



Danish Ministry of the Environment

# Assessment of nanosilver in textiles on the Danish market

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**Title:**

Assessment of nanosilver in textiles on the Danish market

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# Preface

The project "Survey, sampling, analysis and assessment of nanosilver in textiles on the Danish market" has been carried out from September 2011 to March 2012.

The report describes the project results including survey, assessment of development and extrapolation of the market for nanosilver textiles. The results from the chemical analyses of a number of selected products are presented and assessments are made on the exposure and health and environmental risks in connection with the release of silver from the products.

As the start of the project, the Danish market was surveyed for consumer products, which were expected to contain nanosilver, and a representative number of these was selected. The products were subjected to quantitative and migration analyses and washing tests, and finally, a health, environmental, exposure and risk assessment was carried out.

The project has been carried out by Danish Technological Institute with COWI as subcontractor and has been headed by project managers M.Arch. Kathe Tønning and Lic.Scient. Nils Nilsson, quality manager MSc Eva Jacobsen, Head of Laboratory, MSc Nils Bernth and Head of Section Pia Wahlberg.

Health and environmental assessments were carried out by MSc Sonja Hagen Mikkelsen and MSc Jesper Kjølholt from COWI.

To assess the progress and results of the project, a steering committee has been set up with the following members:

Flemming Ingerslev, the Danish EPA  
Poul Bo Larsen, the Danish EPA  
Annette Orloff, the Danish EPA  
Magnus Løfstedt, the Danish EPA  
Jette Rud Larsen Heltved, Danish EPA  
Sonja Hagen Mikkelsen, COWI  
Kathe Tønning, Danish Technological Institute

The project has been financed by the Danish EPA.

# Summary

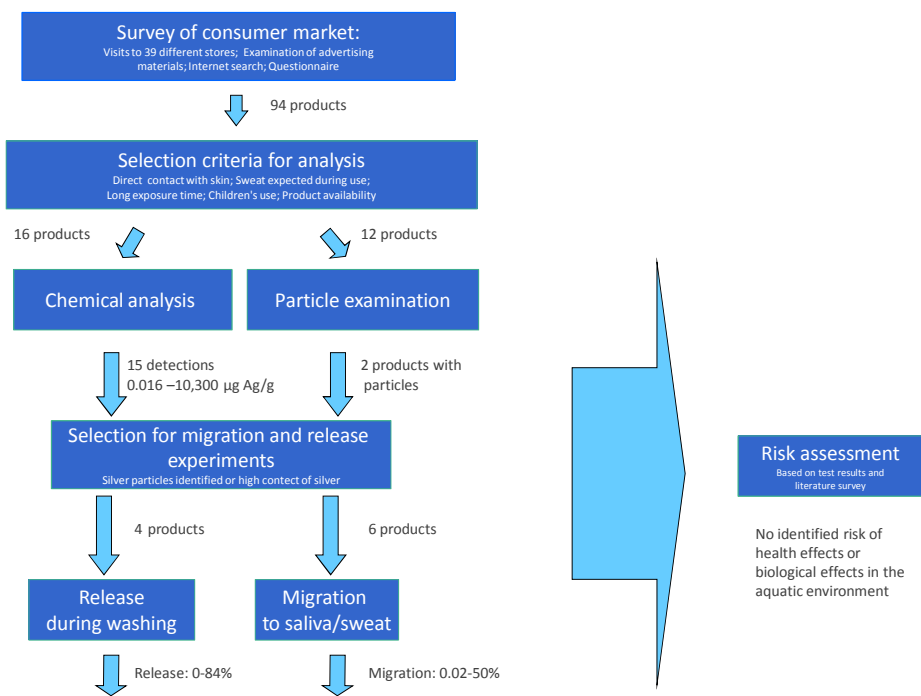
The application of nanosilver attracts great attention. By adding antibacterial agents such as nanosilver in textiles, it is possible to inhibit bacterial growth and thus reduce odour. In the case of nanosilver in textiles there will often be direct skin contact between the fabric and the user with the risk of human exposure. Nanosilver may also enter the human body via oral routes of exposure.

Through washing of clothes containing nanosilver, the nanosilver may be released from the clothes to the sewerage systems and may thereby affect the environment.

These concerns have formed the basis for our survey on a number of consumer products in the Danish market.

A brief overview of the different elements of the survey is illustrated in Figure **Error! Reference source not found.** and summarised in the following.

FIGURE 1 OVERVIEW OF THE SURVEY



## Survey

The survey comprises alone products marketed in Denmark or sold on Danish/Danish language internet pages.

The survey was limited to include textiles referred to as:

- Containing nanosilver
- Containing silver
- With antibacterial effect

Not only the retail shops, but also the internet shops were visited in connection with this survey.

In total, 39 different shops were visited. We registered only few products which according to the labelling contained silver, but there was no information about in which form the silver was present in the product. Several of the products registered were marked with antibacterial effect.

In general, the staff of the various shops informed us that customers do not specifically ask for products with antibacterial effect or the like, but merely ask for look, colour, brand, functionality and material, and when it comes to shoes, shock-absorbance. Most often, the staff did not even know whether the textiles had got an antibacterial treatment.

The survey on the internet registered a few textile products, which according to the web page, contained nanosilver. A number of products was registered to contain silver, but had no information about the form of the silver. More products were registered, which according to the web page information had an antibacterial effect.

Totally 94 products were registered in this survey. According to the information provided by the importers/producers/retailers 7 products contained silver in nanoform, 9 products contained silver ions/salts, 52 products contained threads/macrosopic silver and 17 products had got antibacterial treatment.

A part of the survey was subsequently to contact importers/producers/retailers by mail/letter to enquire about the form of the silver in the individual products.

Seen in the light of the intense discussions in various media and blogs, we expected to find a larger number of this type of products than was actually possible in this present, systematic project.

The results of this study indicates that there are only very few textile products on the Danish market which the companies declared to contain nanosilver (7 products were registered), but since the research also established that importers and dealers lack knowledge about the actual content of their products, some products might be “hidden” and used more widespread than it appears from the responses. Although many textiles are imported from countries outside the EU, it is worth noting that according to Swiss data<sup>1</sup> there is only limited use of nanosilver in textiles within the EU.

### **Chemical analysis and particle examination**

With background in the survey, 16 textile products were chosen for further examination by means of chemical analyses. Only seven products were registered, which according to the received information contained silver in nanoform. From these products, six were selected for analysis – the seventh product is not being manufactured anymore. To be able to compare the level of silver concentration in textiles with and without silver in nanoform, also products registered as containing silver and with antibacterial effect were selected for analysis. By the selection of specific textiles, focus has been

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<sup>1</sup> HSR, UMTEC: Entsorgung nanosilberhaltiger Abfälle in der Textilindustrie – Massenflüsse und Behandlungsverfahren -

directed on issues as direct contact with skin, sweat expected during use, long exposure time, products for both children and adults and release to the environment.

The textiles were on basis of information from the importers/producers/retailers as follows:

- Textiles with nanoparticles: Running T-shirt, fitness and running T-shirt, microfibre cloth, shower curtains, insoles and insoles in a sandal
- Textiles with silver ions/silver salts: Ski underwear/T-shirt, hat/scarf, patterned
- Textiles with silver threads: Piece goods, cuddly toy, sheet for cot, body-stocking
- Textiles with silver form not identified: Tank top for children, running socks, top mattress
- Textiles with antibacterial effect: Nursing pad

The analysis program was initiated with a quantitative analysis of selected textiles for determination of the total amount of silver with the purpose of subsequently being able to select relevant textiles for further examination of determination of particle sizes (SEM/EDX), migration of silver in saliva and sweat simulants, and release of silver in washing experiments.

The quantitative analyses for silver were carried out by means of inductively coupled plasma mass spectrometry (ICP-MS) and inductively coupled plasma atomic emission spectrometry (ICP-AES). The quantification limits have been validated to 0.03 µg/g and 1.5 µg/g, respectively.

Quantitative analysis of the 16 textiles for determination of the total amount of silver showed very varying contents of silver from 0.016 µg/g to 10,300 µg/g. In one product (fitness and running T-shirt) no silver was detected. The relative standard deviation for quantitative analysis of double determinations of the textiles varied from 0.11% to 120%, which indicates an inhomogeneous distribution of silver in some of the products. Running socks had the highest relative standard deviation. All products with silver threads have very low relative standard deviations (0.11% – 2.3%), indicating a homogenous distribution of the silver content.

In the products, where the silver form, according to the information in the survey is identified as nano, the highest content is proved to be in the microfibre cloth (270 µg/g) and in the insole (19 µg/g). A low content is shown in a running t-shirt (0.049 µg/g), a shower curtain (0.016 µg/g) and in the upper side of the sole in a sandal (0.037 µg/g).

In products with a silver form identified as something else than nano, the highest contents of silver have been detected in products containing silver threads, piece goods (7,650 µg/g) and cuddly toys (10,300 µg/g), respectively. The other two products with silver threads had a much lower concentration of silver, sheet for cot (7.0 µg/g) and body-stocking (12 µg/g), respectively. In products where the silver form according to the information in the survey is identified as silver ions/silver salts, silver form not identified, or as antibacterial, the determined silver was in the low concentrations (0.047 µg/g to 20 µg/g). Only one product, a top mattress, had a higher concentration (270 µg/g).



12 products were selected for particle studies. By selection of specific textiles, focus has been directed at products, where the silver form, according to the information in the survey, is identified as nano, selecting four products. Four products, where the silver form was not identified or registered as antibacterial, were selected for identification of the silver form. Two products with expected content of silver ions/salts and two products with expected content of silver threads were selected.

The surface of the samples is examined in scanning electronic microscope (SEM) with facilities for X-ray microanalysis (EDX). For samples with satisfactory electrically conducting properties, the SEM technique has a dissolving power of approx. 4-5 nm. Therefore, it is not possible to localize the particles with diameters below 5 nm. The resolution for the EDX technique is approximately 1  $\mu\text{m}$  and allows detection of silver down to 0.1 w/w % (1 mg/g). A single silver nanoparticle down to 5 nm cannot be detected by EDX, but if silver nanoparticles are clustered together or are present on the surface in concentrations larger than 0.1 w/w % EDX can detect the silver nanoparticles.

The particle examination shows that only two textiles (microfibre cloth and insole) are coated with silver particles. The particles appear individually and with a relatively large distance to the sample fibres. The silver particles have diameters from approx. 0.2 to 1.0  $\mu\text{m}$  (200 to 1000 nm), which is larger than the applied definition of the size of nanoparticles (1-100 nm) in this report. The dealers of two products explain that they contain silver nanoparticles, which indicates that they apply another definition for the size of nanoparticles (e.g. 1-1000 nm).

It cannot be excluded that the sandal and the running t-shirt contains silver nanoparticles, which were not detected during the SEM analysis if the diameters of the particles is below 5 nm. During the ICP-MS analysis, the content in these products is determined to be between 0.035 and 0.058  $\mu\text{g/g}$ , which is below the detection limit for EDX. The detection limit for EDX is 0.1 w/w % (1 mg/g) in single spots or areas larger than 1  $\mu\text{m}^2$ . The rubber material of the sandal contains a lot of very small particles.

In two textiles (cuddly toy and top mattress), interwoven silver threads with a rough surface have been detected.

No silver nanoparticles, silver particles or silver threads have been detected in 8 out of 12 textile samples.

From the results of the quantitative determination of the total content of silver and particle examinations, a series of products has been selected for migration examinations. Importance has been attached to including products with silver particles (microfibre cloth and insoles). Furthermore, products with a proved high content of silver have been included as well for comparison matters (hat/patterned textile, cuddly toy, body-stocking and tank top).

Migration examinations were performed for artificial sweat and artificial saliva and, subsequently, ICP-MS analysis was carried out.

The lowest migration to sweat and saliva simulant is detected from products declared to contain nanosilver (0.02% for the microfibre cloth and 8% for the

insole) and silver threads (0.09% for the cuddly toy), and the highest from products containing silver ions (50% for the tank tops and 24% for hat/scarf).

From the results from the quantitative determination of the total amount of silver and particle examinations, a series of products has been selected for washing examinations. Importance has been attached to including products with silver particles (microfibre cloth). Furthermore, products containing a high amount of silver and which are expected to be washed often have also been included (hat/patterned textile, cuddly toy and body-stocking).

A release of 15 % was detected in the microfibre cloth, 32 % in the cuddly toy and 84 % in the hat/scarf. No release was detected from the body-stocking.

## **Human health assessment**

### **Exposure scenarios**

Exposure scenarios are developed to illustrate and assess dermal and oral exposure to silver, migrating from the selected textiles to artificial sweat and saliva. The primary route of exposure to silver in the nanosize region from the identified consumer textiles is the dermal route, whereas oral exposure in particular is relevant in relation to children sucking on e.g. bed sheets, hats or cuddly toys with antibacterial properties. Exposure may also occur through inhalation when dust is released from textiles in use. However, this exposure route is not expected to make a significant contribution from the product types tested in this survey and therefore not included in the exposure scenarios.

Based on the migration results, exposure scenarios were selected involving dermal exposure to insoles (adults) and tank tops (children) which gave rise to the highest migration to sweat. With regard to oral exposure, children's exposure to silver via cuddly toys was selected as the migration to saliva was higher compared to the migration from the hat and because it was considered more likely that children would suck on the cuddly toy for a longer time compared to the hat. The selection of exposure scenarios thus considered both the amount of silver migrating from the tested products and the relevance of the scenarios. Only the insole represented a scenario with positive identification of nanosize particles although the particle examination concludes that the particles had diameters from 0.2 to 1  $\mu\text{m}$ .

The dermal exposure scenarios were calculated for adults in contact with insoles 8 hours per day and children in contact with a tank top 16 hours per day. The oral exposure scenario was calculated for 2 hours' daily contact with a cuddly toy.

Dermal absorption data from the literature indicate that absorption is limited through both intact and abraded skin. However, due to limitations with regard to characterisation of nanosilver and studies investigating dermal absorption, an absorption of 100% was used as a default value for a first calculation followed by a calculation with a presumed conservative absorption of 0.1%, which was also used in the US EPA risk assessment of nanosilver in relation to the registration of nanosilver as a textile preservative.

### **Toxicity of nanosilver**

There are in general few relevant studies available on the acute toxicity of nanosilver. It is speculated, but not clarified, if toxicity is directly proportional to the rate of release of monovalent silver ions. No major signs of toxicity have been observed in rodents in acute toxicity studies with oral and dermal

exposure to silver in the nanoform. In guinea pigs there are indications of slight liver, spleen, and skin damage following exposure to >0.1 mg/kg of silver nanoparticles by dermal route. Case reports have demonstrated skin discolouration (argyria) and elevated liver enzyme levels in patients with burns treated with wound dressings containing nanosilver. There are no indications of irritation or sensitisation from dermal contact to nanosized colloidal silver.

There is some indication of slight liver, spleen, and skin damage from repeated dermal exposure to nanoparticles. Oral administration of silver nanoparticles (42 nm) to mice for 28 days at dose levels up to 1 mg/kg bw/day resulted in elevated liver enzymes in the highest dose group indicating induction of liver toxicity and a NOAEL of 0.5 mg/kg bw/day.

Based on *in vitro* studies, it is suggested that nanosilver may cause mutagenicity/genotoxicity via an indirect threshold mechanism driven by formation of ROS (reactive oxygen species). No studies investigating the carcinogenicity of silver nanoparticles have been identified. However, as signs of genotoxicity have been observed, carcinogenicity cannot be excluded and needs further investigation. A few *in vitro* toxicity studies with relevance for male reproductive toxicity have been identified and indicate that nanosilver may have reproductive effects, however, no firm conclusions can be drawn.

The elevated liver enzymes observed in the oral repeated dose toxicity study in mice are selected as critical endpoint of relevance for dermal and oral consumer exposure and NOAEL based on this effect is used for the calculation of the risk characterisation ratio.

Overall, nanosilver seems to exert the same relatively low toxicity as silver by the oral and the dermal route but to be more toxic through inhalation. This exposure route is though not considered relevant for the products in question. Discolouration of the skin (argyria) as a result of excessive exposure to silver is also observed in patients with burns treated with wound dressings containing nanosilver.

### **Health risk assessment**

The calculated risk ratios relevant for the three selected exposure scenarios, i.e. adults in dermal contact with insoles 8 hours per day, 3-9-year old children in contact with a tank top 16 hours per day, and finally 2-year old children in oral contact with a cuddly toy 2 hours per day, indicate that the risk from each individual scenario is very low. Also combined exposures from different sources with similar migration patterns are not likely to pose an unacceptable risk to consumers based on the findings in this study.

The two dermal scenarios were, as mentioned, calculated based on a conservative estimate of 0.1% dermal absorption. Yet, there are indications that the absorption is much lower, both through intact and abraded skin, and therefore the risk quotients may be even lower than indicated by the calculations in this study.

The overall assessment is that there is no identified risk from dermal and oral contact with the products tested in this survey, based on the available literature findings and assumptions made on this basis.

## **Environmental assessment**

### **Exposure scenarios**

During the service life of textiles containing nanosilver, the only potentially significant pathway of environmental exposure is considered to be release to the sewerage system, primarily in connection with wash of textiles or shower bathing in private households. Following treatment of the sewage at a sewage treatment plant (STP), the effluent is discharged to a surface water body, while the resulting sludge may be spread on e.g. agricultural soil.

The exposure scenario for environment was developed based on the ECHA guidance document on environmental exposure and is therefore based on the inputs from a population of 10,000 (10,000 PE) to the public sewerage system. The 10,000 PE are presumed to be distributed on 2,500 standard households each consisting of 4 persons. Each PE contributes with 200 L water/day and 0.011 kg sludge/day.

The daily input of nanosilver from a family (household) to the sewerage system was calculated based on an estimated number of machine washes per day and likewise, an estimated number of showers per day.

Based on the results of the chemical analyses of textiles described above and the standard parameters selected for the release to the sewerage system, the total daily release of nanoAg from one family to the sewerage system is estimated to approx. 15 mg/family/day of which about 80 % originates from washing of textiles.

### **Ecotoxicity of nanosilver**

A review and assessment of recent scientific literature on the ecotoxicity of nanosilver was carried out as part of the study. The most sensitive, reliable standard short-term endpoint identified was the EC<sub>50</sub> = 0.040 mg Ag/l (40 µg/l) for *Daphnia pulex*, whereas no standard long-term NOECs were identified. A 21-day LOEC (growth) of 0.005 mg Ag/l (5 µg/l) for *D. magna* has recently been reported.

Silver is a well-known environmental pollutant that has been investigated in many details. An overview of available data is provided in the background report describing how the Danish EQS for silver is set (Miljøministeriet 2009). In the report, the most sensitive endpoints used for the environmental risk assessment were a short-term EC<sub>50</sub> of 0.24 µg Ag/l for crustaceans (*D. magna*) and a chronic NOEC value of 0.09 µg Ag/l for fish (*O. mykiss*). Given the great amount of data, the Danish EQS for silver is set using a low uncertainty factor resulting in an AA-EQS of 0.017 µg/l for freshwater and 0.2 µg/l for marine waters (dissolved fraction; added to the natural background level). Thus, silver ions appear to be more toxic than nanosilver in the aquatic environment.

### **Environmental risk assessment**

#### *Sewage effluent*

Using the result of the environmental exposure scenario, the concentration of nanosilver in the raw sewage will, when applying standard REACH conditions, be approx. 0.019 mg/l (19 µg/l). Literature data suggest that approx. 10 % of the nanosilver in the raw sewage can be assumed to remain in the water phase after treatment at the STP, and a standard dilution factor of 10 is applied for the initial dilution of the effluent in the receiving surface

water body. Thus, the concentration in the effluent will be 1.9 µg/l, and 0.19 µg/l in the receiving surface water body at the border of the mixing zone.

This level is approx. 200 times lower than the lowest EC<sub>50</sub> value and 25 times lower than the lowest chronic NOEC value stated for the aquatic toxicity of nanosilver. The concentration is, however, only slightly below the lowest chronic NOEC for (free) silver, 0.24 µg/l and about 11 times higher than the Danish freshwater EQS for (dissolved) silver. However, only a fraction of the originally released nanosilver is believed to be present as free Ag<sup>+</sup> in the water phase, while the majority will be either nanosilver or species resulting from transformation reactions in the sewage system such as silver complexes or silver sulfide.

In conclusion, based on the existing (albeit incomplete) data on the ecotoxicity of nanosilver and the scenario for environmental exposure through discharge of sewage effluent from STPs, there appears to be little risk of biological effects in the aquatic environment arising from the use of nanosilver in textiles.

Possible cumulative effects in the aquatic environment due to the presence of other forms and sources of silver have not been considered.

#### *Sewage sludge*

According to the exposure scenario for STPs, 90 % of the nanosilver in the untreated wastewater will be distributed to the sewage sludge. This leads to an estimated concentration of nanosilver in the sludge of approx. 300 mg nanosilver/kg sludge dw. and a resulting soil concentration after application of sludge to farmland of approx. 150 mg/m<sup>2</sup> or 0.1 mg/kg soil dw. This estimated soil concentration is much lower than the existing soil quality criteria (50 mg Ag/kg soil dw), which, however, is not based on assessments of toxicity to soil-dwelling organisms. It is not considered feasible to conduct an ecotoxicological risk assessment of the terrestrial environment based on the available effect data on nanosilver.

