corresponding to average release constant of 0.0050 1/min.

Von Goetz *et al.* (2013) model is based on Ag leaching to artificial sweat and sweat generation that human generates during activity. The model calculates dermal exposure as Ag mass normalized with body weight by considering male and female physiological parameters (Table S1, *External data*; Koivisto *et al.*, 2024). Von Goetz *et al.* (2013) calculated dermal exposure for 60-min sport scenarios for male and female during wear of T-shirt and trousers having dermal contact area of 0.69 and 0.345 m^2, respectively. Average dermal exposure was 1235 and 659 µg for male and female, respectively, without body weight normalization.

We reproduced the sport scenario for female by using average release constants and assuming that the textiles and skin are sweaty during the 60-min period (Figure 1 and Table S1, *External data*; Koivisto *et al.*, 2024). Dermal exposure was 2848 µg after 60-min exposure which is 4.3 times higher than average exposure calculated for von Goetz *et al.* (2013). Dermal intake was calculated by assuming that Ag absorption is the same as Bianco *et al.* (2014) reported for fresh skin. The intake was 2.6 ng corresponding to 0.043 ng/kg-bw (60-kg bw) corresponding to RCR of 4.3×10^{-6} (DNEL of 0.01 mg/bw-kg/day). Differences in dermal exposure are related to different approaches. Von Goetz *et al.* (2013) associate release to sweat generation while here the skin and textiles are considered as

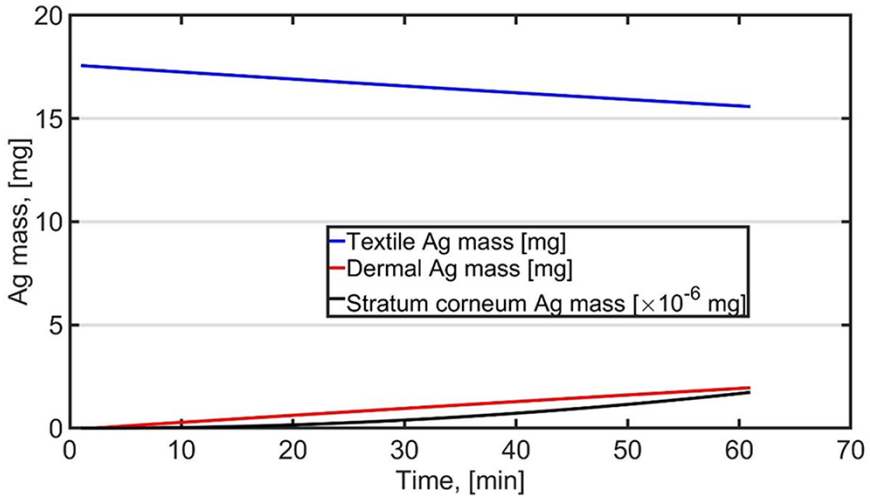


Figure 1. Dermal exposure and intake for female when wearing T-shirt and trousers during 60-min sport activity as presented by von Goetz *et al.* (2013).

sweaty all times and release is independent of amount of sweat generated.

3.2 Soft abrasion release test

Release rate constant is calculated using Equation (3), where time is 10 sec, m corresponds to the initial Ag concentration and m_x to the release for unwashed textiles. Concentration is equivalent to mass, as surface area is not changing through the release or washing. Release constants for unwashed textiles were 0.086, 0.12, and 0.15 1/min for AgCur, AgHEC6.4, and AgHEC textiles, respectively (Table 3). The release constants obtained here are 25 to 44 times higher than average release rate constant measured by von Goetz *et al.* (2013) for acidic and alkaline sweat (0.0034 1/min). This can be explained by different textiles, coating techniques, and release test. It is also expected that the manufactured textile batch curation time was too short due to the manufacturing conditions that were designed primarily for evaluating process emissions and workers exposure under different operational conditions (unpublished).

3.3 Dermal exposure and intake via stratum corneum

Average release rate constants for unwashed textiles were used to calculate the intake and risk during wear of nano-Ag coated textiles (Table 1). Calculation parameters for all scenarios are presented in Table S1 as *Extended data* (Koivisto *et al.*, 2024).