#### **Overall health and environmental assessment**

No specific risk of health effects or biological effects in the aquatic environment arising from the use of silver in textiles was identified based on the existing data on (nano)silver (eco) toxicity, test results, and the selected exposure scenarios. It should however be emphasized that this study did not find silver falling within the definition of nanosilver proposed in the EU, in any of the tested products. Furthermore the conclusion must be seen in the light of the limited information database and the need for more studies in order to make firm conclusions regarding the risk of exposure to nanosilver compared to the risk from conventional forms of silver. For the aquatic environment, though, the available information indicates that dissolved silver (ions) are more toxic than silver in the form of nanoparticles.

# Sammenfatning

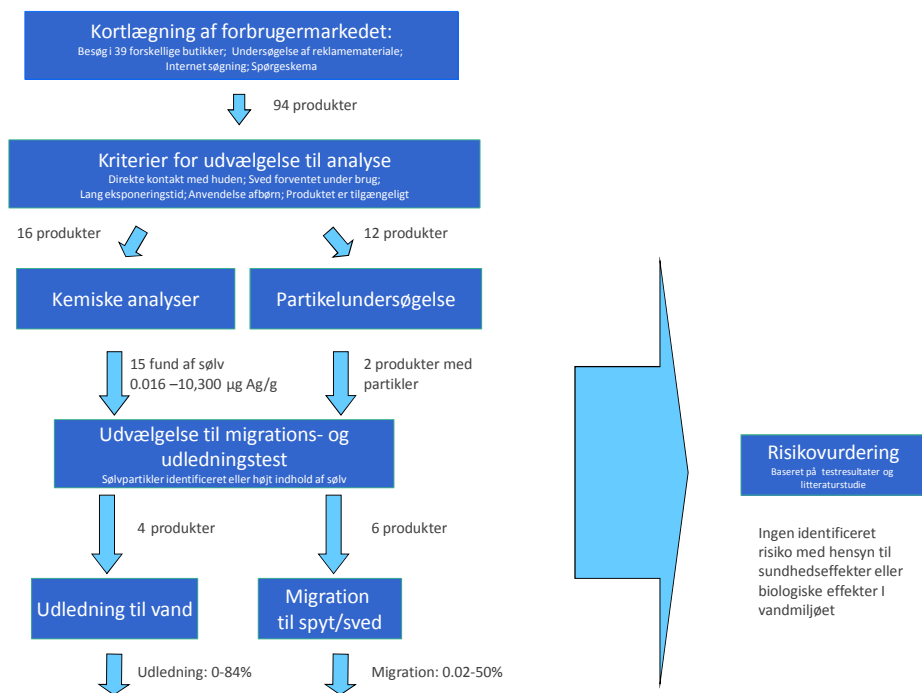
Anvendelsen af nanosølv har stor bevågenhed. Ved at tilsætte antibakterielle midler som nanosølv til tekstiler kan man hæmme bakterievæksten og dermed mindske lugtgener. I forbindelse med nanosølv i tekstiler vil der i mange tilfælde forekomme direkte hudkontakt mellem tekstilet og brugeren med potentiel risiko for human udsættelse. På samme måde kan nanosølv optages i kroppen via oral eksponering.

Med hensyn til miljøpåvirkning, vil nanosølv kunne påvirke miljø og vandrensningssystemer, når nanosølvet udvaskes i forbindelse med tøjvask.

På denne baggrund er der gennemført en undersøgelse af en række forbrugerprodukter på det danske marked.

En oversigt over de enkelte elementer i kortlægningsprojektet er vist i Figur og opsummeret i det følgende.

FIGUR 2 OVERSICHT OVER KORTLÆGNINGEN



## Kortlægning

Kortlægningen har alene omfattet produkter, der markedsføres i Danmark eller forhandles på danske/dansksprogede internetsider.

Kortlægningen er afgrænset til at omhandle tekstilprodukter, der blev omtalt som:

- Indeholdende nanosølv
- Indeholdende sølv
- Havende antibakteriel virkning.

Både detailhandelsbutikker og internetbutikker blev besøgt i forbindelse med kortlægningen.

I alt 39 forskellige butikker blev besøgt. Der blev ved disse butiksbesøg ikke registreret produkter, der blev markedsført som indeholdende nanosølv, eller hvor det fremgik produktmærkater, at produktet indeholdt nanosølv. Der blev registreret få produkter, som ifølge mærkningen indeholdt sølv, men det fremgik ikke på hvilken form, sølvet indgik i produktet. Der blev registreret en del produkter, der var mærket med antibakteriel virkning.

Generelt blev det oplyst i de besøgte butikker, at kunderne ikke efterspørger, at produkterne er antibakterielt behandlet eller lignende. Kunderne interesserer sig for udseende, farve, brand, funktionalitet samt materiale og (for fodtøjs vedkommende) stødabsorption. Ofte vidste personalet i de besøgte butikker ikke, at tekstilet var antibakterielt behandlet.

Der blev ved kortlægning via internetsider registreret enkelte tekstilprodukter, som ifølge oplysninger på internetsiden indeholdt nanosølv. Der blev registreret en række produkter, som ifølge oplysninger på internetsiden indeholdt sølv, men det fremgik ikke, på hvilken form sølvet indgik i produktet. Der blev registreret en del produkter, som ifølge oplysninger på internetsiden havde en antibakteriel virkning.

Der blev registreret i alt 94 produkter ved kortlægningen. Syv produkter, der ifølge det oplyste indeholder nanosølv; ni produkter, der indeholder sølvioner eller sølvsalte; 52 produkter, der indeholder sølvtråde/makroskopisk sølv og 17 produkter der har fået antibakteriel behandling.

Som en del af kortlægningen blev importører/producenter/forhandlere efterfølgende kontaktede via mail eller brev med forespørgsel om, i hvilken form sølvet indgik i de enkelte produkter.

I forhold til den diskussion, der har fundet sted i diverse medier og blogs på nettet, var det forventet, at det ville være muligt at finde mange flere tekstilprodukter, der indeholder nanosølv, end tilfældet har været ved nærværende, systematiske kortlægning.

Undersøgelsens resultater tyder på, at der kun er ganske få tekstilprodukter (der blev registreret syv produkter), der ifølge leverandøren indeholder nanosølv, på det danske marked. Man skal dog være særdeles påpasselig med en sådan konklusion, da undersøgelsen samtidig viser, at der stor mangel på viden hos importører og forhandlere om det konkrete indhold i tekstilet, hvorfor anvendelsen kan være ”skjult” og mere udbredt, end det umiddelbart fremgår af besvarelsene. Selvom mange tekstiler importeres fra lande uden fra EU, er det er dog værd at notere sig, at det ud fra schweiziske oplysninger<sup>2</sup> samlet set kun er et meget begrænset forbrug af nanosølv til tekstiler i EU.

### **Analyser og undersøgelse af partikler**

Med baggrund i kortlægningen blev 16 produkter valgt til kemiske analyser. Kun syv produkter indeholdt sølv i nanoform i henhold til de modtagne informationer. Af disse produkter blev seks valgt til analyse – det syvende produkt forhandles ikke længere. For at kunne sammenligne niveauet af sølvkoncentrationer i tekstiler med og uden sølv i nanoform, blev der til

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<sup>2</sup> HSR, UMTEC: Entsorgung nanosilberhaltiger Abfälle in der Textilindustrie – Massenflüsse und Behandlungsverfahren -

analyserne også medtaget produkter registeret indeholdende sølv på anden form og med antibakteriel virkning. Ved valg af specifikke tekstiler var fokus på tekstiler, hvor der er direkte kontakt til hud, hvor der kan forventes sved ved anvendelse, lang anvendelsestid, produkter for børn og voksne, og hvor der kan forventes udvaskning til miljøet.

Tekstilerne var på basis af informationerne fra importører/producenter/forhandler følgende:

- Tekstilprodukter, som indeholder nanopartikler: Fitness T-shirt, indlægssål, sål i en sandal, badeforhæng og mikrofiberklud
- Tekstilprodukter, som ifølge det oplyste indeholder sølvioner/sølvsalte: Skjorte, skiundertøj, "hue", der kan anvendes som hue, pandebånd og/eller halstørklæde
- Tekstilprodukter, som ifølge det oplyste indeholder sølvtråde/makroskopisk sølv: Metervare, krammedyr, lagen, bodystocking
- Tekstilprodukter, hvor sølvformen ikke er identificeret: Børnetanktop, løbesokker, topmadras
- Tekstilprodukter, som ifølge det oplyste har fået en antibakteriel behandling: Ammeindlæg

Analyseprogrammet blev indledt med en kvantitativ analyse af udvalgte tekstiler for bestemmelse af total mængde af sølv med det formål at kunne udvælge relevante tekstiler for yderligere undersøgelser i form af bestemmelse af partikelstørrelse, migration af sølv i spyt- og svedsimulant og frigivelse af sølv i vaskeeksperimenter.

Analyserne for sølv er udført ved henholdsvis induktivt koblet plasma massespektrometri (ICP-MS) og induktivt koblet plasma atomemissionsspektrometri (ICP-AES). Kvantificeringsgrænserne blev valideret til henholdsvis 0,03 µg/g and 1,5 µg/g.

De kvantitative analyser for bestemmelse af det totale indhold af sølv for de 16 tekstiler viste meget varierende indhold af sølv fra 0,016 µg/g to 10.300 µg/g. I et produkt (fitness T-shirt) blev der ikke påvist sølv. Den relative standardafvigelse for de kvantitative analyser af dobbeltbestemmelserne af tekstilerne varierede fra 0,11 % til 120 %, hvilket indikerer en uhomogen fordeling af sølv i nogle af produkterne. Løbesokkerne havde den højeste relative standardafvigelse. Alle produkter med sølvtråde havde meget lav relativ standardafvigelse (0,11 %-2,3 %), hvilket indikerer en homogen fordeling af sølvindholdet.

I produkter, hvor sølvformen i henhold til informationer i kortlægningen er identificeret som nano, er det højeste indhold påvist i mikrofiberkluden (270 µg/g) og i en sål (19 µg/g). Et lavt indhold er påvist i en løbe T-shirt (0,049 µg/g), badeforhæng (0,016 µg/g) og øverste del af en sål i en sandal (0,037 µg/g).

I produkter, hvor sølvformen er identificeret som andet end nano, er det højeste indhold af sølv blevet påvist i produkter indeholdende sølvtråd, henholdsvis metervare (7.650 µg/g) og krammedyr (10.300 µg/g). De andre to produkter med sølvtråde indeholder meget lavere koncentrationer af sølv, henholdsvis lagen (7,0 µg/g) og bodystocking (12 µg/g).



I produkter, hvor sølvformen i henhold til kortlægningen er identificeret som sølv ioner/sølvsalte, sølvform ikke identificeret eller som antibakteriel, blev der bestemt lave koncentrationer (0,047 µg/g til 20 µg/g). Kun et produkt, en top madras, havde en højere koncentration (270 µg/g).

12 produkter blev udvalgt til partikelundersøgelser. Ved udvælgelse af specifikke tekstiler blev der fokuseret på at vælge produkter, hvor sølvformen i henhold til kortlægningen var identificeret som nano; heraf blev valgt fire produkter. Fire produkter, hvor sølvformen ikke var identificeret eller registreret som antibakteriel, blev valgt for at identificere sølvformen. To produkter, som forventes at indeholde sølvioner/sølvsalte og to produkter med forventet indhold af sølvtråde, blev ligeledes valgt.

Prøvernes overflade undersøges i scanning elektronmikroskop (SEM) med faciliteter til røntgenmikroanalyse (EDX). SEM-teknikken har for prøver med gode elektrisk ledende egenskaber en opløsningsevne på ca. 4-5 nm. Det er derfor ikke muligt at lokalisere partikler med diametre under 5 nm.

Opløsningsevnen for EDX-teknikken er ca. 1 µm og tillader bestemmelse af sølv ned til 0,1 w/w % (1 mg/g). En enkelt sølvnanopartikel ned til 5 nm kan ikke detekteres af EDX, men hvis sølvnanopartiklerne klumper sammen eller er repræsenteret på overfladen i koncentrationer større end 0,1 w/w %, kan EDX detektere sølvnanopartiklerne.

Partikelbestemmelsen viser, at kun to tekstiler (mikrofiberkluden og sålen) er coated med sølvpartikler. Partiklerne forekommer individuelt og med en relativt stor afstand på prøvens fibre. Sølvpartiklerne har diametre fra ca. 0,2 til 1,0 µm (200 til 1000 nm), hvilket er større end den anvendte definition af størrelse af nanopartikler (1-100 nm) i denne rapport. Forhandlerne af de to produkter har oplyst, at produkterne indeholder nanopartikler, hvilket indikerer, at de anvender en anden definition for størrelsen af nanopartikler (f.eks. 1-1000 nm).

Det kan ikke udelukkes, at der i sandal og løbe T-shirt er indhold af sølvnanopartikler, som ikke kan detekteres med SEM-analysen, hvis diameteren af partiklerne er mindre end 5 nm. I forbindelse med ICP-MS-analyserne, er indholdet af disse produkter blevet bestemt til mellem 0,035 and 0,058 µg/g, hvilket også er under detektionsgrænsen for EDX, som er 0,1 w/w % (1 mg/g) i enkeltpunkter. Gummimaterialet af sandalen indeholder mange meget små partikler.

I to tekstiler (krammedyr og topmadras) er der blevet bestemt indvævede sølvtråde med en ru overflade.

Der blev ikke bestemt sølvnanopartikler, sølvpartikler eller sølvtråde i 8 ud af de 12 tekstiler.

Ud fra resultaterne af de kvantitative analyser for totalt indhold af sølv og af partikelundersøgelserne, blev en række produkter udvalgt til migrationsundersøgelser. Der blev lagt vægt på at inkludere produkter med sølvpartikler (mikrofiberkluden og sålen). Desuden blev produkter med højt indhold af sølv udvalgt til sammenligning ("hue", der kan bruges som hue, pandebånd og/eller halstørklæde (mønstrede del), krammedyr, bodystocking og børnetanktop).

Migrationsundersøgelserne blev udført med henholdsvis kunstig sved og kunstigt spyt og efterfølgende analyse ved ICP-MS.

Der blev påvist den laveste migration til sved- og spytsimulanterne fra produkter, som er deklareret med at indeholde nanosølv (0,02 % for mikrofiberkluden og 8 % for sålen) og sølvtråde (0,09 % for krammedyret), og det højeste indhold fra produkter indeholdende sølvioner/sølvsalte (50 % for tanktoppen og 24 % for huen).

Ud fra resultaterne af de kvantitative analyser for totalt indhold af sølv og partikelundersøgelserne blev en række produkter udvalgt til vask. Der blev lagt vægt på at inkludere produkter med sølvpartikler (mikrofiberkluden). Desuden blev valgt produkter med højt indhold af sølv og produkter, som forventes at blive vasket ofte (hue (mønstret del), krammedyr og bodystocking).

En udvaskning på 15 % blev bestemt for mikrofiberkluden, 32 % for krammedyret og 84 % for hue (mønstret del). Der blev ikke påvist udvaskning fra bodystocking.

### **Sundhedsvurdering**

#### **Eksponeringsscenarier**

Eksponeringsscenarierne er udviklet til at illustrere og vurdere dermal og oral eksponering for sølv, der migrerer fra de udvalgte tekstiler til kunstig sved og spyt. Dermal eksponering er den primære eksponeringsvej for sølv i nanostørrelse fra de identificerede forbrugertekstiler, hvorimod oral eksponering i særdeleshed er relevant i forhold til børn, der sutter på fx lagner, huer/tørklæder eller krammedyr med antibakterielle egenskaber. Eksponering kan også forekomme ved indånding af støv, der frigøres fra tekstiler i brug. Inhalation antages dog ikke at udgøre et betydende bidrag fra de produkttyper, der blev testet i denne undersøgelse, og er derfor ikke medtaget i eksponeringsscenarierne.

Baseret på resultater af migrationstestene blev det besluttet at opstille eksponeringsscenarier omfattende dermal eksponering for indlægssåler (voksne) og tank tops (børn), som var de produkter, der gav anledning til størst migration til sved. Med hensyn til oral eksponering, blev børns udsættelse for sølv ved at sutte på krammedyr valgt som scenarie, da migration til spyt var højere for krammedyret sammenlignet med migration fra huen/tørklædet, og fordi det blev anset mere sandsynligt, at børn ville sutte på tøjdyr i længere tid end på huen/tørklædet. Udvalget af eksponeringsscenarier har derfor både taget mængden af sølv migreret fra de testede produkter og relevansen af de mulige scenarier i betragtning. Kun indersålen repræsenterede et scenarie med positiv identifikation af partikler i nanostørrelses regionen, selvom partikelundersøgelsen konkluderer, at partiklerne havde diameter fra 0,2 til 1  $\mu$  m og dermed ikke falder ind under EU's forlag til definition af nanomaterialer.

De dermale eksponeringsscenarier blev beregnet for voksne i kontakt med indlægssåler 8 timer om dagen og børn i kontakt med en tank top 16 timer i døgnet. Det orale eksponeringsscenarie blev beregnet for 2 timers daglig kontakt med et krammedyr.

Dermale absorptionsdata fra litteraturen indikerer, at absorption er begrænset både gennem intakt og beskadiget hud. På grund af begrænsninger med

hensyn til karakterisering af nanosølv og hvad angår undersøgelser af dermal absorption, er der først regnet på en hudoptagelse på 100% som standardværdi efterfulgt af en beregning med en absorption på 0,1%, hvilket vurderes at være en konservativ antagelse. Denne værdi blev også anvendt af US EPA til risikovurdering af nanosølv i forbindelse med registrering af nanosølv som konserveringsmiddel i tekstiler.

### **Toksicitet af nanosølv**

Der er generelt få relevante undersøgelser tilgængelige vedrørende akut toksicitet af nanosølv. Det antages at toksiciteten er direkte proportional med frigivelse af monovalente sølvioner, men det er ikke endelig klarlagt. Der er ikke observeret væsentlige tegn på toksicitet i gnavere i undersøgelser af akut toksicitet med oral og dermal eksponering for sølv i nanoform. I marsvin er der indikationer på svag lever-, milt- og hudskade efter dermal udsættelse for mere end 0,1 mg / kg sølv nanopartikler. Misfarvning af huden (argyria) og forhøjede niveauer af leverenzymen er set hos patienter med brandsår behandlet med bandager, der indeholder nanosølv. Der er ingen tegn på irritation eller sensibilisering på grund af hudkontakt med kolloid sølv i nanostørrelse.

Der er en vis indikation af milde lever-, milt- og hudskader fra gentagen dermal eksponering for nanopartikler. Oral administration af nanosølvpartikler (42 nm) til mus i 28 dage ved dosisniveauer på op til 1 mg / kg legemsvægt / dag resulterede i forhøjede leverenzymen i den højeste dosisgruppe, hvilket indikerer induktion af levertoksicitet og en NOAEL på 0,5 mg / kg legemsvægt / dag.

Baseret på in vitro studier, foreslås det, at nanosølv kan forårsage mutagenicitet / genotoksicitet via en mekanisk baseret dannelse af ROS (reaktiv oxygen) over en bestemt tærskelværdi. Der er ikke identificeret nogen undersøgelser af carcinogenicitet i forbindelse med nanosølvpartikler, men da tegn på genotoksicitet er blevet observeret, kan carcinogenicitet ikke udelukkes, og afklaring heraf kræver yderligere undersøgelser. Et par in vitro toksicitetsundersøgelser med relevans for mandlig reproduktionstoksicitet er blevet identificeret, og indikerer, at nanosølv kan have effekter på reproduktionen. Der kan ikke drages endelige konklusioner på den baggrund.

Forhøjede niveauer af leverenzymen observeret i det orale toksicitetsstudie med gentagen dosering i mus er valgt som kritisk endpoint med relevans for dermal og oral eksponering af forbrugerne og NOAEL baseret på denne effekt anvendes til beregning af risikoen ved eksponering.

Samlet set synes nanosølv at udøve den samme relativt lave toksicitet som sølv ved oral og dermal eksponering, men at være mere toksisk ved indånding. Denne eksponeringsvej er dog ikke betragtet som relevant i forbindelse med de undersøgte produkter. Misfarvning af huden (argyria), som et resultat af overdreven eksponering for sølv, er også observeret hos patienter med brandsår behandlet med bandager, der indeholder nanosølv.

### **Sundhed – risikovurdering**

Den beregnede risikokarakteriseringsratio relevant for de tre udvalgte eksponeringsscenarier, dvs voksne i hudkontakt med indlægssåler 8 timer om dagen, 3-9-årige børn i kontakt med en tank top 16 timer om dagen, og endelig 2-årige børn i oral kontakt med et krammedyr 2 timer om dagen, viser, at risikoen fra hver enkelt scenarie er meget lav. Heller ikke kombineret

belastning fra forskellige kilder med lignende migrationsmønstre antages at udgøre en uacceptabel risiko for forbrugerne baseret på resultaterne i denne undersøgelse.

De to dermale scenarier blev, som nævnt, beregnet på grundlag af et konservativt skøn på 0,1% dermal absorption. Eksisterede undersøgelser tyder dog på, at optagelsen er betydeligt lavere, både gennem intakt og beskadiget hud, og derfor antages risikokarakteriseringsratioen at være endnu lavere end angivet på baggrund af beregningerne i denne undersøgelse.

Den samlede vurdering er, at der ikke er fundet nogen risiko forbundet med dermal og oral kontakt med de testede produkter i denne undersøgelse, baseret på resultater i den tilgængelige litteratur og forudsætninger fastlagt på dette grundlag.

### **Miljøvurdering**

#### **Eksponeringsscenerier**

I forbindelse med brugsfasen for tekstiler, der indeholder nanosølv, anses den eneste potentielt betydelige miljøeksponeringsvej for at være udledning til kloaksystemet, primært i forbindelse med vask af tekstiler eller brusebadning i private husholdninger. Efter behandling af spildevand i et spildevandsbehandlingsanlæg (STP), ledes spildevandet til en overfladerecipient, medens det resulterende slam kan spredes på f.eks. landbrugsjord.

Miljøeksponeringsscenariet er udarbejdet baseret på ECHAs vejledning om miljøeksponering og er derfor baseret på input fra en population på 10.000 personækvivalenter (10.000 PE) til det offentlige kloaksystem. De 10.000 PE antages at være fordelt på 2.500 standard husstande hver bestående af 4 personer. Hver PE bidrager med 200 l vand / dag og 0,011 kg slam / dag.

Det daglige input af nanosølv fra en familie (husstand) til kloaknettet er beregnet på grundlag af et anslået antal af maskinvaske per dag og ligeledes et anslået antal brusebade per dag.

Baseret på resultaterne af de kemiske analyser af tekstiler beskrevet ovenfor, og de faste parametre udvalgt for frigivelse til kloaksystemet, er den samlede daglige frigivelse af nanosølv fra en familie til kloaksystemet estimeret til ca. 15 mg / familie / dag, hvoraf omkring 80% stammer fra vask af tekstiler.

#### **Økotoksicitet af nanosølv**

En gennemgang og vurdering af den seneste videnskabelige litteratur om økotoksicitet af nanosølv blev udført som en del af undersøgelsen. Det mest følsomme og pålidelige standard korttids endpoint, som blev identificeret, var  $EC_{50} = 0,040 \text{ mg Ag / l}$  ( $40 \text{ ug / l}$ ) for *Daphnia pulex*, hvorimod ingen standard langtids NOECs blev identificeret. En 21-dages LOEC (vækst) på  $0,005 \text{ mg Ag / l}$  ( $5 \text{ ug / l}$ ) for *D. magna* er for nylig rapporteret.

Sølv er et velkendt miljøforurenende stof, som er blevet undersøgt i detaljer. En oversigt over tilgængelige data findes i baggrundsrapporten for fastlæggelsen af det danske vandkvalitetskriterie (EQS) for sølv udgivet af Miljøministeriet i 2009. I rapporten, var de mest følsomme endpoints, der anvendes til miljørisikovurderingen en korttids  $EC_{50}$  på  $0,24 \text{ ug Ag / l}$  for krebsdyr (*D. magna*) og en kronisk NOEC-værdi på  $0,09 \text{ ug Ag / l}$  for fisk (*O. mykiss*). I lyset af den store mængde tilgængelige data, er det danske

vandkvalitetskriterie for sølv fastlagt ved hjælp af en lav usikkerhedsfaktor, hvilket resulterer i en AA-EQS på 0,017 µg / l for ferskvand og 0,2 µg / l for havvande (opløst fraktion, føjet til det naturlige baggrundsniveau). Således synes sølvioner at være mere giftige end nanosølv i vandmiljøet.

### **Miljøriskovurdering**

#### *Spildevand*

Med resultatet af det miljømæssige eksponeringsscenarie, vil koncentrationen af nanosølv i det rå spildevand, når der anvendes standard REACH forhold, være ca. 0,019 mg / l (19 µg / l). Data fra litteraturen indikerer, at ca. 10% af nanosølv i det rå spildevand kan antages at forblive i vandfasen efter behandling ved STP, og en standard fortyndingsfaktor på 10 anvendes til den indledende fortynding af udløbet i recipienten. Således vil i koncentrationen i udløbet være 1,9 µg / l, og 0,19 µg / l i recipienten på grænsen til blandingszonen.

Dette niveau er ca. 200 gange lavere end den laveste EC50 værdi og 25 gange lavere end den laveste kroniske NOEC værdi er angivet for den akvatiske toksicitet af nanosølv. Koncentrationen er dog kun lidt under den laveste kroniske NOEC for (fri) sølv, 0,24 mg / l og omkring 11 gange højere end de danske ferskvands EQS for (opløst) sølv. Imidlertid menes kun en brøkdel af vandfasen, mens størstedelen vil være enten nanosølv eller andre former som følge af omdannelsesreaktioner i kloaksystemet, såsom sølv-komplekser eller sølvsulfid.

Baseret på de eksisterende (omend ufuldstændige) data vedrørende økotoksicitet af nanosølv og scenariet for miljømæssig eksponering gennem udledning af spildevand fra renselanlæg, kan det konkluderes at der synes at være lille risiko for biologiske effekter i vandmiljøet som følge af brugen af nanosølv i tekstiler.

Mulige kumulative langtidsvirkninger i vandmiljøet på grund af tilstedeværelsen af andre former og kilder af sølv er ikke blevet vurderet.

#### *Spildevandsslam*

Ifølge eksponeringsscenariet for renselanlæg, vil 90% af nanosølv i urensset spildevand fordeles til spildevandsslam. Dette fører til en anslået koncentration nanosølv i slammet på ca. 300 mg nanosølv / kg slam ts. og en deraf følgende jordkoncentration efter anvendelse af slam på landbrugsjord på ca. 150 mg/m<sup>2</sup> eller 0,1 mg / kg jord ts. Denne estimerede jordkoncentration er meget lavere end de eksisterende jordkvalitetskriterier (50 mg Ag / kg jord TS), hvilket dog ikke er baseret på vurdering af toksicitet for jordbundsorganismer. Det anses ikke for muligt at foretage en økotoksikologisk risikovurdering af det terrestriske miljø baseret på de tilgængelige effektdata om nanosølv.

### **Samlet sundheds-og miljøvurdering**

Der er ikke identificeret nogen speciel risiko for sundhedsmæssige effekter eller biologiske effekter i vandmiljøet som følge af brugen af sølv i tekstiler baseret på de eksisterende data om (nano) sølv (øko) toksicitet, testresultater, og de valgte eksponeringsscenarier. I den forbindelse skal det understreges, at dette studie ikke fandt nanosølv, der faldt ind under den foreslåede definition i EU, i nogen af de testede produkter. Konklusionen skal desuden ses i lyset af begrænsningen i data og behovet for flere undersøgelser for at kunne drage robuste konklusioner vedrørende risikoen for eksponering for nanosølv i

forhold til risikoen ved de konventionelle former for sølv. For vandmiljøet, tyder tilgængelige oplysninger på, at opløst sølv (ioner) er mere giftigt end sølv i form af nanopartikler.

# 1 Introduction

The application of nanosilver has attracted great attention in recent years. Addition of antibacterial agents such as nanosilver in e.g. sportswear, socks/stockings, bed linen, and top mattresses etc. can inhibit bacterial growth and thus reduce odour nuisances. The particles may, however, be released from the clothing to the sweat, just as the nanosilver can be released to the wash water during laundering, and through the waste water treatment plant, it will finally be released into the environment through the purified water.

In the case of nanosilver used in textiles there will often be direct skin contact between the fabric and the user with the risk of human exposure. As the toxicological effect of silver nanoparticles on humans has not yet been mapped out, it is important to have an overview of how and to which extent the Danish consumers are exposed to silver nanoparticles.

Therefore, this project's objective is to analyse a number of consumer products on the Danish market. The products concerned are:

- Sportswear; incl. running clothes, skiing underwear, biking shorts, socks, etc.
- Mattresses and top mattresses
- Shoes and insoles
- Towels (e.g. for use during kayaking and trekking)
- Shower curtains
- Cleaning cloths
- Clothing (underwear and t-shirts for adults and children, shirts)
- Sheets and linen
- Duvets and pillows
- Nursing pads

According to the product specifications, the silver content is described as silver, silver threads, and nanosilver, respectively.

Nanosilver: The nanof orm of silver is often characterised by spherical particles with a size of 1-250 nm<sup>3</sup> but generally less than 100 nm and consisting of approx.. 20-15,000 silver atoms (MST, 2011; Wijnhoven *et al.*, 2009)<sup>4</sup>. Nanosilver is normally sold as powder, flakes, grains, bars, etc. In its pure form nanosilver will aggregate and is therefore often modified on the surface with e.g. dextran, citrate, polysaccharide, hydrocarbons or polyvinyl pyrrolidone. The antibacterial effect of nanosilver appears by blocking the cell respiration or penetrating the outer cell wall dependent on the structure of the cell wall whereby a wide spectrum of Gram-positive and Gram-negative bacteria will be effectively killed (Wijnhoven *et al.*, 2009). The exact mechanism has not been fully clarified.

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<sup>3</sup> The EU has in 2011 in their definition of nanomaterials set an upper limit of the particle size of nanomaterials of 100 nm.

<sup>4</sup> Survey on basic knowledge about exposure and potential environmental and health risks for selected nanomaterials. Environmental Project No. 1370, Miljøstyrelsen, 2011)

Silver, which is not in nanoform, can also have an antibacterial effect because of the released  $\text{Ag}^+$ -ions. The mechanism behind the antimicrobial activity of the silver ion is closely linked to its interaction with thiol (sulfhydryl) groups, though other interactions are also possible (Jung et al., 2008).

Silver threads are often used in the textile industry. The silver threads, which slowly release silver ions, may be coated e.g. with transparent plastic film to prevent that the silver oxidizes and turns black.

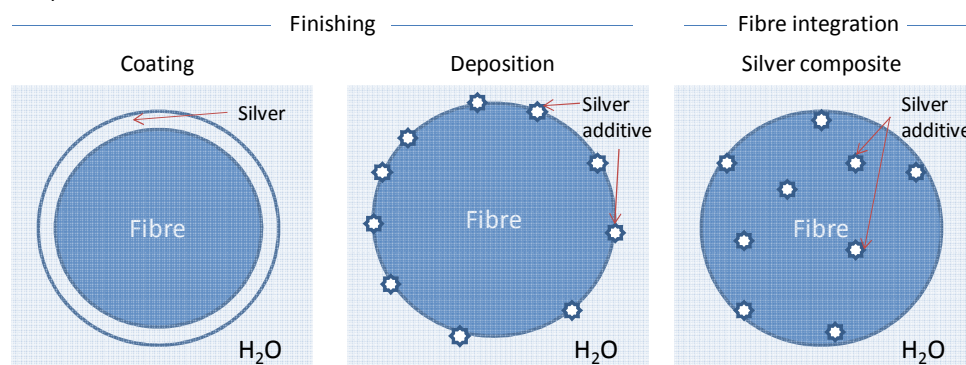
Burkhardt *et al.* (2011) describes three different groups of silver used for antimicrobial treatment of textiles:

- Particulate silver: All silver products present as silver ion exchangers, silver salts and metallic silver.
- Particles with silver: Silver ion exchangers, silver salt and metallic silver-micro composite with up to 10,000 nm particle size, but without the metallic nanosilver.
- Nanosilver: Metallic nanosilver with a size of 1-100 nm in three dimensions, which is present in monodisperse form in the preparation.

Due to size distribution and morphology of the particles, the ISO-definition<sup>5</sup> of nanomaterials does probably not apply to silver ion exchangers, particulate silver chloride and micro-composites with embedded silver. These forms do also not belong under the EU definition of nanomaterials. However, in the literature, the different silver forms are often not fully differentiated and are often just referred to as nanosilver particles and nanosilver (Burkhardt *et al.*, 2011).

Two different methods are applied for refinement of textiles with silver: (1) coating or deposition, or (2) fibre integration as shown in Figure 3.

FIGURE 3 METHODS FOR APPLICATION OF SILVER ON TEXTILE FIBRES (AFTER BURKHARDTS *ET AL.*, 2011)



Which method of refinement is chosen depends on the composition of the textile material and the desired application of the textile.

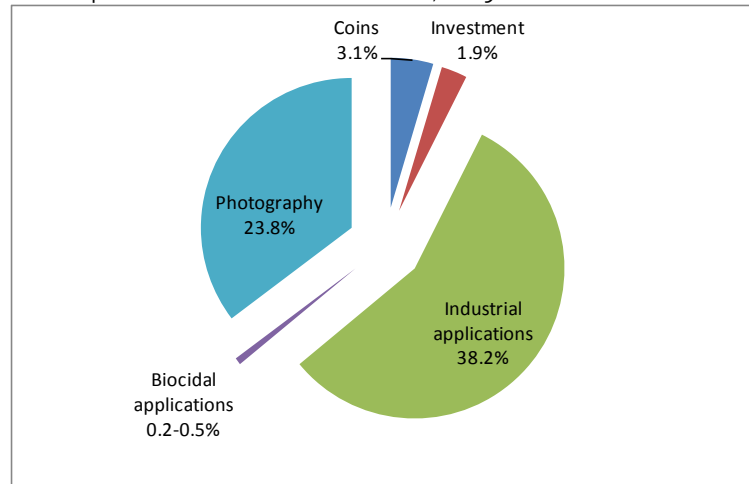
According to information from the Silver Institute, *World Silver Survey 2010 – A Summary*, the global consumption of silver amounted to approximately 28,000 tonnes in 2009. Of this amount, industrial applications, jewellery/silverware and photography account for approximately 95 %. The

<sup>5</sup> Material with any external dimension in the nanoscale or having internal structure or surface structure in the nanoscale ISO/TS 80004-1:2010.



remaining 5 % include coins, investments and biocidal applications, the latter accounting for only 0.2 - 0.5 % of the global consumption, which corresponds to approximately 140 tonnes. Most of the silver used for biocidal purposes is used for disinfection of water, accounting for 75 %, 20 % is used in textiles and the remaining 5 % in polymers and dispersions (Burkhardt *et al.*, 2011).

FIGURE 4 GLOBAL SILVER CONSUMPTION, 2009



As demonstrated by Figure 4, the total amount of silver used for antimicrobial treatment of textiles is small compared to other uses. Burkhardt *et al.* (2011) also refers to another analysis from 2006, estimating the European consumption of silver for biocidal purposes at 110 tonnes and thereby indicating that Europe is the main consumer of silver used in biocidal applications in the world.

## 2 Survey

### 2.1 OBJECTIVE OF THE SURVEY

The objective of the survey was to identify consumer products on the Danish market within the product line nanosilver-containing textiles.

Besides identifying these products, the objective was to assess the development of the market within this area. The assessment is based on the market players' expectations to the market and their judgment of the market development in recent years.

### 2.2 DELIMITATION

The survey comprises alone products marketed in Denmark or sold on Danish/Danish language internet pages.

Initially, the survey was limited to include textiles referred to as:

- Containing nanosilver
- Containing silver
- With antibacterial effect

### 2.3 PROCEDURE

The survey has been implemented during September/October 2011.

A number of retail shops, primarily national chain stores, but also non-chain stores have been visited.

Besides, contact was made to a wide range of internet shops dealing with textile products containing nanosilver, silver and/or products marketed as antibacterial.

Finally, written questionnaires have been submitted to a number of national chain stores.

#### **2.3.1 Shops**

Visits were paid to 39 different shops, including:

- Furniture and interior shops
- Shops for sports and outdoor wear
- Shoe shops
- Department stores
- Supermarkets

In addition, catalogues, advertising brochures etc. were examined.

### **2.3.2 Internet**

Google search was used with different search words such as e.g. "nanosilver, antibacterial, silver, silver thread, textiles, clothing, bathing suits, insoles, mattresses, top mattresses, sportswear, running clothes, nursing pads" and combinations hereof in order to find internet shops selling textile products containing nanosilver, silver and/or textile products marketed as antibacterial.

### **2.3.3 Manufacturers/importers/retailers**

Manufacturers/importers/retailers of the textile products registered during shop visits and via internet pages were contacted through an information letter and a questionnaire. The letter and questionnaire were made in both Danish and English. (Appendix 1, 2 and 3).

Three contact groups were used.

1. Companies selling products which according to the description on the internet or the actual product label contained silver.
2. Companies with products according to the description on the internet or the actual product label with antibacterial effect.
3. General enquiry to companies asking whether they were selling products containing silver and, in the affirmative, in which form.

The general enquiry was made to a number of national chain stores, e.g..

- Coop Danmark A/S
- Dansk Supermarked A/S
- Jysk A/S
- Imerco A/S
- IKEA A/S (Aarhus)
- Kop&Kande
- Inspiration
- Hästens
- Mette Ditmer
- Rikki Tikki Company A/S
- F&H A/S (Södahl)
- Magasin
- Ilva (Aarhus)
- Ecco
- Temprakon
- WeBike Fyn ApS
- Bovictus
- Hansen Møbler.

As mentioned above, three different questionnaires were presented to manufacturers/importers/retailers of textile products.

1. Questionnaire on silver-containing products/products containing nanosilver (see appendix 1)
2. Questionnaire on products with antibacterial effect (see appendix 2)
3. General questionnaire (see appendix 3).

In total we contacted 19 companies within group 1, 19 companies within group 2 and 19 companies within group 3.

Manufacturers/importers/retailers in group 1 were questioned about:

- form of the silver present in the products
- whether they were selling silver-containing products for children
- share of the silver-containing products of the total product line
- whether the sale of silver-containing products had changed during the last 5 years
- the company's expectations for the future sale of silver-containing products (falling, increasing, unchanged).

Additionally, manufacturers/importers/retailers in group 2 were asked whether the products' antibacterial effect was based on silver, and in the affirmative, the form of the silver.

Group 3 was additionally asked whether they were selling silver-containing products to adults and children.

Out of 19 enquiries to companies in group 1, 14 responses were received.

Out of 19 enquiries to companies in group 2, 12 responses were received.

Out of 19 enquiries to companies in group 3, 12 responses were received.

## 2.4 RESULT OF THE SURVEY

### 2.4.1 Shop visits

During the visits to the physical shops, we did not register any products that were marketed as containing nanosilver nor did it appear from the labelling that the products contained nanosilver.

There were a few products which according to the label contained silver, but no information about in which form the silver was present in the product, these were:

- Mattresses/top mattresses
- Skiing underwear

As part of the survey the manufacturers/importers/retailers were subsequently contacted by mail or letter with enquiry about the form of the silver in the individual products.

The shop visits revealed a number of products which were labelled as antibacterial. The label had no information about the cause of the antibacterial effect.

The products concerned were:

- Shower curtains
- Insoles
- Running socks
- Biking shorts
- Tank top (sleeveless) for children and adults
- Footwear (boots)
- Skiing underwear
- Shirts

- Lingerie
- Nursing pads

The labelling of these products only stated that the product had got an antibacterial treatment and/or that the product was treated with one of the below methods:

- NanoGlide®
- Ægis Microbe Shield®
- Polygiene®
- Coolmax® FreshFX™
- Sanitized® Silver

Appendix 4 presents a description of the different treatment methods.

The manufacturers/importers/retailers of products labelled as antibacterial were subsequently contacted by email or letter with enquiry about the cause of the antibacterial effect in the individual products.

There was general consensus among the staff of the shops that customers do not specifically ask for products with antibacterial effect, merely ask for look, colour, brand, functionality, and when it comes to shoes, shock-absorbance.

In one single home textile shop the salesman stated that though the customers do not specifically ask for antibacterial functionalities, the salesmen use it as an extra sales pitch when selling e.g. shower curtains. When first being informed about it, the customers considered this functionality an advantage.

Often the staff did not even know that the textiles had got an antibacterial treatment.

#### **2.4.2 Result of survey via internet pages**

The survey on the internet registered a few textile products, which according to the web page, contained nanosilver. The products were:

- Running t-shirts
- Nano socks
- Cleaning cloths

A number of products was registered to contain silver, but contained no information about the form of the silver, these were:

- Baby underwear
- Boots
- Sheets /bed linen
- Cleaning cloths
- Socks
- Teddy bears
- Mattresses/top mattresses.

As part of the survey, the manufacturers/ importers /retailers were subsequently contacted by email or letter with enquiry about the form of the silver in the individual products.

Further, the internet survey revealed a number of products which according to the web page information had an antibacterial effect, but only in a few cases information about the cause of the effect was available.

The products concerned were:

- Shoes (shoes and sandals - insoles)
- Hats
- Money belt
- Underwear
- Biking shorts
- Biking shoes
- Running socks and other socks
- Duvets and pillows
- Mattress covers
- Nursing pads
- Towels

Also this group was subsequently contacted by email or letter with enquiry about the cause of the antibacterial effect.

### 2.4.3 Result of contact to manufacturers/importers/retailers

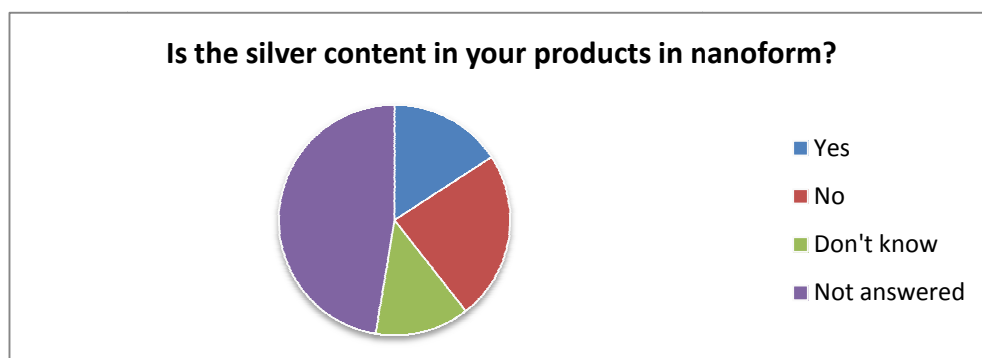
The companies that did not reply to our first enquiry were contacted again. The companies which still not replied received yet another request to fill in the submitted questionnaire.

In total we received 38 responses out of 57 enquiries.