Release and intake of Ag NPs was calculated for 8-h wear of face mask for fresh skin, cryopreserved skin and glyzerolized skin (Figure 2). During the first hour, 99% of the Ag NPs are released from the textile to skin. During 8-h wear in fresh skin 0.0014% is penetrated to *stratum corneum* (Figure 2A). Absorption of Ag NPs in cryopreserved skin was 1.5 times higher than in fresh skin while the absorption for glycerolized skin was 19 times higher than in fresh skin (Figure 2B). In fresh skin and cryopreserved skin, the absorption was in similar range ranging from 11 to 19 µg while for glycerolized skin the absorption was significantly higher ranging from 212 µg (AgCur) to 245 µg (AgHEC6.4 and AgHEC) (Figure 2C). This was mainly caused by higher release rate constants because the average Ag NP initial mass concentrations were similar (1530, 1658 and 1430 ng/cm^2 for AgCur, AgHEC6.4 and AgHEC, respectively). The effect of wear time without hand washing was investigated for fresh skin during 8-h, 2-h and 30-min wear times (Figure 2D). The 8-h and 2-h wear times resulted to similar intake levels and 30-min wear time reduced the intake by 1 ng.

RCR was investigated for 8-h wear of face mask (A=555 cm^2) and full body exposure (A=20 000 cm^2) (Table 4). The highest RCR for face mask was 0.02 and for full body exposure 0.9 for glycerolized skin indicating adequately controlled exposure. Full body exposure for glycerolized skin representing necrotic skin is a worst-case exposure scenario and is an upper limit for exposure (tightly fitted clothing, sweating, and

Table 3. Ag release rate by abrasion for unwashed textiles (textile mass 145 g/m^2, release is for 10 second abrasion).

Sample code	Ag load in textile, ng/cm^2	Release to receptor, ng/cm^2	Release during 10 s, %	k_a, [1/min]
AgCurA	849	17.6	2.1	0.13
AgCurB	1444	12.6	0.9	0.053
AgCurC	1530	19.1	1.2	0.075
AgCurC(P)	1147	17.9	1.6	0.094
AgCurD	1957	27.6	1.4	0.085
AgCurE	2258	31.7	1.4	0.085
Average (standard deviation)	**1531 (471)**	**21.1 (6.5)**	**1.4 (0.4)**	**0.086 (0.022)**
AgHEC6.4A	1149	25.0	2.2	0.13
AgHEC6.4B	1222	33.1	2.7	0.17
AgHEC6.4C	1538	34.1	2.2	0.13
AgHEC6.4C(P)	1529	16.4	1.1	0.065
AgHEC6.4D	1861	40.7	2.2	0.13
AgHEC6.4E	2651	47.3	1.8	0.11
Average (standard deviation)	**1658 (501)**	**32.8 (10.0)**	**2.0 (0.5)**	**0.12 (0.031)**
AgHECC	1311	35.9	2.7	0.17
AgHECC(P)	1406	23.3	1.7	0.10
AgHECD	1574	49.7	3.2	0.19
Average (standard deviation)	**1430 (442)**	**36.3 (8.9)**	**2.5 (0.5)**	**0.15 (0.033)**