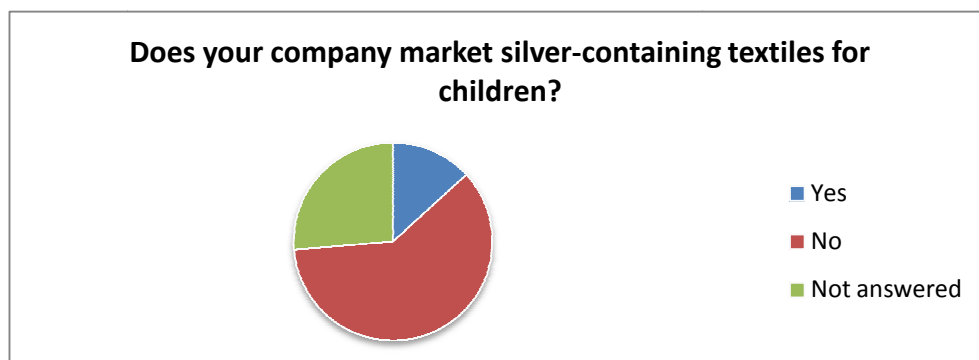
The degree of detail of the questionnaire responses varied a lot. For some of the completed questionnaires only a few of the questions were answered.

The below diagram shows the answers to some of the questions. The answers are illustrated for the total number of returned questionnaires as well as for the questionnaires which answered the actual question.

	Yes	No	Don't know	Not answered
Is the silver content in your products in nanoform?	6	9	5	18



	Yes	No	Not answered
Does your company market silver-containing textiles for children?	5	23	10



#### 2.4.4 Total result of survey in shops, on internet pages and contacts to manufacturers/importers/retailers

The registered textiles are divided into five categories, see below.

The categorization is based either on information from the manufacturers/importers/retailers, from the product label or the product internet page.

- Category 1 – Textile products, containing **silver in nanoform**. This category comprises a limited number of products (7).
- Category 2 – Textile products, containing silver ions/salts. In this category, only a few more products were registered (9).
- Category 3 – Textile products, containing **silver threads/macroscopic silver**. A considerably higher number of products fall into this category (52) – of which 46 products came from one and the same retailer.
- Category 4 – Textile products, where **the form of silver was not identified**. This category numbers 9 products. The lack of identification is due to two factors: the manufacturers/importers/retailers did not know the form of the silver or they did not respond to the questionnaire.
- Category 5 – Textile products which had got an **antibacterial treatment**. This category comprises 17 products.

For 16 out of the 17 products in category 5 it is still not known what the antibacterial treatment consists of, simply because there has been no response from the manufacturers/importers/retailers in spite of more enquiries to them. Besides, more products were registered which were said to be antibacterial (primarily on the internet), but a further examination of the labels and web information revealed that the antibacterial effect was caused by the fabric as such (silk or wool).

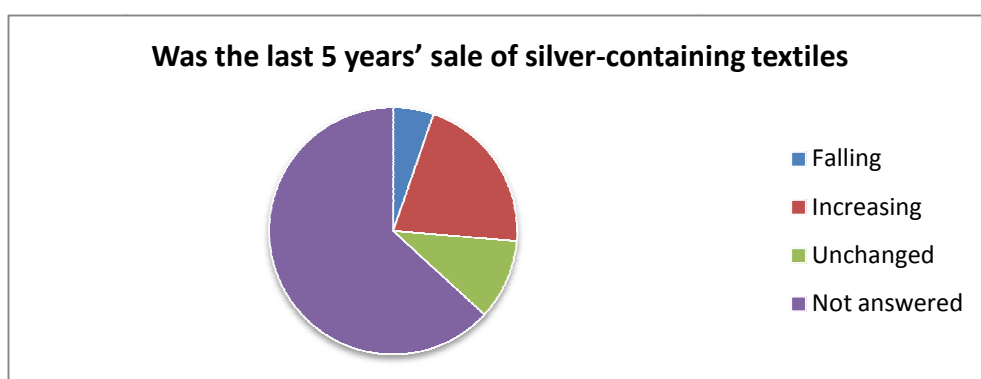
## 2.5 ASSESSMENT OF DEVELOPMENT AND EXTRAPOLATION OF THE MARKET

Only in very few cases, the shop staff knew whether a given product contained silver, thus it is not possible to base the assessment of the market development

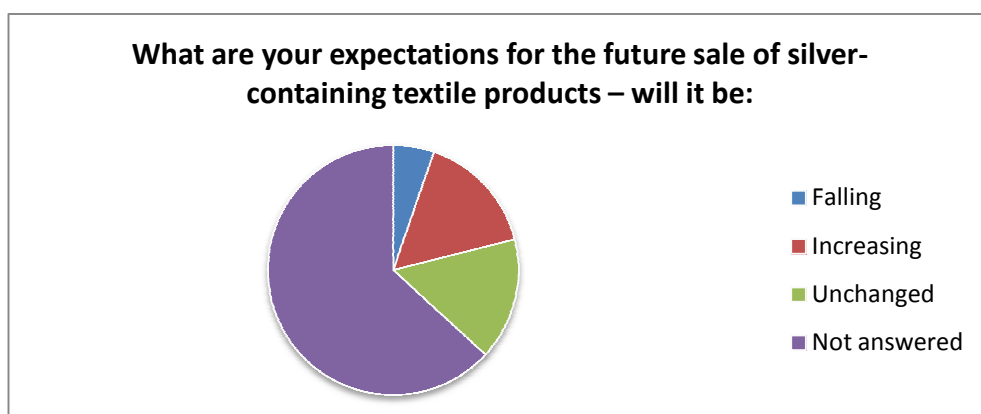
on the shops' experiences. As mentioned before, the staff of the visited shops all agreed that people are looking for brand/make, colour etc., and not for silver content or antibacterial effect.

Enquiries to the manufacturers/importers/retailers did not contribute to a unique estimate of the development of sale of products containing silver or nanosilver. Of the relatively few answers available (14), 8 show that there has been growing sales of products within the last 5 years, 4 that sales were unchanged and 2 that there has been a decrease in the number of products sold.

	Falling	Increasing	Unchanged	Not answered
Was the last 5 years' sale of silver-containing textiles =>	2	8	4	24



	Falling	Increasing	Unchanged	Not answered
What are your expectations for the future sale of silver-containing textile products – will it be =>	2	6	6	24



The summary of the responses is that 6 expect an increasing sale, 6 expect unchanged sale and 2 foresee a falling sale of silver-containing products.



## 2.6 CONCLUSION

In connection with the survey only few textile products were registered in stores and on the internet, which according to the labelling or web information contained nanosilver. Seen in the light of the intense discussions in various media and blogs, we expected to find a larger number of this type of products, than was actually possible in this present, systematic project.

The contact to the manufacturers/importers/retailers showed that the major part has very limited knowledge about the content and/or the form of the silver in their products. In several cases the manufacturers/importers/retailers have replied that their products did not contain silver, despite the information on the product labels or the websites

Looking solely on the research results will give the impression that there are only very few textile products on the Danish market which contain silver, but since the research also established that importers and dealers lacked knowledge about the actual content of their products, it is highly probable that the use of such products might be “hidden” and more widespread than it appears from the responses. Although many textiles are imported from countries outside the EU, it is worth noting that according to Swiss data<sup>6</sup> there is only limited use of nanosilver in textiles within the EU.

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<sup>6</sup> HSR, UMTEC: Entsorgung nanosilberhaltiger Abfälle in der Textilindustrie – Massenflüsse und Behandlungsverfahren -

# 3 Chemical Analyses

With background in the survey, a series of textiles has been chosen for further examination by means of chemical analyses. The results of the analyses have been used in the risk and health assessment and in the environmental impact assessment.

During the survey, only seven products were registered, which according to the received information contain silver in nanoform (category 1). From these products, six were selected for analysis – the seventh product is not being manufactured anymore.

To be able to compare the concentration level of silver in textiles with and without silver in nanoform, also products from the other categories were selected for analysis (category 2-5).

## 3.1 CRITERIA FOR SELECTION OF PRODUCTS FOR ANALYSIS

By selection of specific textiles, focus has been directed to the following conditions:

- Focus on textiles that often are in direct contact with skin
- Focus on textiles used where sweat typically is expected
- Period of use for the textiles (how long and how often are the products expected to be used)
- Products for both children and adults
- Products that are relevant for assessment of environmental impact (leaching)
- Products available in "physical" shops and products available in "internet shops".

The extent of the project has only permitted purchase of a limited number of textile products for further analysis (a total of 16 textile products), which has been carried out with every possible consideration of the above criteria. Thus, it has not been possible to select a number of products from each criteria category for further examination and inspection.

### 3.1.1 Reasons for Choice of Products

In the survey, the registered products are divided into five categories:

Category 1 – Textile products which according to information contain *silver in nanoform*

Category 2 – Textile products which according to information contain *silver ions/silver salts*

Category 3 – Textile products which according to information contain *silver threads/macrosopic silver*

Category 4 – Textile products where *the silver form is not identified*

Category 5 – Textile products which according to information have been subject to *antibacterial treatment*.

**Category 1 – Textile products which according to information contain *silver in nanoform***

Six products were sampled for analysis in this category. The six selected textiles include two T-shirts, one insole, one sole from a sandal, one shower curtain and a microfibre cloth intended for cleaning.

**Category 2 – Textile products which according to information contain *silver ions/silver salts***

A total of nine products are registered in this category. The category includes a shirt and a "hat" which can be used as a hat, a headband and/or a scarf.

The shirt was selected since it gets in direct contact with skin. The shirt has short sleeves, and therefore it is considered to be used mostly in the summer, when sweat is expected to occur. Furthermore, a shirt is supposed to be worn for many hours. The hat is selected, partly due to its availability in junior sizes, and partly due to its supposedly direct contact with skin and mouth/nose, when it is used as a scarf.

**Category 3 – Textile products which according to information contain *silver threads/macroscopic silver***

A total of 52 products are registered in this category. Actually, the category is beyond the project frames, since the textile products contain silver threads. However, four products were sampled from this category, since three of the products are targeted directly at small children (cuddly toys, sheets intended for cots and a body-stocking). The fourth product is piece goods which can be used in many products.

**Category 4 – Textile products *where the silver form is not identified***

Nine products are registered in this category. One tank top, one pair of running socks and one top mattress were selected.

The tank top was selected because it is intended for children, it has directly contact with skin, it is used during the summer where sweat might occur, and because it is expected to be worn for a longer period of time. The running socks were selected because of their direct contact with skin, since they are used where sweat might occur and they possibly are worn for a longer period of time. The top mattress was selected, since it is used for a longer period of time.

**Category 5 – Textile products which according to information have been subject to *antibacterial treatment***

17 products have been registered. Nursing pads were selected due to their direct contact with skin.

### 3.2 ANALYSIS PROGRAM

The analysis program was initiated with a quantitative analysis of selected textiles for determination of the total amount of silver with the purpose of subsequently being able to select relevant textiles for further examinations in the shape of:

- particle examination for identification and determination of particle sizes
- migration examination for liberation of silver for saliva and sweat simulants

- washing experiments for liberation of silver.

### 3.3 QUANTITATIVE ANALYSIS FOR SILVER

During the initial quantitative analysis, the total amount of silver (both nanoparticles and silver on other forms) was analysed from the selected textiles.

#### 3.3.1 Method for Quantitative Analysis for silver, ICP-MS and ICP-AES

The analyses for nanosilver were carried out by means of inductively coupled plasma mass spectrometry (ICP-MS) and inductively coupled plasma atomic emission spectrometry (ICP-AES).

A test portion was sampled from the product and weighed. The weighed test portion was prepared with concentrated nitric acid and hydrogen peroxide in a quartz autoclave by microwave-induced heating, by which means the silver is brought to a dissolved form. The prepared samples were analysed for silver by means of ICP-MS with rhodium (Rh) as internal standard and by means of ICP-AES. The silver content was quantified to traceable standards of silver nitrate. High concentrations of silver were mainly analysed by means of ICP-AES, possibly after dilution of the sample. Double determination was carried out. The results are indicated in  $\mu\text{g}$  silver per g sample ( $\mu\text{g/g}$ ).

Linearity was determined in the measuring range 0.5-50 ng/ml for ICP-MS and 0.05-2.5  $\mu\text{g/ml}$  for ICP-AES. By detection of content above the linear range, the sample was diluted and re-analysed. No silver was detected in the blanks. The detection limit was determined to 0.01  $\mu\text{g/g}$  and 0.5  $\mu\text{g/g}$  by ICP-MS and ICP-AES, respectively. The quantification limits have been set to 0.03  $\mu\text{g/g}$  and 1.5  $\mu\text{g/g}$ , respectively. The uncertainty of the method is less than 2% above the quantification limits. The relative analysis error of double determinations has been indicated for each product.

Examination of recovery percentage has been carried out, since test portions were sampled of selected products, which were spiked with a solution of silver nitrate before the preparation (six determinations on each level). It turned out that the recovery percentage is very different for the three examined products (36492-2, -4 and -12).

The recovery percentage for product no. 36492-2 was determined to be below 30%, both on a low and a high concentration level, which indicates that there are components in the product which absorb the silver, meaning that it is not available for analysis.

The recovery percentage for product no. 36492-4 was determined to be 95-98% at low level (approx. 15  $\mu\text{g/g}$ ), whereas the recovery percentage at higher level (approx. 70 and 140  $\mu\text{g/g}$ ) drops down to 30-40%.

The recovery percentage for product no. 36492-12 was determined to be 80-86% at high level (approx. 40  $\mu\text{g/g}$ ).

Generally, it looks like the recovery percentage depends on the condition of the product and it decreases for silver concentrations in the upper measuring range, resulting in and underestimation of the silver concentration in products with a high silver content. It should, however, be noticed that silver is being

spiked in the form of dissolved silver nitrate, which is far from being representative for the silver that has been released from the product during the preparation and thus can be more available for side reactions, deposits, etc., which influence the recovery. Finally, there might be products that are produced by materials, where the silver is less available under the applied conditions.

### 3.3.2 Results for Quantitative Analyses of Silver

The results of the analyses by ICP-MS and ICP-AES for the total amount of silver (both nanoparticles and silver on another form) from the selected textiles are listed in TABLE 3.1. The table shows the relative standard deviation (%RSD) calculated on the basis of the single determinations. DL means “Detection limit”.

TABLE 3.1 QUANTITATIVE RESULTS FOR SILVER CONTENT IN TEXTILES

Product	Description	Single determination Ag µg/g	Average Ag µg/g	%RSD	DL Ag µg/g	Silver form according to the survey	Comment to sampling, analysis and results
36492-1	Running t-shirt	0.058 0.040	0.049	25	0.01	Nano	High %RSD indicates an inhomogeneous distribution of silver in the product
36492-2	Fitness and running t-shirt	-	-		0.01	Nano	
36492-3	Microfibre cloth	93 37 313 263 246	65 270	61 13	0.5	Nano	Test portions were sampled on two different areas of the product. The last three test portions are sampled close to the sampling for migration and wash test. The result shows that the silver is not homogeneously distributed in the product.
36492-4	Shower curtain	0.017 0.014	0.016	12	0.01	Nano	
36492-5	Insoles	20 18	19	8.7	0.5	Nano	
36492-6	Sandal	0.039 0.035	0.037	6.3	0.01	Nano	Test portion sampled of upper side of sole
36492-7	Ski underwear/ t-shirt	5.1 3.6	4.3	25	0.5	Silver ions/ silver salts (Polygiene)	High %RSD indicates an inhomogeneous distribution of silver in the product
36492-8a	Hat/ scarf/ headband	0.18	0.18	-	0.01	Silver ions/ silver salts (Polygiene)	Consists of two types of fabric material, same product as in 8b, sampling of fleece, single determination
36492-8b	Hat/ scarf/ headband	20.5 20.3	20	0.71	0.5	Silver ions/ silver salts (Polygiene)	Consists of two types of fabric material, same product as in 8a, sampling of plain, patterned fabric
36492-9	Piece goods	7660 7640	7650	0.21	0.5	Silver threads	
36492-10	Cuddly toy	10200 10400	10300	1.3	0.5	Silver threads	

Product	Description	Single determination Ag µg/g	Average Ag µg/g	%RSD	DL Ag µg/g	Silver form according to the survey	Comment to sampling, analysis and results
36492-11	Sheet for cot	6.9 7.2	7.0	2.3	0.5	Silver threads	
36492-12	Body-stocking with long sleeves	10.3 10.3 11.9 11.5 11.4	10 12	0.11 2.3	0.5	Silver threads	Test portions were sampled on two different areas of the product. The last three test portions are sampled close to the sampling for migration and wash test. The result shows a homogenous distribution of the silver.
36492-13	Tank top for children	13 10	12	16	0.5	Silver form not indicated	
36492-14a	Running socks	0.37 -	0.37 -		0.01	Silver form not indicated	Underside, new product bought and analysed, see 36492-14b, since silver was only detected in one of the test portions
36492-14b	Running socks	0.068 0.780	0.43	120	0.01	Silver form not indicated	High %RSD indicates an inhomogeneous distribution of silver in the product, the product is identical with 36492-14a
36492-15	Top mattress	300 240	270	15	0.5	Silver form not indicated	Test portion sampled on upholstery fabric
36492-16	Nursing pad	0.043 0.050	0.047	10	0.01	Antibacterial	

<sup>1,2</sup> Means “not detected above the detection limit”.

### 3.3.3 Conclusion of Results for Quantitative Analysis of Silver

The examined textiles showed very varying contents of silver.

The relative standard deviation for analysis of double determinations varied from 0.11% to 120%, which indicates an inhomogeneous distribution of silver in some of the products. All products with silver threads have a homogenous distribution of silver content.

In the products where the silver form according to the information in the survey is identified as nano, the highest content is proved to be in the microfibre cloth (36492-3) and in the insole (36492-5). A lower content is shown in a running t-shirt, a shower curtain and in the upper side of the sole in a sandal (36492-1, -4 and -6).

In one product, fitness running t-shirt (36492-2), it has not been possible to detect any content of silver by sampling for double determination. There can be several reasons for that:

- The product does not contain silver
- The product has an inhomogeneous distribution of silver, which is not possible to determine visually
- The product absorbs the silver and it is therefore not available at the applied analysis.

In products where the silver form is identified as something else than nano or is not identified, the highest contents of silver have been detected in products

containing silver threads, piece goods and cuddly toys, respectively (36492-9 and -10).

The method validation indicates that results for products with a high silver content could possibly have been underestimated.

### 3.4 PARTICLE EXAMINATION

From the results of the quantitative determination of the total amount of silver (both nanoparticles and silver on another form), textiles have been selected where the textile samples' surfaces are examined for silver nanoparticles. In cases where particle content is demonstrated, the size and distribution of the particles on the surface of the textile have been determined.

In the following, nanoparticles are defined as particles in the size 1-100 nm in accordance with the definition recommended by the European Commission (2011).

12 products were selected for particle studies. By selection of specific textiles, focus has been directed on products, where the silver form, according to the information in the survey, is identified as nano - four products were selected (36492-1, 36492-3, 36492-5, 36492-6). Four products, where the silver form was not identified or were registered as antibacterial, were selected for identification of the silver form (36492-13, 36492-15, 36492-14b, 36492-16). Two products with silver ions/salts and two products with silver threads were selected (36492-7, 36492-8b, 36492-10, 36492-12).

#### 3.4.1 Method for Particle Examination, SEM/EDX

The surface of the samples is examined in scanning electronic microscope (SEM) with facilities for X-ray microanalysis (EDX). By the SEM and EDX examination, particles of the textile surfaces are localized, and the particles' composition of elements is determined. The particle sizes are measured. Particle shapes are documented in SEM-images. For samples with satisfactory electrically conducting properties, the SEM technique has a dissolving power of approx. 4-5 nm. Therefore it is not possible to localize the particles with diameters below 5 nm. The dissolving power for spot analyses carried out with the EDX technique is approx. 1 µm.

The result of the examination is available in pictures describing how and where the silver particles or nanoparticles are located on the surface of the examined textile samples. It was attempted to measure diameters of approx. 20 particles or clusters of particles on the surface of each of the examined samples.

#### 3.4.2 Results of Particle Examinations

In Appendix 5 there are comments to the results for each product. In Appendix 5, a picture has been enclosed for each examined sample (Appendix 5, A1-A12), in which four SEM-images are showing the textile samples' surface. The four SEM-images show enlargements of 100, 500, 1000 and 5000 times, respectively. Details down to 50 nm will easily show up in the images recorded at 5000 times magnification. The samples were examined at magnifications well over 5000 times. The magnifications which give the most representable SEM image of the samples were included in

appendix 5. The SEM-images were recorded with a backscattered electron detector (RBSD).

Backscattered electron signals from the samples' surface will depend on the composition of elements in the chosen picture areas. In SEM-images recorded with the RBSD detector, silver particles and silver threads will appear with a very light grey tone (almost white) compared with the textile fibres which appear with a darker grey tone.

On some of the SEM-images, light spots appear, which is due to the electron charging of the surface. During the SEM examination it was not possible to remove the electrons from the surface of the fibres. They are accumulating and appear as light spots on the surface of the fibres. That the bright spots observed on the fibres is due to this phenomenon is verified during the SEM examination where it is registered that the light spots change place on the samples' surface after each scan with the electron beam.

### **3.4.3 Conclusion of Results of Particle Examinations**

The examination shows that only two textiles (microfibre cloth, 36492-3, Appendix 5, A2 and insole, 36492-5, Appendix 5, A3) are coated with silver particles. The particles appear individually and with a relatively large distance to the textile samples' fibres. The silver particles have diameters from approx. 0.2 to 1.0  $\mu\text{m}$  (200 to 1000 nm), which is larger than the applied definition of the size of nanoparticles (1-100 nm) in this report.

The dealers of two products explain that they contain silver nanoparticles, indicating that they apply another definition for the size of nanoparticles (e.g. 1-1000 nm).

It cannot be excluded that the surface of the insole of the sandal (36492-6, Appendix 5, A4) and the running t-shirt (36492-1, Appendix 5, A1) contains silver nanoparticles which were not detected during the SEM and EDX analysis. SEM can localize particles with a diameter down to 5 nm. During the ICP-MS analysis, the silver content in these products is determined to be between 0.035 and 0.058  $\mu\text{g/g}$ , which is below the detection limit for EDX, which is 0.1 w/w % (1 mg/g) in single spots or areas larger than 1  $\mu\text{m}^2$ . If the silver is distributed evenly in the products, either as ions or as nanoparticles, the concentration will be under the detection limit for the EDX method. When having a concentration of silver that low, the EDX method only allows detection of silver, if silver nanoparticles are clustered together and create clumps which jointly contain silver in an amount above 0.1 w/w%.

The rubber material of the sandal contains a lot of very small particles, but it is not possible to determine whether they consist of silver.

In two textiles (cuddly toy, 36492-10, Appendix 5, A7, and top mattress, 36492-15, Appendix 5, A10), interweaved silver threads with a rough surface have been detected.

No silver nanoparticles, silver particles or silver threads have been detected in eight out of 12 textile samples.



### 3.5 MIGRATION EXAMINATIONS

From the results of the quantitative determination of the total content of silver and particle examinations, a series of products has been selected for further examinations. Importance has been attached to including products with silver particles (36492-3 and 36492-5). Furthermore, products with a proved high content of silver have been included as well for comparison matters.

TABLE 3.2 SELECTION OF PRODUCTS FOR MIGRATION EXAMINATIONS

Product	Description	Migration sweat	Migration saliva	Migration time hours
36492-3	Microfibre cloth	X		2
36492-5	Insoles	X		8
36492-8	Hat/patterned textile		X	2
36492-10	Cuddly toy		X	2
36492-12	Body-stocking with long sleeves	X		16
36492-13	Tank top for children	X		16

#### 3.5.1 Method for Migration Examinations

Migration examinations were performed for artificial sweat and artificial saliva and subsequently, ICP-MS analysis was carried out. The migration tests were carried out at a temperature of 37 °C, since this is close to the body temperature. During the performance of the migration examinations, the simulant is pre-heated before being connected to the products.

The selected artificial sweat simulant is described in ISO 105-E04:2008<sup>7</sup>, which is also used in connection with examinations of Øko-Tex® products. The sweat simulant in DS/EN ISO 105-E04 consists of 1-histidine-mono-hydrochloride-1-hydrate, sodium chloride, sodium dihydrogen phosphate and sodium hydroxide for adjustment of pH to 5.5.

The selected artificial saliva simulant has previously been used in the Danish Ministry of the Environment's project concerning two-year old children's exposure to chemical matters and is described in the JRC report<sup>8</sup> and ISO 105-F04 respectively. The artificial saliva consists of sodium chloride, potassium chloride, sodium sulphate, ammonium chloride, lactic acid, urea and hydrochloric acid for adjustment of pH to 6.8.

A test portion of the product was sampled and weighed, and the surface was estimated. The test portion was placed in the pre-heated simulant in a temperature-controlled heating chamber and with static contact to the simulant for the number of hours which are established in the exposure scenarios. Double determinations were carried out.

The test portion is removed from the simulant and analysed to determine the content of silver by means of ICP-MS after adjustment with nitric acid. Traceable standards of silver nitrate prepared in the simulant adjusted with nitric acid are used for the quantification. The results have been reported as µg/g and ng/cm<sup>2</sup>.

Linearity was determined in the measuring range 0.5-50 ng/ml for the simulants artificial saliva and sweat by means of ICP-MS. At demonstration of contents above the linearity area, the sample was diluted and re-analysed.

<sup>7</sup> Textiles - Tests for colour fastness - Part E04: Colour fastness to perspiration

<sup>8</sup> 20001 EUR 19826 EN

There was not detected any silver in the simulant-blanks. The detection limit was determined to 0.01 µg/g during CP-MS. The quantification limit has been set to 0.03 µg/g. The uncertainty of the method is less than 2% above the quantification limit. The relative analysis error of the double determinations has been indicated for each product.

Examination of the recovery percentage was carried out, as test portions of selected products were sampled (hat/scarf, 36492-8, for artificial saliva and body-stocking, 36492-12, for artificial sweat), which were spiked with a silver solution before placement in pre-heated simulants for two hours (six determinations on each level, 200 and 500 ng/ml, respectively in saliva and 10 and 500 ng/ml in sweat), after which the saliva/sweat solution was segregated and analysed for silver by ICP-MS.

The recovery in sweat for body-stocking was determined to be 0% on both concentration levels, and the recovery in saliva for hat/scarf was determined to be 25-135% (an average of 82%) at 200 ng/ml and 0-21% (an average of 6%) at 500 ng/ml. It should be noticed that it is possible to produce standards of silver nitrate in both simulants from 0.5 to 50 ng/ml without any difficulties. Not even traces of precipitated silver compounds were observed during the analysis, which could indicate that all added silver on dissolved form must have been precipitated/ absorbed in the textile. The result of the recovery experiments only demonstrates that the addition of dissolved silver is not a suitable way to simulate recovery under these conditions.

### 3.5.2 Results of Migration Examinations

The results of the migration examinations for sweat simulant and saliva simulant appear from

TABLE 3.3, TABLE 3.4 and

TABLE 3.5. The tables show the relative standard deviation (%RSD) calculated on the basis of the single determinations.

TABLE 3.3 SURFACE/MASS RATIO

Lab. mark	Sample mark	Medium	Cut out cm	2 x area cm <sup>2</sup>	Weight g	cm <sup>2</sup> /g
36492-3	Microfibre cloth	Sweat	5 x 5	50	0.42	119
36492-5	Insoles	Sweat	4.5 x 1.5	13.5	1.05	12.9
36492-12	Body-stocking	Sweat	7.0 x 6.0	84	1.05	80.0
36492-13	Tank top for children	Sweat	5 x 5	50	0.75	66.7
36492-8	Hat/patterned textile	Saliva	5 x 5	50	0.41	122
36492-10	Cuddly toy	Saliva	5 x 5	50	0.40	125

TABLE 3.4 RESULTS OF MIGRATION EXAMINATIONS FOR ARTIFICIAL SWEAT

Lab. mark.	Sample mark	Total <sup>1)</sup> Aver. Ag µg/g	Migration sweat Ag µg/g	Migration sweat Aver. Ag µg/g	Migration sweat Aver. Ag ng/cm <sup>2</sup>	%RSD	Migration sweat Aver. %	Migration time hours
36492-3	Microfibre cloth	270	0.050 0.066	0.058	0.49	20	0.02	2
36492-5	Insoles	19	1.57 1.41	1.5	120	7.6	8	8
36492-12	Body-stocking with long sleeves	10.3	- -	-	-		-	16
36492-13	Tank top for	12	5.91	6.0	89	1.1	50	16

Lab. mark.	Sample mark	Total <sup>1)</sup> Aver. Ag µg/g	Migration sweat Ag µg/g	Migration sweat Aver. Ag µg/g	Migration sweat Aver. Ag ng/cm <sup>2</sup>	%RSD	Migration sweat Aver. %	Migration time hours
	children		6.00					

<sup>1)</sup> Result from quantitative double determination

‘.’ Means “not detected above the detection limit”.

TABLE 3.5 RESULTS OF MIGRATION EXAMINATIONS FOR ARTIFICIAL SALIVA

Lab. mark	Sample mark	Total <sup>1)</sup> Aver. Ag µg/g	Migration saliva Ag µg/g	Migration saliva Aver. Ag µg/g	Migration saliva Aver. Ag ng/cm <sup>2</sup>	%RSD	Migration saliva Aver. %	Migration time hours
36492-8	Hat/patterned textile	20.4	4.7 5.2	4.9	40	8.0	24	2
36492-10	Cuddly toy	7650	7.24 7.19	7.2	58	0.52	0.09	2

<sup>1)</sup> Result of quantitative double determination

### 3.5.3 Conclusion of Results of Migration Examinations

Only limited migration of silver to sweat simulant has been detected, 0.02% for the microfibre cloth and 8% for the insole, respectively.

For tank tops for children a migration of 50% was detected for sweat simulant, and for hat/scarf, the migration detected was 24% for saliva simulant. Both products contain silver ions.

During the examination of the cuddly toy, a migration of 0.09% was detected to saliva simulant.

As to the body-stocking, no migration was detected; however, see method description for further comments.

Thus, the lowest migration is detected from products declared to contain nanosilver and silver threads, and the highest from products containing silver ions.

The results were applied at the health and risk assessment.

### 3.6 WASHING EXPERIMENT FOR RELEASE OF SILVER

From the results from the quantitative determination of the total amount of silver and particle examinations, a series of products has been selected for further examinations. Importance has been attached to including products with silver particles (36492-3 and 36492-5). Furthermore, products containing a high amount of silver and which are expected to be washed often have been included as well.

TABLE 3.6 PRODUCTS SELECTED FOR WASHING EXPERIMENTS

Lab. mark	Sample mark	Declared washing temperature of the product (°C)	Applied washing temperature (°C)
36492-3	Microfibre cloth	90	60
36492-8	Hat/scarf, patterned textile	40	40
36492-10	Cuddly toy	40	40

Lab. mark	Sample mark	Declared washing temperature of the product (°C)	Applied washing temperature (°C)
36492-12	Body-stocking with long sleeves	30	30

### 3.6.1 Description of Washing Experiment

Danish Technological Institute's accredited washing laboratory was used for carrying out the washing experiment. The laboratory has five identical washing machines, Miele Novotronic W 375. The machines have a capacity of 5 kg and several programs. Washing is carried out in accordance with DS/EN 60456:2005<sup>9</sup>.

Choice of washing program took place in accordance with the informative label of the product and expected consumption pattern, since the 60 degrees program was chosen instead of 90 degrees for one of the products. During the washing test, one partial sample was taken (double determination) of the product for quantitative analysis by means of ICP-AES. Subsequently, one partial sample (approx. 10 x 10 cm<sup>2</sup>) was washed and then dried at ambient temperature. One partial sample (double determination) was taken from the washed and dried product and analysed quantitatively by means of ICP-AES.

### 3.6.2 Parameters Registered during the Washing Experiment

The products were washed separately in one of the laboratory's washing machines, Miele Novotronic W 375. The washing ballast (pillow case) and detergent are as specified in EN 60 456. Washing and rinsing water comes from local water supply, adjusted to hardness 2.5 mmol/litre with reverse osmosis water. The partial samples of the products were sewn on a pillow case along one of the edgings to obtain a typical washing influence. The washing temperature is measured using a temperature data unit sewn in the ballast cloth. The remaining water after centrifugation was determined for the ballast clothes. Application/determination of detergent, water hardness, water volume, duration of washing and remaining water is accredited according to DANAK, reg. no. 300. The temperature determination is not comprised by the accreditation.

TABLE 3.7 PARAMETERS REGISTERED DURING WASHING EXPERIMENT

Sample mark	Washing program	Washing program duration min.	Washing ballast gr	Detergent gr	Washing/ rinsing water litre	Max. Washing temperature °C	Remaining water %
36492-12	30°C easy-care	81	2512	26.2	59.8	29	73
36492-10	40°C easy-care	82	2500	26.2	61.2	40	71
36492-8b	40°C easy-care	82	2512	26.2	59.0	40	73
36492-3	60°C easy-care	82	2500	26.2	61.3	60	72

### 3.6.3 Results

The results of the washing experiment for release of silver appear from the following table. The table shows the relative standard deviation (%RSD) calculated on the basis of the single determinations.

<sup>9</sup> Washing machines for clothes for domestic use – methods for measurement of functional properties

TABLE 3.8 RESULTS OF WASHING EXPERIMENT

Lab. Mark	Sample mark	Before wash Aver. Ag µg/g	After wash Ag µg/g	After wash Aver. Ag µg/g	%RSD	Leaching degree Aver. %
36492-3	Microfibre cloth	270	203 249	230	14	15
36492-8	Hat/scarf, patterned textile	20.4	3.224 3.220	3.2	0.088	84
36492-10	Cuddly toy	10300	7100 6800	7000	3.1	32
36492-12	Body-stocking with long sleeves	11.6	12.7 11.9	12	4.6	-

<sup>1)</sup> Result from quantitative double determination

<sup>2)</sup> Means "leaching not detected"

### 3.6.4 Conclusion of Washing Experiment

A leaching of 15 % was detected in the microfibre cloth, 32 % in the cuddly toy and 84 % in the hat/scarf.

No leaching was detected from the body-stocking. The difference of the results before and after wash is due to the sampling and the uncertainty of the analysis method.

The results are applied in the environment assessment.

### 3.7 OVERVIEW OF SURVEY RESULTS

Quantitative analysis of the 16 textiles for determination of the total amount of silver showed very varying contents of silver from 0.016 µg/g to 10300 µg/g. In one product no silver was determined. The highest contents of silver have been detected in products containing silver threads.

The particle examination shows that only two textiles (microfibre cloth and insole) are coated with silver particles. In two textiles (cuddly toy and top mattress) interweaved silver threads with a rough surface have been detected. No silver nanoparticles, particles or silver threads have been detected in eight out of 12 textile samples.

The lowest migration to sweat and saliva simulant is detected from products declared to contain nanosilver (0.02% for the microfibre cloth and 8% for the insole) and silver threads (0.09% for the cuddly toy), and the highest from products containing silver ions (50% for the tank tops and 24% for hat/scarf).

A leaching of 15 % was detected in the microfibre cloth, 32 % in the cuddly toy and 84 % in the hat/scarf. No leaching was detected from the body-stocking.

In TABLE 3.9 appear the results of the chemical analyses and examinations which are applied in the health, risk and environment assessment.

TABLE 3.9 RESULTS OF THE CHEMICAL ANALYSES AND EXAMINATIONS

Product	Description	Silver form according to the survey	Silver form according to SEM and EDX	Total silver content Average Ag µg/g	Migration to sweat or saliva %	Leaching degree %
36492-1	Running T-shirt	Nano	-	0.049	x	x

Product	Description	Silver form according to the survey	Silver form according to SEM and EDX	Total silver content Average Ag µg/g	Migration to sweat or saliva %	Leaching degree %
36492-2	Fitness and running T-shirt	Nano	x	-	x	x
36492-3	Microfibre cloth	Nano	Particles	270	0.02	15
36492-4	Shower curtain	Nano	x	0.016	x	x
36492-5	Insoles	Nano	Particles	19	8	x
36492-6	Sandal	Nano	-	0.037	x	x
36492-7	Ski underwear/T-shirt	Silver ions /silver salts (Polygiene)	-	4.3	x	x
36492-8b	Hat/scarf, patterned	Silver ions /silver salts (Polygiene)	-	20	24	84
36492-9	Piece goods	Silver threads	x	7650	x	x
36492-10	Cuddly toy	Silver threads	Silver threads	10300	0.09	32
36492-11	Sheet for cot	Silver threads	x	7.0	x	x
36492-12	Body-stocking With long sleeves	Silver threads	-	12	-	-
36492-13	Tank top for children	Silver form not identified	-	12	50	x
36492-14b	Running socks	Silver form not identified	-	0.43	x	x
36492-15	Top mattress	Silver form not identified	Silver threads	270	x	x
36492-16	Nursing pad	Antibacterial	-	0.047	x	x
Detection limit			SEM 5 nm EDX 1 mg/g	ICP-MS 0.01 µg/g ICP-AES 0.5 µg/g	ICP-MS 0.01 µg/g	ICP-AES 0.5 µg/g

'x' Means "not examined".

'-' Means "not detected above the detection limit". By SEM it means that neither particles, nor threads have been detected.

# 4 Human health and environmental assessment

## 4.1 INTRODUCTION

The antibacterial effect of silver has been utilised for centuries and the silver ion is known to be effective against a broad range of microorganisms. Nano-silver is a more powerful antimicrobial than bulk silver and appears to be more efficient at generating silver ions (Wijnhoven, et al. 2009). The antimicrobial activity increases with decreasing particle size which has been associated with the increasing surface area-to-mass ratio (Loeschner *et al*, 2011). In addition to the greater release of silver ions, nano-silver appears to present new properties, including the ability to cross biological barriers, increased production of reactive oxygen species, and capacity to efficiently deliver silver ions to the surface of bacteria (Marambio-Jones and Hoek, 2010)

The biocidal properties are used for medical purposes like wound treatment and increasingly in consumer textiles like socks, sports textiles, sheets and linen, cleaning wipes and toys where nanosilver inhibits the growth of odour-causing bacteria through slow release of silver ions.

Silver in general is one of the most widely used nanoparticles in consumer products (Wijnhoven et al., 2009) and especially the uses in textile products and in personal care products may lead to human and environmental exposures. However, as indicated in chapter 1, silver for biocidal applications still constitutes a minor part of the global silver market and of silver ending up in consumer products.

The health and environmental risk related to the use of biocidal nanosilver in certain consumer products has been evaluated or is focus for evaluation by different authorities and institutes. In the US the USEPA has granted the first registration of nanosilver in December 2011, a conditional registration of a preservative for textiles under the FIRA pesticide regulation. The basis for the conditional registration is among others that use of the product will not cause unreasonable adverse effects on the environment during the period when newly required data are being developed.

In Germany, the Federal Institute for Risk Assessment (BfR) published an opinion in December 2009, recommending that nano-silver is not used in foods and everyday products until sufficient data are available to allow a conclusive risk assessment which would ensure that products are safe for consumer health (BfR, 2009). The recommendation caused a strong reaction from industry and other groups, including the Silver Nanotechnology Working Group (SNWG), asking BfR to reconsider their position. BfR then organised a workshops in February 2010 to consider alternative views on nanosilver. Another scientific workshop was organised in February 2012 to discuss the safety of nanosilver in consumer products. The workshops showed that there is significant progress in the area of analysis and physicochemical characterisation of nanomaterials although improvements are still required in relation to validation, standardisation and provision of reference materials.

In the US, biocidal nanosilver is regulated as a pesticide under the FIFRA Regulatory Framework (Federal Insecticide, Fungicide, and Rodenticide Act) regulation. In 2008 the US EPA received the first application to register a product known to contain nanosilver from HeiQ and in December 2011 the USEPA granted a four-year conditional registration for the product, AGS-20. AGS-20, is a nanosilver-silica composite where the nanosilver active ingredient is sintered onto amorphous silicon dioxide having typical particle diameters of one micrometer. The antimicrobial pesticide product, HeiQ AGS-20 is a silver-based product for use as a preservative for textiles (USEPA, 2011). As a condition of registration, EPA is requiring additional data on the product to confirm EPA's assessment that the product will not cause unreasonable adverse effects on human health or the environment, the general standard for a registration under the Federal Insecticide, Fungicide, and Rodenticide Act.

The USEPA risk assessment is based on data from the scientific literature on nanosilver. The daily dose to consumers, was calculated based on an assumption that all of the silver found in the wash water from colour-fast testing of AGS-20 treated textiles and during drying of AGS-20 treated textiles consisted of nanosilver, which would overestimate the daily dose of nanosilver from wearing AGS-20 treated textiles (USEPA, 2011).

Because of the limitations of the database, the USEPA used a maximum 10-fold database uncertainty factor when evaluating the risk from exposure to the nanosilver that might break away from AGS-20.

The assessment of impact to the environment was based on ecotoxicity studies available in the scientific literature for analogous forms of nanosilver and environmental exposure was assessed assuming that 300 million people (U.S. population) each purchased one t-shirt treated with AGS-20 and that all the silver in those t-shirts was released as nanosilver within the first year (USEPA, 2011).

Based on these assumptions the USEPA concluded that for the period of conditional registration there is a low probability of adverse risk to children and the environment from textiles treated with AGS-20. However, because of a risk concern related to occupational use of AGS-20, the label must require workers to wear personal protective equipment. The USEPA is expecting that the use of AGS-20 will lead to less environmental loading of silver compared to currently registered products with the same use pattern, due to the efficiency based on slow release of silver ions from nanosilver compared to the rapid release of silver ions from products containing silver salts (USEPA, 2011).

In 2011 the Danish EPA issued a Survey on basic knowledge about exposure and potential environmental and health risks for selected nanomaterials (Mikkelsen *et al.*, 2011) covering seven different nanomaterials including nanosilver. The background information and findings of relevance for health and environment in this study are summarised in section 4.3 and 4.4 and results from newer literature are added.

The Danish EPA survey and other recent reviews of nanosilver toxicity have suggested that nanosilver may not be more hazardous to humans than other forms of silver and may result in low internal exposure. However, because



data are insufficient to carry out a full risk assessment and because of potential risks related to possible increased resistance of microorganisms against silver, the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) has been requested to provide a scientific opinion on nanosilver: safety, health and environmental effects and role in antimicrobial resistance, by early 2013 (SCENIHR, 2011).

## 4.2 ANTIMICROBIAL RESISTANCE

The antibacterial mode of action of silver ions is only partially understood and different bactericidal mechanisms have been suggested. It is suggested that silver nanoparticles anchor to and penetrate the cell wall of Gram-negative bacteria causing structural changes in the cell membrane and increased permeability which can lead to uncontrolled transport through the cytoplasmic membrane followed by cell death (Wijnhoven, 2009).