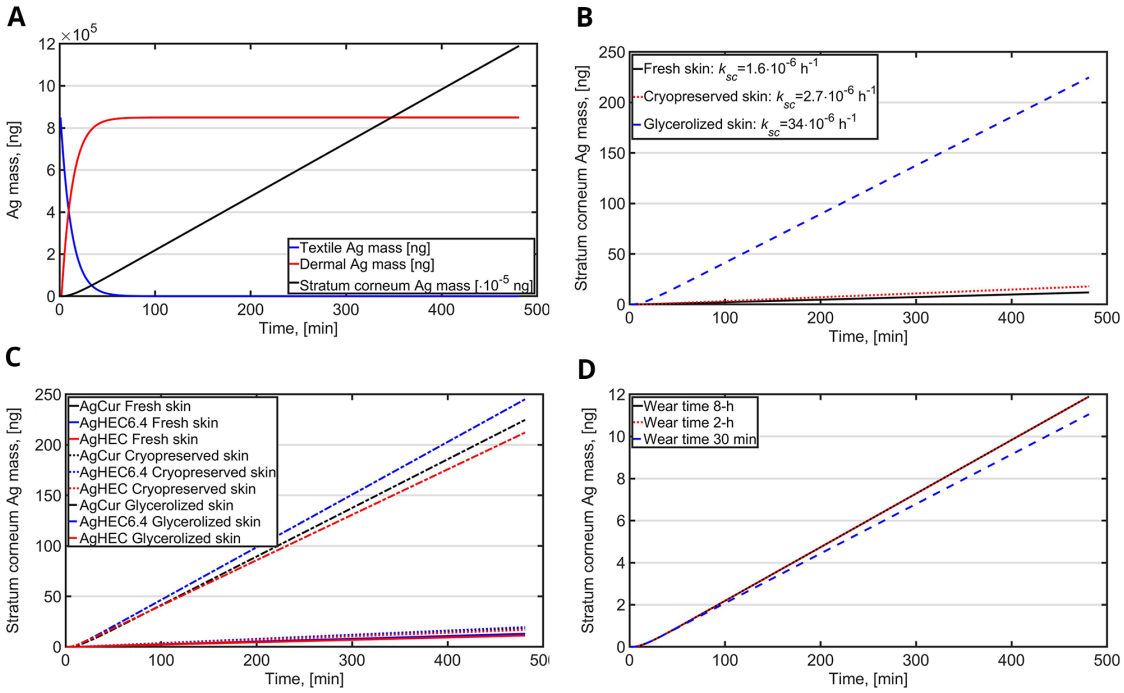


Figure 2. Dermal exposure and intake for face mask with surface area of 555 cm². A) An example of Ag release form AgCur coated textile to dermis and penetration to *stratum corneum* through fresh skin (healthy skin) during 8-h wear, **B**) An example of the effect of skin type on dermal penetration of Ag to *stratum corneum* for AgCur coated textile during 8-h wear, **C**) Dermal intake via *stratum corneum* during 8-h exposure for different textiles and for different skin types, and **D**) The effect of wear time (dermal exposure) for AgCur coated textile within 8-h duration for 8-h continuous wear, 2-h wear, and 30 min wear (fresh skin).

Table 4. Ag intake via *stratum corneum* during 8-h exposure and RCRs for different textiles and for different skin types. RCRs are calculated by using a DNEL of 0.01 mg/bw-kg/day (bw = 60 kg) for the general population.

Textile	Skin type			RCR (Intake/DNEL)		
	Fresh skin, [µg]	Cryopreserved skin, [µg]	Glycerolized skin, [µg]	Fresh skin	Cryopreserved skin	Glycerolized skin
Face mask, A = 555 cm²						
AgCur	12	18	225	0.001	0.002	0.02
AgHEC6.4	13	19	245	0.001	0.002	0.02
AgHEC	11	17	212	0.001	0.002	0.02
Full body, A = 20 000 cm²						
AgCur	429	643	8099	0.04	0.06	0.8
AgHEC6.4	468	702	8835	0.05	0.07	0.9
AgHEC	405	607	7647	0.04	0.06	0.8

through-body abrasion with 10 N force for 8-h). Thus, it can be concluded that the Ag NP coated textile wear does not cause a risk related to Ag NP penetration through *stratum corneum*.

3.3 Inadvertent oral intake

Inadvertent oral intake was investigated by assuming 1-h wear of AgCur treated gloves followed by finger mouthing three fingertips with surface are of 11.5 cm². During 1-h glove wear all Ag mass is released from the textile and the Ag mass in the three fingertips would be 1.8 µg. During the three fingertips mouthing and assuming complete NP transfer (k_o = 1 1/min) and gut absorption the RCR would be 0.0002. Compared to Ag dermal absorption via hands (900 cm²; fresh skin), the 1-h glove wearing via *stratum corneum* would be 0.4 µg

(RCR = 4×10^{-5}) the finger mouthing cause 5 higher risk than dermal penetration. This demonstrates the importance of good hygiene practices.

3.4 Recommendations for development of dermal exposure assessment

NP release from textiles depends for example on the NP properties, application technique, e.g., incorporation or impregnation, textile type, concentration, and leaching media and mechanical stress (Koivisto *et al.*, 2017). In addition to synthetic sweat, other relevant exposure medias are saliva for oral exposure assessment and washing detergents for environmental and dermal exposure assessment. The absorption of Ag NPs differed significantly between skin conditions. Cryopreserved skin exhibited 1.5 times higher absorption compared to fresh skin, while glycerolized skin showed a much higher absorption rate, 19 times higher than fresh skin, attributed to higher release rate constants, despite similar initial mass concentrations of Ag NPs in the textiles. Overall, these findings highlight the complex interplay between release rates, skin condition, wear duration, and intake levels of Ag NPs from Ag coated textiles during wear. Distinction between dermal exposure and dermal intake should be made clear; von Goetz *et al.* (2013) reported dermal exposure normalized with body weight while here is reported dermal intake normalized by body weight that can be compared with the DNEL value when assuming that intake results to complete absorption to systemic circulation.