Another suggestion is that the mechanism is related to the formation of free radicals which induce membrane damage. The silver ions can enter the cells and interact with thiol groups of vital enzymes and possibly interact with DNA. This has however, not been proved. Other recent evidence suggests that silver nanoparticles may modulate the phosphotyrosine profile of putative bacterial peptides that could affect cellular signalling and inhibit the growth of bacteria (Wijnhoven, 2009).

Studies are available that discuss the mechanisms of silver resistance in different bacterial strains. The two most likely mechanisms of silver resistance as suggested by Chopra (2007) are plasmid acquisition and gene mutation to decrease silver ion uptake and promote efflux. Genetic linkage of silver resistance genes and antibiotic resistance genes has been reported on plasmids mediating silver resistance.

The risk of developing resistance in the clinical setting is considered low due to the lethal concentrations used. However, concerns are raised that the widespread use of nanosilver in consumer products releasing sublethal levels of silver over a long time period may result in development of bacteria resistance (USEPA, 2011).

To study if silver nanoparticles released into estuarine environments resulted in increased antibiotic resistance within the natural bacterial population in estuarine sediments, a 50-day microcosm exposure experiment was carried out. Sediment samples were screened at the end of the exposure period for the presence of bacteria resistant to eight different antibiotics. Statistical analyses showed that there was no increase in antibiotic resistance amongst the bacterial population in the sediment due to dosing of the microcosms with silver nanoparticles. This study indicated that, under the tested conditions, nanosilver released into the coastal marine environment did not increase antibiotic resistance among naturally occurring bacteria in estuarine sediments (USEPA, 2010).

## 4.3 HUMAN HEALTH ASSESSMENT

### 4.3.1 Toxicity of silver and nanosilver

#### 4.3.1.1 Toxicity of silver

Although silver has a long history of clinical and industrial use, the toxicological database is limited. Most information is available for elemental

silver and the monovalent silver ion which are also the most common forms of silver. Drake and Hazelwood (2005) have reviewed the exposure-related health effects of silver and silver compounds and concluded that the body's uptake of silver is usually higher from oral exposure compared to occupational exposure by inhalation. Soluble silver compounds can bind to proteins and are more readily absorbed and seem to cause toxic effects at lower concentrations than metallic silver and insoluble silver compounds. Wijnhoven (2009) has formulated the hypothesis that toxic effects of silver substances are proportional to the rate of release of free silver ions from them.

Soluble silver compounds can enter the blood stream resulting in deposition of silver-protein complexes in body tissues. These protein complexes can be reduced to metallic silver by exposure to UV light or by reductases in the internal organs resulting in discolouration of the skin. The deposition of silver in the skin is known as 'argyria' and deposition in the eyes is termed 'argyrosis', conditions which are characterised by a blue-grey pigmentation of skin and mucous membranes, respectively. Local discolouration can also result from direct contact with skin and conjunctiva. Historically, medical exposure has been the main cause of argyria, but occupational exposure could also lead to the condition. In recent years, ingestion of unregulated silver-containing supplements, including colloidal silver solutions, has increased with the rise of alternative medical practices (Bowden *et al.*, 2011). Silver in doses below levels that cause argyria or argyrosis are generally considered to be relatively non-toxic (Wijnhoven *et al.*, 2009).

Chronic symptoms from prolonged intake of low doses of silver salts are fatty degeneration of liver and kidneys and changes in blood cells. Long term exposure may also result in accumulation of small amounts in the brain and muscles. However, argyria or argyrosis is considered the most notable adverse effect of silver (Drake and Hazelwood, 2005).

#### 4.3.1.2 Toxicity of nanosilver

A number of recent reviews have summarised the existing literature on nanosilver toxicity and discussed the relevance for risk assessment. The following summary of the toxicological effects of nanosilver is based on reviews in Mikkelsen *et al.* (2011), Christensen *et al.* (2010) and USEPA (2010), and in addition relevant information from more recent literature is added. A key issue in relation to some of the older toxicological literature is the lack of proper characterisation of nanosilver. The composition of the nanosilver particles may therefore differ considerably with regard to size, size distribution, solubility, and aggregation and may affect the results of the studies (USEPA, 2010).

#### Absorption, distribution and elimination:

Nanoparticles can enter the body via the inhalatory, oral and dermal routes of exposure. Absorption of nanoparticles by the oral and inhalatory routes has been demonstrated in *in vivo* studies in rats. Silver was measured in blood and found in low concentrations in different organs such as liver, kidney and brain. However, it is unclear in which form (as particles, free ions, silver ions or complexes) nanosilver is absorbed and distributed to target organs. For uptake via the oral route it is likely that some of the uptake occurs as ions. Smaller particles appear to exhibit higher toxicity compared to larger particles (Mikkelsen *et al.*, 2011).

Dermal absorption is an important potential exposure route for nanosilver in antibacterial textiles. Increased levels of silver in plasma and urine have been measured, proving the absorption of silver following application of nanosilver-containing wound dressings used for treatment of burned skin. Serum levels increased with increasing exposure time peaking after 9 days and returning to normal 6 months after exposure. However, as in the case of oral absorption, it is not clear whether silver is absorbed as silver ions released from nanosilver or as nanosilver particles. If silver ions alone enter the body, toxicological evaluations should focus on the rate and release of ions from nanosilver whereas in the case that nanosilver itself enters the body, toxicity studies should need to consider nanosilver as a new chemical entity (Mikkelsen *et al.*, 2011; Wijnhoven, 2009).

In an *in vitro* diffusion cell system silver nanoparticles (70 µg cm<sup>-2</sup>, for 24 hours) were able to pass through the skin preparations but at a very low absorption rate (0.00066% of applied dose with intact skin) but detectable and the penetration was 5 times greater in damaged skin (0.0033%) (Mikkelsen *et al.*, 2011).

Nanosilver absorbed through lung epithelia, intestinal lining or dermis, is transported with the blood and distributed throughout the body. Due to the specific properties of nanosilver including the small diameter, nanosilver can penetrate deep into the lungs and diffuse to the high lung surface area in the alveolar region (Wijnhoven, 2009). Silver has been detected in the following secondary organs: liver, kidney, spleen, heart, olfactory bulb, brain, testes and skin (discolouration of the skin), with liver and skin being the likely major secondary organ sites of accumulation (Mikkelsen *et al.*, 2011).

Little information is available regarding elimination of nanosilver. Reports of elevated silver levels in urine following exposure to nanosilver in wound dressings indicate that renal elimination is a likely excretion route. Other excretion routes, e.g. via bile and subsequent elimination via feces, excretion via sweat, lungs and saliva are other possible elimination routes which need further investigation (Wijnhoven, 2009).

#### Acute toxicity:

There are few relevant studies available on the acute toxicity of nanosilver. It is discussed but not confirmed that the toxic effects (especially acute) of silver-containing materials are directly proportional to the rate of release of monovalent silver ions (USEPA, 2010). The rate and extent of release of silver ions from nanosilver at the site of entry is therefore focus for future research (Wijnhoven, 2009).

Evidence of inflammation (lymphocyte influx) for both particle sizes (greater response for the nanoform) was reported in mice that received a single high dose of silver in the form of nano- (15 nm) and micro-particles (2 - 3.5µm). Because of the high concentration the study is considered of limited relevance for consumer risk assessment.

Acute oral and dermal toxicity of nano-sized colloidal silver was studied in Sprague Dawley rats according to OECD guidelines No. 423<sup>10</sup> and 402<sup>11</sup>, and GLP. The average particle size was 10.0 nm and the mean surface area was

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<sup>10</sup> OECD Guideline No. 423: Acute Oral Toxicity – Acute Toxic Class Method

<sup>11</sup> OECD Guideline No. 402: Acute Dermal Toxicity

$3.18 \times 10^2 \text{ mm}^2/\text{particle}$  ( $54.88 \text{ m}^2/\text{g}$ ). None of the rats showed any abnormal signs or mortality at dose levels up to 2000 mg/kg (Kim, 2012).

A recent study of acute dermal toxicity in guinea pigs exposed to 100, 1000, and 10000  $\mu\text{g}/\text{mL}$  of colloidal nanosilver performed in compliance with the OECD guideline no. 402 did not demonstrate any significant changes in organ weight or result in major macroscopic changes. Dose-dependent histopathologic abnormalities were seen in skin, liver, and spleen of all test groups and the results indicated that exposure to  $>0.1 \text{ mg}/\text{kg}$  of silver nanoparticles may result in slight liver, spleen, and skin damage (Korani, 2011).

Case reports have demonstrated skin discolouration (argyria) and elevated liver enzyme levels in patients with burns treated with wound dressings containing nanosilver. Elevated enzyme levels indicated liver injury or stimulation, but returned to normal after treatment was stopped (Christensen *et al.*, 2010).

No acute inhalation toxicity studies with nanosilver have been identified.

#### Irritation, corrosion and sensitisation:

Kim *et al.* (2012) studied acute eye and dermal irritation and corrosion using rabbits exposed to nano-sized colloidal silver (same material as used for the acute oral and dermal tests) but found no significant clinical signs or mortality and no acute irritation or corrosion.

Kim *et al.* (2012) also studied skin sensitisation in a guinea pigs maximization test according to OECD guideline no. 406<sup>12</sup> using the same nano-sized colloidal silver. At 24 and 48 hours after removing the challenge patch, one animal (1/20) showed discrete or patchy erythema and the nanosilver test substance was categorised as a weak skin sensitiser. However, as wound dressings containing silver nanoparticle are routinely applied and none of the studies investigating this application have reported sensitisation, nanosilver particles are generally not considered dermal sensitisers. Regarding possible sensitisation following inhalation, further proof/investigation is required.

#### Repeated dose toxicity:

Repeated dose toxicity in rats from 28 days and 90 days oral exposure to nanosilver particles is reported in Mikkelsen *et al.* (2011) from studies carried out according to OECD guidelines. The 28 days study with rats exposed to relatively high doses up to 1000 mg/kg bw/day showed dose-dependent toxicity in the liver, but no NOAEL could be established based on this study due to limited histopathology. In the 90 days study with rats dosed at 0, 30, 125 and 500 mg/kg bw/day, the liver was also the target organ with significant dose-related changes in alkaline phosphatase and cholesterol levels of male and female rats at and above 125 mg/kg bw/d, indicating slight liver damage. A NOAEL of 30 mg/kg bw/day and LOAEL of 125 mg/kg bw/day were suggested based on liver effects. Dose-related increase of silver deposition was observed in testes, liver, kidneys, brain, lungs and blood of treated rats.

Repeated dose toxicity in mice following 28 days oral exposure to nanosilver particles (42 nm) in doses of 0.25 mg/kg bw, 0.5 mg/kg bw and 1.00 mg/kg bw resulted in elevated liver enzymes and slight cell infiltration in the cortex of

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<sup>12</sup> OECD Guideline No. 406: Skin sensitisation

the kidneys in the high-dose group. These findings indicate adverse impacts on liver and kidney (Park *et al.*, 2010).

The results of a study investigating the distribution of silver after 28 days oral administration to rats of nanosilver particles and silveracetate showed presence of nanosized silver granules containing selenium and sulphur in the intestinal wall of rats exposed to either of the silver forms suggesting a similar organ distribution and a common mechanism of their formation (Loeschner *et al.*, 2011). Additional studies are needed to further elucidate the underlying mechanisms of the granule formation.

Results from two 28 days inhalation studies in rats exposed to nanosilver particles showed slight hepatic necrosis at the highest dose level in one study but did not demonstrate any distinct toxicity (Christensen *et al.*, 2010).

Results from 90 days inhalation exposure to nanosilver particles studied in rats showed lung inflammation and alterations in lung function parameters, and liver effects at the highest dose. A LOAEL of 49  $\mu\text{g}/\text{m}^3$  based on the lung effects and NOAEL of 133  $\mu\text{g}/\text{m}^3$  based on the liver effects were suggested to be used for risk assessment purposes (Christensen *et al.*, 2010). Another conclusion from the authors was that surface area seemed to be an important parameter for nanosilver toxicity and that the nanosilver particles were shown not to aggregate/agglomerate during the study. Since gold nanoparticles, which are not likely to be ionised, also have been shown to cause lung inflammation, the authors also hypothesize that effects from silver nanoparticles are not only due to ionisation of silver from the surface of silver nanoparticles, but may originate (at least in part) from direct effects of nanoparticles (Kim *et al.*, 2010).

Results from a recent study with mice exposed to 3.3  $\text{mg}/\text{m}^3$  nanosilver 4 hours/day for 10 days showed minimal pulmonary inflammation or cytotoxicity. It was also reported that nanosilver did not dissolve in artificial interstitial fluid (Stebounova *et al.*, 2011).

A study with guinea pigs exposed daily to an aqueous solution of nanoparticles to shaved skin for 13 weeks showed dose and exposure duration-dependent histopathological abnormalities of the skin, liver, and spleen. The results indicated that exposure to > 0.1  $\text{mg}/\text{kg}$  could result in slight liver, spleen, and skin damage. The authors further conclude that tests involving lower doses and different time periods are required and that the effect of particle shape and size on the toxicity profile of nanosilver administered by different routes should be determined in the future (Korani *et al.*, 2011).

#### Mutagenicity and genotoxicity:

Kim *et al.* (2012) did not observe any mutagenic effect in an Ames test carried out in accordance with OECD guideline no. 471<sup>13</sup> and no chromosomal abnormalities in an *in vitro* chromosomal aberration test according to OECD guideline no. 473<sup>14</sup>.

Flower *et al.* (2012) evaluated genotoxicity of synthesized nanosilver particles in human peripheral blood cells using the alkaline comet assay. Results

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<sup>13</sup> OECD Guideline No. 471: Bacterial Reverse Mutation Test

<sup>14</sup> OECD Guideline No. 473: In Vitro Mammalian Chromosome Aberration Test

indicated that nanosilver (50 and 100µg/mL) caused DNA damage following a 3h treatment and subsequently, also a short treatment of 5minutes.

Li et al. (2011) found an increased micronucleus frequency in an *in vitro* micronucleus assay with human lymphoblastoid TK6 cells treated with 30µg/ml nanosilver. The result was characterised as a weak positive response. No positive response was found in an Ames test at doses that could be assayed and the authors suggest the *in vitro* micronucleus assay may be more appropriate than the Ames test for evaluating the genotoxicity of the nanosilver.

In a study mimicking the repair status of human testicular cells vs oxidative damage, thus being a suitable model for human male reproductive toxicity, Asare *et al.* (2012) found that silver nano- and submicron-particles (AgNPs) are more cytotoxic and cytostatic compared to TiO<sub>2</sub>-NPs, causing apoptosis, necrosis and decreased proliferation in a concentration- and time-dependent manner. 200 nm nanosilver particles in particular appeared to cause a concentration-dependent increase in DNA-strand breaks in NT2 cells, whereas the latter response did not seem to occur with respect to oxidative purine base damage analysed with any of the particles tested.

It is suggested that nanosilver may cause mutagenicity/genotoxicity via an indirect threshold mechanism driven by formation of ROS (reactive oxygen species) (Christensen *et al.*, 2010). However, further studies are needed in this area.

#### Carcinogenicity and reproductive toxicity:

No studies investigating the carcinogenicity of silver nanoparticles have been identified. However, as signs of genotoxicity have been observed, carcinogenicity cannot be excluded and needs further investigation.

A few *in vitro* toxicity studies with relevance for male reproductive toxicity have been identified and indicate that nanosilver may have reproductive effects. Concentration-dependent toxicity was observed in both a mouse spermatogonial stem cell line and in human testicular cells. However, firm conclusions can not be drawn from these studies due to uncertainty regarding nanosilver toxicokinetics (Christensen *et al.*, 2010; Asare *et al.*, 2012).

Size- and dose-dependent mortality, uptake, and embryonic malformation were observed in zebrafish embryos exposed to colloidal silver. The relevance for humans is uncertain (Christensen *et al.*, 2010).

#### Mechanism of toxicity:

*In vitro* studies have shown that the main mechanism of silver nanoparticle toxicity seems to be mediated by an increase in ROS production, stimulating inflammation and genotoxic events and apoptosis or necrosis. The concentration of the administered nanoparticles is able to influence the toxicity. Specifically, at low levels of oxidative stress a protective response is initiated which progresses to a damaging response with increasing particle concentration, and therefore oxidant levels. It is thus relevant to consider that the toxicity of silver nanoparticles follows a threshold mode of action.

It is still unclear whether nanosilver is more toxic than bulk silver after oral exposure, especially after long term exposure. The toxicity of silver after oral exposure is low, however after long term exposure both nano and bulk silver

can accumulate in the skin and different organs, which can result in a bluish-grey colouring of the skin (argyria) or deposition in the eyes (argyrosis). This discolouration is primarily a cosmetic problem and not a life-threatening condition. The effect on other organs after long term exposure of silver in different forms is still unknown (Mikkelsen *et al.*, 2011).

#### 4.3.1.3 Critical endpoints for human health assessment

Lung, liver and skin appear to be the potential target organs in relation to nanosilver exposure and toxicity. Exposure related to nanosilver in textiles is primarily through dermal contact but also oral exposure may be relevant, especially in relation to children's toys. Argyria is the most frequently reported effect of oral and dermal exposure to large amounts of silver particles, but potential target organs include the liver. The amounts causing argyria and argyrosis largely exceed realistic exposure from consumer products. Dermal absorption primarily occurs through damaged skin, but more information with regard to the absorption through intact skin is required to establish a dermal absorption factor.

Until more such dermal toxicity studies are available, information from other exposure routes must be used for risk assessment and default absorption rates must be applied.

According to Wijnhoven *et al.* (2009) the need for further toxicological information to be used for risk assessment depend on answers to questions regarding characteristics of nanosilver in relation to release of silver-ions, and absorption and toxicity of nanosilver, obtained from *in vivo* studies. If only silver ions cross the port of entry, existing toxicological data on silver can be used in the risk assessment of silver.

Available data for risk assessment are presented in Table 4.1.

TABLE 4.1 AVAILABLE MAMMALIAN TOXICITY DATA FOR RISK ASSESSMENT OF NANOSILVER

Exposure route	LOAEL	NOAEL	Basis
Inhalation	49 µg/m <sup>3</sup>	-	Based on lung inflammation and alteration of lung function parameters following 90 days inhalation exposure of rats. <sup>15</sup>
Inhalation	-	133 µg/m <sup>3</sup>	Based on liver effects following 90 days inhalation exposure of rats. <sup>16</sup>
Oral	125 mg/kg bw/day	-	Based on changes in alkaline phosphatase and cholesterol levels following 90 days oral exposure of rats. <sup>17</sup>
Oral	-	30 mg/kg bw/day	Based on changes in alkaline phosphatase and cholesterol levels following 90 days oral exposure of rats. <sup>18</sup>
Oral	-	0.5 mg/kg bw/day	Based on adverse liver and kidney effects determined by blood chemistry and histopathological analysis from 28 days exposure of mice to 42 nm nanosilver particles. <sup>19</sup>

<sup>15</sup> Reported in Christensen *et al.* 2010

<sup>16</sup> Reported in Christensen *et al.* 2010

<sup>17</sup> Reported in Mikkelsen *et al.* 2011

<sup>18</sup> Reported in Mikkelsen *et al.* 2011

<sup>19</sup> Park *et al.*, 2010

Exposure route	LOAEL	NOAEL	Basis
Dermal	-	0.5 mg/kg bw/day	No NOAEL values are available from repeated dose toxicity studies. A NOAEL from the oral study above is selected by route-to-route extrapolation. <sup>20</sup>

In addition to the direct human health endpoints mentioned above, development of bacterial resistance to silver, preventing the efficient use in treatment of wounds may also be considered a critical endpoint. However, sufficient information is not available to evaluate the consequences of increased use of sublethal levels of nanosilver in consumer products, and further evaluation is not within the scope of this report.

#### 4.3.2 Exposure of humans

Based on the survey, direct exposure of humans to nanosilver from contact with textiles can be expected from sports clothes, socks, insoles and cleaning cloths. Exposure to silver possibly in the nano-form can also be expected from mattress covers, hats, nursing pads and cuddly toys, based on information on antibacterial properties. Other products with less direct contact where nanosilver has been identified from the survey include shower curtains.

Dermal contact is expected to be the primary route of exposure and will be including different parts of the body. Oral exposure is possible with children sucking on the cuddly toy. Exposure may also occur through inhalation when dust is released from textiles in use. However, this exposure route is not expected to make a significant contribution from the products tested in this survey.

Exposure scenarios are developed in accordance with the REACH Guidance document: ‘Guidance on Information Requirements and Chemical Safety Assessment. Chapter R.15 - Consumer exposure estimation’, version 2 from April 2010 (ECHA, 2010a) and include exposure of both children and grown ups.

Algorithms and equations for dermal exposure are based on the models shown in section R.15.3.2.2 in the guidance, which is dealing with dermal exposure in scenarios concerning non-volatile substance migrating from an article and on R.15.3.3 which deals with oral exposure.

Realistic ”worst case” scenarios will also consider combined exposure from different sources including e.g. simultaneous contact with the body stocking and shoe soles. In addition the exposure scenarios will consider different absorption from contact with both intact and damaged skin.

An overview of proposed exposure times for the ”worst case” calculations is presented in Table 4.2.

TABLE 4.2  
OVERVIEW OF ”WORST CASE” EXPOSURE TIMES FOR THE SELECTED PRODUCTS

Product	Description	Silver form according to the survey	Silver form according to SEM/EDX	Migration sweat	Migration saliva	Migration time hours

<sup>20</sup> USEPA, 2011 (Pesticide registration)



36492-3	Microfibre cloth	Nano*	Particles	X		2
36492-5	Insoles	Nano*	Particles	X		8
36492-8	Hat/patterned textile	Ions/salts	-		X	2
36492-10	Cuddly toy	Silver threads	Silver threads		X	2
36492-12	Body-stocking with long sleeves	Silver threads	-	X		16
36492-13	Tank top for children	Not identified	-	X		16

\*: Particle examinations indicate that the nanoparticles have diameters from approx. 0.2 to 1.0 µm (200 to 1000 nm) which is larger than the applied definition of nanoparticles.

Dermal exposure scenarios are calculated for adults in contact with insoles 8 hours per day and children in contact with a tank top 16 hours per day. Migration to the sweat simulant measured for a microfibre cleaning cloth was considerably lower than the migration from the insole and the tank top, and this scenario is therefore not included.

Dermal absorption data indicate that absorption is limited through both intact and abraded skin. Results from a study investigating the penetration of polyvinylpyrrolidone coated nano silver particles on intact and damaged skin in an *in vitro* diffusion cell system, showed that the absorption through intact skin was very low (0.0006%) and five times greater in damaged skin.

However, due to limitations with regard to characterisation of nanosilver and studies investigating dermal absorption, an absorption of 100% is used as a default value for a first Tier 1 calculation assuming that all silver from the product is absorbed and secondly using available information about absorption to refine the calculations.. In addition the dermal absorption that would result in an unacceptable risk is calculated.

An oral exposure scenario is calculated for contact with a cuddly toy. Migration from a hat to saliva simulant was also measured, but migration was lower compared to migration from the cuddly toy for which potential oral exposure is also expected to be higher.

### ***Dermal exposure***

In order to calculate the external dermal exposure of consumers (i.e. the amount that comes in contact with skin), values are used for the total migration to sweat per unit surface and unit time, amount of substance applied to the skin per cm<sup>2</sup> and exposure time per occurrence and number of events..

The calculations are based on the exposure time shown in Table 4.2. In addition the maximum migration found in the migration tests for the tested products is used.

The dermal load is calculated as the external dermal dose (dermal exposure of the skin) per skin area (cm<sup>2</sup>) within the prescribed period for "worst case" scenarios, based on measurements of migration to sweat simulant. Equation R.15-8 (ECHA, 2010) establishes the dermal load as follows:

$$L_{der} = \frac{Q_{prod} \cdot F_{c,prod} \cdot F_{c,migr} \cdot F_{c,contact} \cdot T_{contact}}{A_{skin}}$$

where  $Q_{prod}$  is the weight of the product,  $F_{c_{prod}}$  is the proportion of nanosilver in the product, and  $F_{c_{migr}}$  is the rate at which the nanosilver migrates from the product to sweat.  $A_{skin}$  is the area of contact between the skin and the product, and  $F_{contact}$  the proportion of that area in contact with the skin in cases where the skin is only partially in contact with the product. The default value for  $F_{contact}$  is 1 and will be used here.  $T_{contact}$  is the time the skin is in contact with the product.

Parameters and symbols used in the model formulas from the guidance document are explained in Table 4.3.

TABLE 4.3  
EXPLANATION OF SYMBOLS USED FOR DERMAL SCENARIO (BASERET PÅ ECHA, 2010a)

Input parameter	Description	Unit
$Q_{prod}$	Amount of product used	mg
$F_{c_{prod}}$	Weight fraction of substance in product	mg/mg produkt
$F_{c_{migr}}$	Rate (fraction) of substance migrating to skin per unit time	mg/mg/hours/day
Migr.	Amount of substance migrating to skin per surface and time unit	mg/cm <sup>2</sup> /hours/day
$F_{contact}$	Fraction of contact area for skin (default = 1)	cm <sup>2</sup> /cm <sup>2</sup>
$T_{contact}$	Contact duration	hours/day
$A_{skin}$	Area of contact between product and skin	cm <sup>2</sup>
$C_{der}$	Dermal concentration of substance on skin	mg/cm <sup>3</sup>
BW	Body weight	kg
n	Mean number of events per day	d <sup>-1</sup>
$F_{abs}^*$	Dermal absorption	%
Output parameter	Description	Unit
$L_{der}$	Dermal load on the skin that is expected due to migration	mg/cm <sup>2</sup>
$D_{der}$	Dermal dose per day and body weight	mg/kg lgv/d

For assessment of exposure from the selected products, the migration (Migr.) of (nano)silver per surface and time unit to a sweat simulant is used to calculate the dermal load as follows:

$$L_{der} = Migr \cdot F_{contact} \cdot T_{contact}$$

where the measured migration to sweat per surface and time unit is assumed to be equivalent to the amount of nanosilver which migrate to the skin, expressed as follows in the formulas:

$$Migr \cong \frac{Q_{prod} \cdot F_{c_{prod}} \cdot F_{c_{migr}}}{A_{skin}}$$

The external dermal dose (dermal exposure of the skin) in mg per kg body weight (bw) is based on measurements of migration to sweat simulant, and is calculated as follows:

$$D_{der} = \frac{L_{der} \cdot A_{skin} \cdot n}{BW}$$

equivalent to:

$$D_{der} = \frac{Migr \cdot A_{skin} \cdot T_{contact} \cdot F_{contact} \cdot n}{BW}$$

If the absorption through the skin is inserted in the formula, the internal dermal dose (the amount that may get into the bloodstream) in mg (nano)silver per kg body weight is calculated as:

$$D_{der,int} = \frac{Migr \cdot A_{skin} \cdot T_{contact} \cdot F_{contact} \cdot F_{abs} \cdot n}{BW}$$

Parameters used for the calculation are shown in Table 4.4.

TABLE 4.4 DERMAL EXPOSURE PARAMETERS

Consumer products	Parameter	Value
Insole	<i>Migr.</i>	120 × 10 <sup>-6</sup> mg / cm <sup>2</sup> / 8 hours
Insole	<i>A<sub>skin</sub></i> (adult)	300 cm <sup>2</sup> (estimated)
Insole	BW (adult)	70 kg
Insole	<i>T<sub>contact</sub></i>	8 hours
Tank top	<i>Migr.</i>	89 × 10 <sup>-6</sup> mg / cm <sup>2</sup> / 16 hours
Tank top	<i>A<sub>skin</sub></i> (3-9 years)	0.234 m <sup>2</sup>
Tank top	BW (3-9 years)	16.3 kg (Nanex, WP4)
Tank top	<i>T<sub>contact</sub></i>	16 hours
	<i>F<sub>contact</sub></i>	1 (default)
	<i>n</i>	1
	<i>F<sub>abs</sub></i>	Tier 1: 100% (worst case) Tier 2: 0.1% (USEPA, 2011)
	<i>D<sub>der</sub></i>	Calculated dermal dose per day and body weight in mg/kg bw/day (see Table 4.7).

### ***Oral exposure***

Oral exposure is the result of swallowing saliva containing silver migrated from the cuddly toy to the saliva. Oral exposure expressed as the internal dose will depend on the amount of silver migrating to the saliva and the amount of saliva swallowed during use of the cuddly toy. It is assumed that all silver migrating to the saliva simulant is swallowed by the child.

Based on the analysed migration of silver from the cuddly toy to the saliva (*Migr.*) per surface unit for a given time period, the daily internal dose is calculated based on the equation R.15-11 in the ECHA guidance document on Consumer exposure estimation (ECHA, 2010).

$$D_{oral,int} = \frac{Q_{prod} \cdot F_{C_{prod}} \cdot n \cdot}{BW}$$

As this equation is based on direct ingestion of a substances/mixture, it is modified as follows to reflect the actual scenario where a child is swallowing saliva containing silver migrating from the cuddly toy:

$$D_{oral,int} = \frac{Migr \cdot T_{contact} \cdot S_{toy} \cdot F_{contact} \cdot n \cdot F_{abs}}{BW}$$

Where the measured migration (*Migr*) corresponds to to the amount of silver migrating from the cuddly toy per unit area and migration time.

Parameters used for the calculation are shown in Table 4.5.

TABLE 4.5 ORAL EXPOSURE PARAMETERS

Consumer products	Parameter	Value
Cuddly toy	<i>Migr.</i>	58 ng / cm <sup>2</sup> / 2 hours
Cuddly toy	<i>S<sub>toy</sub></i>	10 cm <sup>2</sup> (estimated)
Cuddly toy	BW (2 year-old)	13.7 kg (EFSA)
Cuddly toy	<i>T<sub>contact</sub></i>	2 hours
	<i>F<sub>contact</sub></i>	1 (default)
	<i>n</i>	1
	<i>F<sub>abs</sub></i>	100% (worst case)

### ***Derivation of a DNEL***

For the purpose of the risk assessment, a Derived No-effect Level (DNEL) is defined for dermal and oral effects, based on the available data for nanosilver. The DNEL-value is derived from repeated exposure data and for systemic effects from oral exposure. No adequate results are available from dermal exposure.

Risk assessment is made by comparing the calculated exposure in defined realistic worst case scenarios with the DNEL which indicates the exposure level below which no damaging health effects are expected.

The risk assessment is based on the NOAEL derived from the critical effect on the liver from 28-days repeated oral exposure, and application of assessment factors.

The endpoint-specific DNEL is based on the following equation:

$$\text{Endpoint – specific DNEL} = \frac{NOAEL_{corr}}{AF_1 \times AF_2 \times \dots \times AF_n} = \frac{NOAEL_{corr}}{\text{Overall AF}}$$

NOAEL<sub>corr</sub> is the corrected NOAEL value, and assessment factors applied are listed in the table below. The assessment factors have been established on the basis of the principles in the REACH guidelines.

TABLE 4.6  
ASSESSMENT FACTORS APPLIED FOR DETERMINATION OF A DNEL FOR NANOSILVER

Parameter	Value	Assessment factor
Interspecies	Allometric scaling. Corrections for differences in metabolic rate per kg body weight.	AS: 4 for rats 7 for mice
Interspecies	Remaining differences between different species	2,5
Intraspecies	Individual differences	10
Dose-response	LOAEL to NOAEL if LOAEL is applied because NOAEL is not established	3

A NOAEL of 0.5 mg/kg bw/day is based on a 28-days oral study in mice where the critical effects on liver and kidney were determined by blood chemistry and histopathological analysis (Park *et al.*, 2010).

The total assessment factor is 175 based on a factor of 2.5 for general interspecies differences, 7 for allometric scaling between mice and humans and 10 for intra species differences. The assessment factor for rats has not been used as it is not considered relevant here. (Assessment factors are presented in Guidance on information requirements and chemical safety assessment, Chapter R.8 Table R8-3. May 2008(ECHA, 2008).

Calculation of the DNEL:

$$DNEL = \frac{0.5 \text{ mg/kg bw/day}}{7 \times 2.5 \times 10} = \frac{0.5 \text{ mg/kg bw/day}}{175} = 0.0029 \text{ mg/kg bw/day}$$

DNEL for nanosilver is thus 0.0029 mg/kg bw/day.

This DNEL will be used for comparison with the estimated dermal and oral exposure.

### 4.3.3 Human health risk assessment

The risk characterisation ratio, RCR, shows as mentioned the relation between the calculated absorption of the substance (the internal dose) and the DNEL which is the exposure level below which no damaging health effects are expected. When RCR is below 1, the exposure alone is considered not to cause any risk.

## Dermal exposure

TABLE 4.7 CALCULATION OF THE RISK CHARACTERISATION RATIO FOR DERMAL EXPOSURE TO SILVER IN TEXTILES

Output parameter	Insoles Worst case scenario mg/kg bw/d	Tank top Worst case scenario mg/kg bw/d
$D_{der}$	$420 \times 10^{-6}$	0.0127
$D_{der, int}$ (100% absorption)	$420 \times 10^{-6}$	0.0127
$D_{der, int}$ (0.1% absorption)	$420 \times 10^{-9}$	
DNEL	0.0029	0.0029
RCR ( $D_{der} / \text{DNEL}$ ) – 100% absorption	0.145	4.14
RCR ( $D_{der} / \text{DNEL}$ ) – 0.1% absorption	0.000145	0.00414

The result of the Tier 1 risk assessment based on 100% dermal absorption shows that only the exposure scenario involving dermal contact with the tank top raises immediate concerns, as its RCR exceeds 1. However, assuming a 100% dermal absorption is far from a realistic scenario. In order to reach a RCR below 1 the dermal absorption should be below 24%.

$$\frac{D_{der, int}}{DNEL} = 1 = \frac{Migr \cdot A_{skin} \cdot T_{contact} \cdot F_{contact} \cdot F_{abs} \cdot n}{BW \cdot DNEL}$$

⇔

$$F_{abs} = \frac{BW \cdot DNEL}{Migr \cdot A_{skin} \cdot T_{contact} \cdot F_{contact} \cdot n} = \frac{16.3 \text{ kg} \times 0.0029 \text{ mg/kg bw/d} \times 16 \text{ h}}{89 \times 10^{-6} \text{ mg/cm}^2 \times 0.234 \text{ m}^2 \times 16 \text{ h} \times 1 \times 1}$$

⇔

$$F_{abs} = 0.23 = 23\%$$

Although data on dermal absorption is limited, the available *in vitro* data suggest a very low dermal absorption through both intact and abraded skin (0.00066% and 0.0033% respectively). In addition the US EPA has estimated a conservative dermal absorption factor of 0.1% based on a human clinical study and the available *in vitro* data, and applied this in risk assessment of biocidal nanosilver (USEPA, 2011).

When calculating the risk based on a more realistic dermal absorption estimate of 0.1% as suggested in the USEPA risk assessment, the conclusion is that the exposure does not result in an unacceptable risk from the selected exposure scenarios. The scenario based on adults in contact with insoles 8 hours per day gives the following result:

$$RCR_{insoles} = \frac{D_{der, int}}{DNEL} = \frac{420 \times 10^{-6} \text{ mg/kg bw/day} \times 0.1\%}{0.0029 \text{ mg/kg bw/day}} = 0.000145$$

The scenario based on a 3-9 year old child wearing a tank top 16 hours per day gives the following result:

$$RCR_{tank\ top} = \frac{D_{der, int}}{DNEL} = \frac{0.012 \text{ mg/kg bw/day} \times 0.1\%}{0.0029 \text{ mg/kg bw/day}} = 0.00414$$

## Oral exposure

TABLE 4.8 CALCULATION OF THE RISK CHARACTERISATION RATIO FOR ORAL EXPOSURE TO NANOSILVER IN TEXTILES

Output parameter	Cuddly toy Worst case scenario mg/kg bw/d
$D_{oral, int(100\% \text{ absorption})}$	$42.3 \times 10^{-6}$
DNEL	0.0029
RCR ( $D_{oral} / \text{DNEL}$ ) – 100% absorption	0.015

The calculation of the RCR for the oral scenario for a 2-year old sucking on a cuddly toy for two hours per day is as follows:

$$RCR_{oral, cuddly\ toy} = \frac{D_{oral, int}}{DNEL} = \frac{42.3 \times 10^{-6} \text{ mg/kg bw/day}}{0.0029 \text{ mg/kg bw/day}} = 0.015 < 1$$

### Combined exposure

As indicated by the calculated risk ratios, even combined exposures from more sources with similar releases of silver as obtained from this study, are not likely to cause an unacceptable risk.

## 4.4 ENVIRONMENTAL ASSESSMENT

### 4.4.1 Environmental toxicity of silver and nanosilver

#### 4.4.1.1 Environmental toxicity of silver

Ratte (1999) has reviewed available information about bioaccumulation and environmental toxicity of silver, not least in the aquatic environment. Data on a considerable number of freshwater and marine species representing various groups of organisms and different trophic levels are included. Most of the data presented are for silver nitrate ( $\text{AgNO}_3$ ) and the silver ion ( $\text{Ag}^+$ ) but also a number of other silver compounds have been investigated.

The most sensitive endpoints among the data referred by Ratte (1999) within the main categories of organisms are presented in Table 4.9 and Table 4.10 below.

TABLE 4.9 ECOTOXICITY OF SILVER COMPOUNDS TO FRESHWATER ORGANISMS. MOST SENSITIVE ENDPOINTS SUMMARIZED FROM RATTE (1999).

Test organism	Compound	Test	Endpoint	Value (mg/l)
<i>Pimephales promelas</i>	$\text{Ag}^+$	96h, static	LC50	0.0025
<i>Pimephales promelas</i>	$\text{AgNO}_3$	96h, static	NOEC	0.010
<i>Pimephales promelas</i>	$\text{NaAgS}_2\text{O}_3$	10 wk, flow-through	NOEC	5
<i>Pimephales promelas</i>	$\text{AgNO}_3$	30d, flow-through	MATC	0.0004-0.0007
<i>Daphnia magna</i>	$\text{AgNO}_3$	48h, mortality	LC50	0.0005
<i>Daphnia magna</i>	$\text{AgNO}_3$	21d, reprod.	EC50	0.0029
<i>Cerodaphnia dubia</i>	$\text{AgNO}_3$	Reproduction	NOEC	0.0045
<i>Selenastrum capricornutum</i>	$\text{AgNO}_3$	?	EC50	>0.125

\* The toxicity of the different silver compounds depends on the concentration of silver ions being generated. AgNO<sub>3</sub> is considered to be fully dissociated while dissolution of NaAgS<sub>2</sub>O<sub>3</sub> only partially leads to full dissociations as free silver ions. This is reflected in the lower toxicity of the latter compound.

In addition to the results presented in the table above, Ratte (1999) presents data for a number of amphibian species, mainly frogs. These are also very sensitive to silver with LC50 values from 0.24 mg/l down to 0.01 mg/l (*Rana palustris*, *Rana pipiens*, *Gastrophryne caroliensis*).

Generally, freshwater fish and invertebrate species were very sensitive to exposure to silver nitrate and free silver ions but less sensitive to silver sulfide (Ag<sub>2</sub>S) and other less soluble silver compounds.

Pronk *et al.* (2009) report short-term effect concentrations from 0.0034 mg Ag/l for algae (EC50 (growth rate) for *P. subcapitata*) to >10 mg Ag/l for fish (juvenile *P. promelas*) and chronic NOEC values ranging from 0.00024 mg Ag/l for fish (*O. mykiss*) to 0.0016 mg Ag/l for invertebrates (*D. magna*).

TABLE 4.10 ECOTOXICITY OF SILVER COMPOUNDS TO MARINE ORGANISMS. MOST SENSITIVE ENDPOINTS SUMMARIZED FROM RATTE (1999).

Test organism	Compound	Test	Endpoint	Value (mg/l)
<i>Onchorhynchus mykiss</i>	AgNO <sub>3</sub>	96h, 25‰ sal.	LC50	0.402
<i>Oligocottus maculosus</i>	AgNO <sub>3</sub>	96h, 25‰ sal.	LC50	0.331
<i>Paralichthys dentatus</i>	Ag <sup>+</sup>	?	Threshold	0.0047
<i>Homarus americanus</i>	Ag <sup>+</sup>	Stress enzymes	Threshold	0.006
<i>Crassostrea virginica</i>	AgNO <sub>3</sub>	48h	LC50	0.025
<i>Ilyanassa obsoleta</i>	Ag <sup>+</sup>	Embryonic development	Threshold	<0.001
<i>Gymnodium</i> sp.	Ag <sup>+</sup>	48h	NOEC	0.002-0.010

Hogstrand & Wood (1998) report the toxicity of silver (added as easily soluble salts such as e.g. silver nitrate) to fish to be considerably lower in seawater (96h LC50 range 330-2,700 µg Ag/l) than in freshwater (96h LC50 range 5-70 µg Ag/l). This is mainly attributable to complexation of the silver ion in seawater.

The Danish annual average Environmental Quality Standard (AA-EQS) for dissolved silver is 0.017 µg/l in freshwater and 0.2 µg/l in marine waters (added to the natural background level). The corresponding MAC-EQS values are 0.36 µg/l and 1.2 µg/l, respectively<sup>21</sup>. The endpoints used for derivation of the EQS was an acute EC<sub>50</sub> of 0.24 µg/l for *D. magna* and a long-term NOEC of 0.09 µg/l for fish (*O. mykiss*) (Miljøministeriet 2009).

<sup>21</sup> Ministry of Environment (2010). Statutory Order No. 1022 of 25/08/2010 on Environmental Quality Standards for the aquatic environment and requirements to discharge of pollutants to water courses, lakes or the sea.



#### 4.4.1.2 Environmental toxicity of nanosilver

A recent survey on basic environmental properties of nanosilver and selected other nanomaterials (Mikkelsen *et al.*, 2011) included an ecotoxicological profile for nanosilver focusing on endpoints of relevance for hazard classification and risk assessment. The following data (Table 4.11) are summarized from the survey.

TABLE 4.11 SUMMARY OF AQUATIC EXOTOXICOLOGICAL DATA ON NANOSILVER. SUMMARISED FROM MIKKELSEN *ET AL.* (2011).

Test organism	Stage	Test	Endpoint	Value (mg/l)
<i>Danio rerio</i>	Adult	Static, 48h	LC50	7.07
<i>Danio rerio</i>	Embryo	Static, 48h	Sublethal effects	0.01-0.02
<i>Daphnia pulex</i>	Adult	48h	EC50	0.040
<i>Ceriodaphnia dubia</i>	Neonate	48h	EC50	0.067
<i>Pseudokirchneriella subcapitata</i>	-	96h	EC50	0.19
<i>Chlamydomonas reinhardtii</i>	-	5h	EC50	0.092
Nitrifying bacteria	-	?	86% inhibition	1.0

In the study on *Chlamydomonas reinhardtii* (by Navarro *et al.* 2008) the results were also expressed in terms of the free silver ion. Expressed as a function of free Ag<sup>+</sup>, EC50 was estimated to range from  $3.6 \pm 0.5$  ug/l after 1 hour, to  $0.9 \pm 0.08$  ug/l after 5 hours. This study is considered important because it was shown that the toxicity of AgNP could not solely be explained by the toxicity of the free ion (Ag<sup>+</sup>).