NP penetration through healthy skin is not a relevant exposure mechanism as compared to unintentional (peri-)oral exposure. Relevant and tailored experimental set-ups for measuring NP release from textiles and products are needed to understand which fraction can cause exposure and intake/uptake via oral route or through damaged skin. For face masks, it is expected that Ag intake via direct oral route is the main exposure pathway. Release to synthetic sweat is recommended to be used as surrogate source in dermal exposure assessment (Koivisto *et al.*, 2017). However, it is not clear how well the standards designed for textile wear resistance are applicable for exposure assessment. For example, it is expected that ISO 105 X12:2016 - Part X12: Colour fastness to rubbing does not represent typical use conditions when textiles/personal protective equipment are worn by workers or consumers. More realistic release test methods should be developed for mimicking typical/occupational use scenario. A systematic review of worst-case and RWC use conditions and hygiene practices would provide a better understanding of potential oral intake. Currently, the main development need in dermal exposure models are the contact surface area and retention efficiency of the pollutants (Bogen *et al.*, 2020).

Environmental exposure was not considered here. Washing experiments leachate Ag NP concentrations can be used to estimate environmental exposure over the textiles lifecycle (Wimmer *et al.*, 2019).

Conclusions

This study investigates Ag NP release potential during washing and wearing. A mass balance model was used to describe the Ag NP dynamics between textile, dermis, stratum corneum, and (peri-)oral exposure. This model was applied for consumer exposure scenario but is also applicable for occupational exposure assessment via dermal contact of contaminated surfaces. The effect of Ag NP type, skin type and exposure duration to intake and risk was demonstrated. The modeling methods introduced here can be used to calculate realistic exposure estimates, which are relevant for example when estimating general population exposure for epidemiological studies. Ot the other hand, the model can be used to estimate realistic worst-case conditions to provide conditions of use for textiles containing NPs. Currently, the main limitation is to have a release test setup for NPs in textiles that mimic more realistic use conditions. The major risk associated with wear of Ag NP containing textiles was associated to gloves wear followed by unintentional finger mouthing.

Ethics and consent

Ethical approval and consent were not required.

Data availability

Zenodo: Supplementary Material for "Exposure Assessment & Risks associated to wearing silver nanoparticle-coated textiles". https://zenodo.org/doi/10.5281/zenodo.10604889 (Koivisto *et al.*, 2024)

This repository contains the following:

- Rate constants, exposure and intake calculation parameters, and model source code for Matlab R2018a (can be translated to Python).
- Supplementary material

Data are available under the terms of the Creative Commons Attribution 4.0 International license (CC-BY 4.0).

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Open Peer Review

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Version 2

Reviewer Report 18 December 2024

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✔ **Daniela Montalvo**
Trace Elements and Nanomaterials, Sciensano, Leuvensesteenweg, Tervuren, Belgium

This study provides a model that can be used to assess exposure to Ag NPs via dermal penetration or oral intake. The authors considered a realistic exposure scenario that includes tear and wear conditions.

- In general the methods are well describe and it is possible to replicate the study. However what I think is missing is information on the characterization of the textiles coated with Ag NPs. For example is it not indicated in the text the size of the Ag NPS capped with cellulose and the particles coated with curcumin. Also an explanation why different capping agents were investigated? Were particles detected in the coated textiles? Electron microscopy imagines of the coated textiles can be included to support the data.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and does the work have academic merit?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
Not applicable

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Environmental chemistry, nanoparticles

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 04 December 2024

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The authors conducted a study aimed at characterising the risks associated with the use of Ag NP-containing textiles. The authors developed an exposure model for three scenarios i) face mask for general population, ii) face mask and a full-body suit for occupational exposure and iii) inadvertent oral intake (and combined scenario, see points 3 and 4). Subsequently, they carried out the pertinent risk characterisation. It is considered positive that multiple coatings were used to represent the variability of textiles available in the modelled scenarios. The article improves the knowledge of exposure assessment modelling and contributes to the risk characterization of nanoparticles in consumer products.

Minor comments are provided below:

1. Conclusions (both abstract and main text). Please, consider to conclude on the basis of the quantitative RCR values. Please, report whether the risk was considered to be acceptable.
2. Page 4/17. Dermal exposure and NP size. Please, define Dp abbreviation. Insert Dp in “Dp < 4 nm …“ and in “Dp > 45 nm … “
3. Page 5/17. Paragraph starting with “In this study, a more realistic approach …”. State that you performed simulations for a hand-to-mouth scenario. E.g. *The simulations are performed for RWC for i) … … ii) … … and iii) inadvertent oral intake* **…** .
4. Page 5/17. Consider to simulate for combined exposure scenario consisted on dermal plus oral intake and report the outcome where corresponds.
5. Page 5/17. 2.1.3 Mass transfer via stratum corneum. Please, replace “kss” with “ksc”.
6. Page 6/17: Consumers finger “mounting” is studied only for children and infants. Please, replace “mounting” with “mouthing”.
7. Page 7/17. Table 2. Please, define R2R.
8. Page 7/17. Typo: replace “was doneunder stirring at 300 rpm for 30 min” with “was done under”.
9. Page 8 / 17. Regarding “Intake is normalized using 60 kg body weight (bw) according to the

U.S. EPA (2011)", please, include as well the EU guideline "Default human factor values for use in exposure assessments for biocidal products" from 2017. Web link: https://echa.europa.eu/documents/10162/1154636/recom_14+_default+human_factor_values_biocidal+products_en.pdf/888a3a-475a-9c7d-d8ef8088d004

10. Page 8/17. Typo: replace "bw-kg"with "kg-bw", i.e. *"...(DNEL of 0.01 mg/* kg-bw */day)"*.
11. Fig 1. Please, consider to add a figure caption. For example, Dermal exposure and intake were calculated by using the dermal exposure model developed in this study. For details, see section 3.1 Model comparison.
12. Page 9/17. Regarding the sentence *"The highest RCR for face mask was 0.02 and for full body exposure 0.9 for glycerolized skin indicating adequately controlled exposure"*, it is debatable that a RCR of 0.9 indicate an adequately controlled exposure. Please, consider to split the sentence in two, one for face mask and another one for glycerolized skin.
13. Pages 9 and 10/17. Regarding necrotic skin, "Full body exposure for glycerolized skin representing necrotic skin is an unrealistic exposure occupational scenario". Please, consider to elaborate on worst-case assumptions leading to RCR values of 0.8-0.9. Regarding the sentence "Thus, it can be concluded that the Ag NP coated textile wear does not cause a risk related to Ag NP penetration through stratum corneum", consider to replace "not cause a risk" with "causes an acceptable risk".
14. Page 10/17. Figure 2C. The type of the line (dotdash) is barely seen in the legend. Improve reporting of legend.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and does the work have academic merit?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Exposure assessment, nanoparticles, biocides assessment

We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 10 October 2024

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The previously mentioned revisions have been made. The manuscript can be accepted in its current form.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Textile chemistry, nanotechnology, microencapsulation, fishing nets, ecological textiles

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 09 July 2024

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1. Introduction and Background:
The introduction provides a comprehensive overview of the context and importance of the study. The relevance of investigating textiles coated with silver nanoparticles is well established, given their increasing use and potential health implications.

Recommendation:
Although the introduction is comprehensive, it could benefit from a more detailed discussion on the regulatory landscape and existing guidelines or standards for silver nanoparticles in textiles. This would provide a clearer picture of the current framework in which this study is taking place.

2. Methodology:
The methodology section is well detailed, describing the experimental design, sample preparation and analytical techniques used. The use of multiple assessment methods improves the reliability of the findings.

Recommendation:
Ensure that reproducibility of methods is well documented. Providing supplementary material with detailed protocols or referring to standard procedures would be beneficial for other investigators wishing to replicate the study.

3. Exposure Assessment:
Exposure assessment is exhaustive, using both in vitro and in vivo models to assess potential risks. The choice of models and endpoints is appropriate for assessing dermal exposure to nanoparticles.

Recommendation:
The study should be strengthened by including a wider range of exposure scenarios, such as different lengths and frequencies of textile use. In addition, considering the possibility of nanoparticle release during washing and subsequent exposure could provide a more comprehensive view of the risks.

4. Risk Assessment:
The risk assessment framework is solid, incorporating hazard identification, dose-response assessment, exposure assessment and risk characterization. The use of quantitative approaches to estimate risk levels is laudable.

Recommendation:
To improve risk assessment, probabilistic hazard assessment should be considered. This would take into account variability and uncertainty in exposure and toxicity data, providing a more qualitative understanding of potential risks.

5. Results and Discussion:
The results are clearly presented, with appropriate use of tables and figures to illustrate key findings. The discussion effectively interprets the results in the context of the existing literature, highlighting the novel contributions of the study.

Recommendation:
Make sure that the discussion section critically evaluates the limitations of the study, such as

possible confounding factors or uncertainties in the data. In addition, suggesting specific directions for future research based on the study's findings would be valuable in advancing the research field.

6. Conclusion:
The conclusion briefly summarizes the main findings and their implications.
It effectively communicates the significance of the study and its contribution to the understanding of nanoparticle exposure and risk.

Recommendation:
The conclusion could be expanded to include recommendations based on the findings. This addressed to stakeholders, such as manufacturers, regulators and consumers as well. Providing actionable insights may enhance the practical impact of the report.

7. General Comments:
- Innovation:
The study approaches a key gap in the literature regarding the safety of textiles coated with silver nanoparticles, showing innovative approaches to exposure and risk assessment.

Clarity and Readability:
The manuscript is well written and structured, making it accessible to a broad reading group, including researchers, policy makers and industry stakeholders.

Aspects Not Evaluated:

Supplementary Materials:
If the study includes supplementary materials, they were not reviewed as part of this assessment. Ensuring that these materials are complete and accessible is important for full transparency and reproducibility.
Long Term Exposure:
The assessment focuses on acute and sub-chronic exposure scenarios. Long-term exposure effects, including possible chronic health outcomes, were not evaluated and represent an important subject area for future research.
Environmental Impact:
The study focuses mainly on risks to human health. Assessing the environmental impact of the release of silver nanoparticles from textiles during use and disposal is another crucial aspect that deserves further investigation.
As a whole, the study is a highly valuable contribution to the area of research, providing valuable insights into the risks associated with textiles coated with silver nanoparticles. Addressing the recommendations provided may further improve the robustness and impact of the research.

References
1. Ferdous Z, Nemmar A: Health Impact of Silver Nanoparticles: A Review of the Biodistribution and Toxicity Following Various Routes of Exposure.*Int J Mol Sci*. 2020; **21** (7). PubMed Abstract | Publisher Full Text
2. Salleh A, Naomi R, Utami ND, Mohammad AW, et al.: The Potential of Silver Nanoparticles for Antiviral and Antibacterial Applications: A Mechanism of Action.*Nanomaterials (Basel)*. 2020; **10** (8). PubMed Abstract | Publisher Full Text

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and does the work have academic merit?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: ImmunochemistryInflammationImmunology of Infectious DiseasesNatural Product ChemistryNatural Product PharmacologyExtraction of Natural ProductsMedical NanotechnologyAnalytical ToxicologyAcute Toxicity TestsEcotoxicologyEnvironmental NanotechnologyFood NanotechnologyAntioxidantsSecondary Metabolites

We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however we have significant reservations, as outlined above.

Reviewer Report 03 July 2024

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As a reviewer for this article on exposure assessment and risks associated with wearing silver nanoparticle-coated textiles, here are some suggestions for corrections and comments on the article in general: Provide more details on the methodology used for exposure assessment, including the sampling techniques, analytical methods, and any limitations encountered during

the study. Clarify how the exposure levels were measured and assessed in relation to silver nanoparticle-coated textiles. Discuss the implications of the findings in relation to existing literature on the topic and highlight any novel contributions of the study. Please add more recent articles about silver nanoparticles in textile. Provide a comprehensive discussion on the potential health risks associated with wearing silver nanoparticle-coated textiles, considering both dermal and inhalation exposure pathways. Summarize the key conclusions of the study and highlight the significance of the research in the field of nanotechnology and textile safety. The article could benefit from more detailed information on the role of silver nanoparticles in textiles, their potential release mechanisms, and their interaction with the human body. Consider expanding the discussion on the regulatory aspects related to the use of nanomaterials in consumer products and the need for standardized safety assessments. Overall, the article provides valuable insights into the exposure assessment and risks associated with silver nanoparticle-coated textiles. By addressing the suggested corrections and enhancing the clarity and depth of the content, the article can further contribute to the understanding of nanotechnology applications in the textile industry and their implications for human health.

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and does the work have academic merit?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Partly

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Textile chemistry, nanotechnology, microencapsulation, fishing nets, ecological textiles

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.