In the study with nitrifying bacteria no correlation between nanoparticle size and bacteria inhibition in respirometry tests was found. However, a correlation was found between inhibition and the fraction of nanoparticles with sizes less than 5 nm, indicating that this fraction might have a stronger effect on respiration than larger size fractions. Furthermore it was found that, at the same total concentration of Ag, Ag nanoparticles caused a greater inhibition than the free Ag<sup>+</sup>.

SRU (2011) summarises in a nanomaterials management strategy report some of the same data and find that nanosilver is often, but not always, less toxic than easily soluble silver salts or ions, and that nano-specific mechanisms of toxicity seem to exist.

Pronk *et al.* (2009) summarise the results of short-term ecotoxicity tests with nanosilver on fish, invertebrates and algae. The EC50/LC50 values range from 0.04 mg Ag/l for invertebrates (*D. pulex*, adults) to 7.2 mg Ag/l for fish (*D. rerio*, juveniles). They found that long-term NOECs were not available.

A search for ecotoxicological data on nanosilver published later than the above survey by Mikkelsen *et al.* (2011) has resulted in identification of a number of new articles or reports of relevance, which are summarized in the following.

Griffitt *et al.* (2012) found that for the sheepshead minnow (*Cyprinodon variegatus*), chronic exposure to low levels of AgNP (10 µg/l nominal) induced significant adverse (sublethal) effects in both juvenile and adult specimens,

effects that did not appear to be attributable to the release of silver ions through particle dissolution.

Zhao & Wang (2011) investigated the acute and chronic toxicity of AgNP to *Daphnia magna* and compared it to that of silver nitrate ( $\text{AgNO}_3$ ). No acute mortality was observed at 500  $\mu\text{g Ag/L}$  as AgNP whereas an acute  $\text{LC}_{50} = 2.51 \mu\text{g/L}$  was found for the free silver ion (added as  $\text{AgNO}_3$ ). The 21-day chronic effect of waterborne AgNP on daphnia growth was higher than that of  $\text{AgNO}_3$  while dietborne influence on reproduction was more pronounced for  $\text{AgNO}_3$  than for AgNP.  $\text{AgNO}_3$  was significantly reprotoxic at 0.1  $\mu\text{g}$  dissolved Ag/L while the LOEC of AgNP on growth and reproduction, respectively, was 5  $\mu\text{g/L}$  and 50  $\mu\text{g/L}$ . Thus, Zhao & Wang (2012) observed higher toxicity of the free silver ion to *D. magna* than that of nanosilver.

Gaiser *et al.* (2012) studied the toxicity of nano-sized (35nm) and micro-sized (600-1,600 nm) silver to *D. magna* and the common carp *Cyprinus carpio* and found the nano-sized material to be more toxic than the micro-sized material.

Lee *et al.* found powder-type AgNP suspensions to be more toxic to *D. magna* than sol-type AgNP suspensions (48h  $\text{EC}_{50} = 0.75 \mu\text{g/L}$  and  $7.98 \mu\text{g/L}$ , respectively, in terms of total Ag, and  $0.37 \mu\text{g/L}$  and  $0.88 \mu\text{g/L}$ , respectively, in terms of dissolved Ag). However, according to Lee *et al.* it remains unclear whether the major toxic effect was governed by silver ions or by AgNPs themselves.

McLaughlin & Bonzongo (2012) found AgNP suspended in natural water with a low ionic strength/DOC ratio to be less toxic to the crustacean *Ceriodaphnia dubia* and the green alga *Pseudokirchneriella subcapitata* than AgNP suspended in natural water with high ionic strength/DOC ratio. The  $\text{LC}_{50}$ s for *C. dubia* were 221 and  $0.443 \mu\text{g/L}$ , respectively, in the two media, while the  $\text{IC}_{50}$ s for *P. subcapitata* were  $1,600 \mu\text{g/L}$  and  $22.6 \mu\text{g/L}$ , respectively.

Som *et al.* (2011) refer to "careful studies" showing that most of the effect of nano-Ag is due to the release of silver ions. Further, they refer to recent research showing that under real-life environmental exposure conditions nano-Ag is rapidly converted to silver sulfide having no measurable impact on wastewater treatment plants and being much less toxic than ionic silver.

In summary, the newest publications on the ecotoxicity of nanosilver do not basically change the quantitative data basis provided by Mikkelsen *et al.* (2011) but rather add some details on the influence of various external factors and on toxic mechanisms that appear to be nano-specific, and thus contributing to the already multi-faceted picture of the aquatic toxicity of nanosilver.

#### 4.4.1.3 Critical endpoints for the environmental risk assessment

For nanosilver the most sensitive standard short-term endpoint seem to be the  $\text{EC}_{50} = 0.040 \text{ mg Ag/l}$  for *Daphnia pulex* whereas no standard long-term NOECs have been identified. A 21-day LOEC (growth) of  $0.005 \text{ mg Ag/l}$  for *D. magna* was recently reported by Zhao & Wang (2012).

For silver (silver nitrate or free silver ion) the most sensitive endpoints are difficult to establish from the review by Ratte (1999) because of the large span in test conditions and very limited level of details on the studies performed.

If instead the more recent review by Pronk *et al.* (2009) is used as basis, the lowest short-term EC50 is 0.0034 mg Ag/l for algae (*P. subcapitata*) while the lowest chronic NOEC value is 0.00024 mg Ag/l for fish (*O. mykiss*).

The Danish AA-EQS for silver of 0.017 ug/l for freshwater and 0.2 ug/l for marine waters (dissolved fraction; added to the natural background level) is also considered in the risk assessment.

#### **4.4.2 Environmental exposure**

This section establishes the exposure scenario for the subsequent environmental risk assessment of nanosilver in textiles (consumer products).

During the service life of textiles containing nanosilver, the only potentially significant pathway of environmental exposure is considered to be release to the sewerage system, primarily from households. The sewage is transported to the sewage treatment plant (STP) where partitioning of the nanosilver occurs between the solid phase (sludge) and the aqueous phase. Following this, the treated effluent is discharged to a surface water body while the sludge may be spread on e.g. agricultural soil.

Two main types of releases to the sewerage system have been identified based on the outcome of the survey of nanosilver-containing textiles on the Danish market: (1) from the washing of a variety of textiles and (2) from releases during shower bathing (from shower curtains).

The standard European Union STP has, according to the ECHA Guidance on Environmental Exposure Estimation (Chapter R.16.5.5.4), a capacity of 10,000 PE. Each PE contributes with 200 L water/day and 0.011 kg sludge/day.

The exposure scenario for environment is therefore based on the inputs from a population of 10,000 to such an STP. The 10,000 PE are presumed to be distributed on 2,500 standard households each consisting of 4 persons.

The daily input of nanosilver from a family (household) to the sewerage system is calculated as follows:

Amount/family/day =  $\Sigma$  (Daily amount from textile washing + Daily amount from shower bathing)

where

Daily amount per family from textile washing =  
No. of machine washes/day · Weight of clothes/wash · Fraction of clothes containing nanosilver · Fraction of nanosilver in nano-clothes · Fraction of nanosilver released/wash

and

Daily amount per family from shower bathing =  
No. of showers/day · Amount of nanosilver/shower.

### ***Textile washing:***

The symbols and input values used for the textile washing scenario are shown in Table 4.12. The input-values are suggested to represent a realistic worst case scenario for the input from washing of textiles in a family of four persons (2 adults and 2 children), all assumed to be physically active people:

TABLE 4.12  
SYMBOLS AND INPUT PARAMETERS USED FOR THE TEXTILE WASHING SCENARIO

<b>Input parameter</b>	<b>Description</b>	<b>Input value</b>
W	Weight of clothes per wash	6 kg
N <sub>tex</sub>	Number of textile washes per day per household	1
F	Fraction of clothes containing nanosilver (default = 1)	0.10
C	Average fraction (relative concentration) of nanosilver in the textiles that contain nanosilver	0.0002
R	Fraction of nanosilver released per wash	0.10 <sup>22</sup>
<b>Output parameter</b>	<b>Description</b>	<b>Unit</b>
A <sub>tex</sub>	Daily amount of nanosilver per family from textile washing	mg/day

### ***Showering:***

With regard to the releases from shower bathing, the parameters shown in Table 4.13 are proposed:

TABLE 4.13  
SYMBOLS AND INPUT PARAMETERS USED FOR THE SHOWERING SCENARIO

<b>Input parameter</b>	<b>Description</b>	<b>Input value</b>
Q <sub>curtain</sub>	Amount of nanosilver in shower curtain	80 mg
R <sub>bath</sub>	Fraction of nanosilver released per shower	0.01
A <sub>bath, person</sub>	Daily amount of nanosilver per person from showering	800 µg <sup>23</sup>
N <sub>tex</sub>	Number of showers per day per family	4
<b>Output parameter</b>	<b>Description</b>	<b>Unit</b>
A <sub>bath, family</sub>	Daily amount of nanosilver per family from showering	µg

<sup>22</sup> The results obtained in the study were highly variable, from 0 % to 84 % released. This was also observed by others, e.g. Gottschalk *et al.* (2010) (1% to 45 % lost in one wash), who for modeling purposes use a fraction released of 5 %. Here 10% is chosen.

<sup>23</sup> No data on this have been obtained in this study as no shower curtains containing nanosilver were identified. If a shower curtain has a size of 4 m<sup>2</sup> (2x2 m) and a weight of 1 gram per 100 cm<sup>2</sup> (as found in this study for a number of other textiles), the total weight is 400 grams. If the content of nanosilver is 0.2 mg/g (as used for the risk assessment of textile washing), the total amount in the curtain becomes 80 mg. The release per shower is probably lower than for machine washing of clothes and is proposed to be 1% per shower. This results in a release of 0.8 mg nanoAg/shower.

### ***Derivation of PEC for surface water and soil:***

The daily input of nanosilver to the STP will be 2,500 times the amount calculated for the standard four member family.

For the determination of the environmental exposure,  $PEC_{SW}$  and  $PEC_{SOIL}$ , the following input values will be used:

- Distribution between water phase and solid phase at STP: 10 : 90<sup>24</sup>.
- Dilution in receiving water = 10 times (REACH standard)
- Sludge production: 0.011 kg/person/day (REACH standard)
- Application rate for sludge on agricultural soil = 5,000 kg dw/ha/year.

### **4.4.3 Environmental risk assessment**

The exposure scenarios for textile washing and shower bathing presented in the preceding section will, if using the proposed values for the various factors in the exposure equations, result in the following daily amount per family from textile washing ( $A_{tex}$ ):

Textile wash:

$$A_{tex} = N_{tex} \times W \times F \times C \times R_{tex} = 1 \text{ wash/day} \times 6 \text{ kg/wash} \times 0.1 \times 0.002 \times 0.1$$

$$A_{tex} = 12 \text{ mg Ag/day}$$

Shower bathing:

$$A_{bath, family} = N_{bath} \times R_{bath} = 4 \text{ showers/day} \times 0.8 \text{ mg nanoAg/shower}$$

$$A_{bath, family} = 3.2 \text{ mg nanoAg/day}$$

The total daily release of silver from one family ( $A_{tot}$ ) to the sewage system from textile washing and showers:

$$A_{tot} = A_{tex} + A_{bath, family} = 15.2 \text{ mg Ag/family/day}$$

### ***Aquatic environment***

The resulting concentration of silver in the raw sewage will, when applying standard REACH conditions (200 litres of water per PE), be approx. 0.019 mg/l (19 µg/l).

According to the assumptions for calculation of the resulting concentration of silver in the STP effluent approx. 10 % of the silver will remain in the water

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<sup>24</sup> This figure is based e.g. on Som *et al.* (2011) referring a study where more than 90 % of nano-Ag was removed during wastewater treatment. A recent evaluation of data from more than 10 years of monitoring at Danish STPs (Kjølholt *et al.* 2011) indicates that this order of magnitude is probably to be anticipated. Burkhardt *et al.* (2012) report even higher removal efficiencies (94-99 %).

phase at the STP<sup>25</sup> and a dilution factor of 10 can be used for the initial dilution of the effluent in the receiving surface water body.

This means that the concentration of silver in the effluent will be 1.9 µg/l, and in the receiving surface water body at the border of the mixing zone be 0.19 µg/l i.e. 100 times lower than in the influent to the STP.

This level is approx. 200 times lower than the lowest EC50 value and 25 times lower than the lowest chronic NOEC value proposed in section 4.4.1.3 for the aquatic toxicity of nanosilver. The concentration is, however, only slightly below the lowest chronic NOEC for dissolved silver, 0.24 µg/l, mentioned in the same section and about 11 times higher than the Danish freshwater EQS for (dissolved) silver. However, only a fraction of the originally released silver will be present as free Ag<sup>+</sup> in the water phase, while the majority is believed to be either nanoAg or species resulting from transformation reactions in the sewage system such as silver complexes or silver sulfide.

Som *et al.* (2011) refer modelling results according to which expected concentrations of nanoAg in treated wastewater are likely in the range of 0.03-0.13 µg/l i.e. about 15-60 times lower than calculated here using the input values proposed above, which were generally considered to be "worst case". The results referred by Som *et al.* (2011) indicate that this appears to be the case. If the values mentioned by Som *et al.* are used then nanoAg discharged with treated sewage effluent will not reach a level in surface waters at or above the freshwater AA-EQS for dissolved silver.

In conclusion, based on the existing (albeit incomplete) data on the ecotoxicity of nanosilver and the theoretical scenario for environmental exposure through discharge of treated sewage effluent from ETPs there appears to be little risk of biological effects in the aquatic environment arising from the use of nanosilver in textiles.

Possible cumulative effects in the aquatic environment due to the presence of other forms and sources of silver have not been considered.

### ***Sewage sludge***

According to the exposure scenario for STPs, 90 % of the silver in the untreated wastewater will be distributed to the solid fraction, i.e. the sewage sludge.

In the sludge scenario this corresponds to 13.5 mg/family/day in 4 x 0.011 kg sludge/family/day or a concentration of nanosilver in the sludge of approx. 300 mg Ag/kg sludge dw.

At an application rate onto agricultural land of 5,000 kg sludge/ha = 0.5 kg sludge/m<sup>2</sup> this concentration corresponds to approx. 150 mg Ag/m<sup>2</sup> or 0.1 mg/kg soil dw if assuming incorporation into the upper 10 cm and a soil density of 1500 kg/m<sup>3</sup>.

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<sup>25</sup> Burkhardt *et al.* (2012) recently investigated the fate of nanosilver particles in wastewater treatment plants and found a removal efficiency of 94-99% and that nano-particles did not occur in the effluent. The nanosilver appears to dissolve in the wastewater stream where the vast majority subsequently is transformed into poorly soluble silver sulfide while only a minor fraction remains in solution.

No ecotoxicological soil quality criteria for silver (or nanosilver) have been identified. The Danish soil QC for silver is 50 mg Ag/kg soil but has been established only considering toxicity to mammals and risk related to exposure of humans. The Dutch "intervention value" for contaminated soil is 15 mg/kg soil dw ([http://en.wikipedia.org/wiki/Dutch\\_standards/](http://en.wikipedia.org/wiki/Dutch_standards/)) while in Canada the "remediation criterion" for silver is 20 mg/kg soil for the land uses "agricultural" and "residential/parkland" (Environment Canada 2007).

Thus, the estimated soil concentration from application of silver to farmland with sewage sludge is much lower than the existing soil quality criteria, which, however, are not based on assessments of toxicity to soil-dwelling organisms. It is not considered feasible to conduct an ecotoxicological risk assessment of the terrestrial environment based on the available effect data on nanosilver.

#### 4.5 CONCLUSION

##### *Human health*

The risk ratio was calculated based on three selected exposure scenarios, i.e. adults in dermal contact with insoles 8 hours per day, 3-9 year old children in contact with a tank top 16 hours per day, and finally 2-year old children in oral contact with a cuddly toy 2 hours per day. The results indicate that the risk from each individual scenario is very low and that combined exposures from different sources with similar migration patterns are not likely to pose an unacceptable risk to consumers.

The two dermal scenarios are calculated based on a conservative estimate of 0.1% dermal absorption used in the USEPA risk assessment for the registration of a nanosilver product. There are however indications that the absorption is much lower, both through intact and abraded skin and therefore the risk may be even lower than indicated by the calculations in this study. However, it should also be stressed that characterisation of nanosilver species has been a weak point in many studies until today, and results must be seen in the light of this limitation.

##### *Environment*

Based on the existing data on the ecotoxicity of nanosilver and the theoretical scenario for environmental exposure through discharge of treated sewage effluent from ETPs, there appears to be little risk of biological effects in the aquatic environment arising from the use of nanosilver in textiles. Silver ions appear to be more toxic in the aquatic environment than nanosilver.

Possible cumulative effects in the aquatic environment due to the presence of other forms and sources of silver have not been considered.

It is not considered feasible to assess the risk to the terrestrial environment resulting from application of nanosilver-containing sewage sludge to farmland.

## 5 Conclusion

The results of the survey indicated that there was little or no information available for the consumers about silver or nanosilver content in textiles in the 39 shops visited as part of the survey. Where silver was mentioned it was not specified in which form it was present. A few products identified on the internet did specify content of nanosilver but also here products were mentioned to contain silver without specification of the form.

Silver particles were only positively identified in two out of 12 products selected for particle examination (microfibre cloth and insole) and these particles were larger than the nanorange as defined in the EU-recommendation for definition of nanomaterials. In another two products (sandal and running T-shirt) the silver content was below the detection limit for EDX particle analysis and therefore content of nanosilver in these products cannot be excluded.

**Since no nanosilver was found in any of the products, the results of this survey did not support the hypothesis that nanosilver treated textiles are very common on the Danish market.**

To provide data for the dermal exposure route, four products were selected for test of migration to artificial sweat (microfibre cloth, insoles, body stocking with long sleeves and tank top for children). The migration to artificial sweat varied from 0 to 50%. Migration to artificial saliva was measured for two products (hat/scarf, patterned textile and cuddly toy) at 24% and 0.09% respectively with an exposure time of 2 hours.

Washing experiments were carried out for four products (microfibre cloth, hat/scarf- patterned textile, cuddly toy, and body stocking with long sleeves). Release from the products was 15%, 84%, 32% and 0% respectively.

**In conclusion, the results of the migration and washing tests revealed that the release of silver from the products was varying but could be substantial.**

The migration and washing results were used as realistic worst case data to estimate the exposure of humans and the environment in the following scenarios:

- Dermal exposure to insoles, 8 hours per day (adult)
- Dermal exposure to tank top, 16 hours per day (3-9 year old child)
- Oral exposure to cuddly toy, 2 hours per day (2-year old child)
  
- Washing textiles, daily amount per family (1 wash, 6 kg textiles)
- Shower bathing, daily amount per family (1 shower/person/day)

The calculated risk ratios relevant for the three selected human exposure scenarios indicate that the risk from each individual scenario is very low. Also combined exposures from different sources with similar migration patterns are



not likely to pose an unacceptable risk to consumers based on the findings in this study.

The two dermal scenarios were calculated based on a conservative estimate of 0.1% dermal absorption. There are however indications that the absorption is much lower, both through intact and abraded skin and therefore the risk-quotients may be even lower than indicated by the calculations in this study. The overall assessment is that the risk from dermal and oral contact with the products identified and tested in this survey is low based on the available literature findings and assumptions made on this basis.

Using the results of the washing test and the environmental exposure scenario, the concentration of silver in the raw sewage will, when applying standard REACH conditions, be approx. 0.019 mg/l (19 µg/l). Using standard assumptions regarding dilution, concentrations in effluent will be 1.9 µg/l, and 0.19 µg/l in the receiving surface water body at the border of the mixing zone. This level is approx. 200 times lower than the lowest EC50 value and 25 times lower than the lowest chronic NOEC value stated for the aquatic toxicity of nanosilver. The concentration is, however, only slightly below the lowest chronic NOEC for (free) silver, 0.24 µg/l and about 11 times higher than the Danish freshwater EQS for (dissolved) silver. However, only a fraction of the originally released silver is believed to be present as dissolved silver in the water phase, while the majority will be either nanoAg or species resulting from transformation reactions in the sewage system such as silver complexes or silver sulfide.

In conclusion, the scenario for environmental exposure through discharge of sewage effluent is not likely to pose a risk of biological effects in the aquatic environment arising from the use of nanosilver in textiles based on the measured results and the assumptions that are introduced.

According to the exposure scenario for STPs, 90 % of the nanosilver in the untreated wastewater will be distributed to the sewage sludge. This leads to an estimated concentration of nanosilver in the sludge of approx. 300 mg Ag/kg sludge dw. and a resulting soil concentration after application of sludge to farmland of approx. 150 mg/m<sup>2</sup> or 0.1 mg/kg soil dw. This estimated soil concentration is much lower than the existing soil quality criteria (50 mg Ag/kg soil dw). It was not considered feasible to conduct an ecotoxicological risk assessment of the terrestrial environment based on the available effect data on nanosilver.

#### *Conclusion –health and environmental assessment*

**Based on the existing data on nanosilver (eco)toxicity and the selected exposure scenarios, there appear to be no risk of health effects or biological effects in the aquatic environment arising from the use of nanosilver in the textiles identified and tested in this survey. With regard to health, nanosilver seems to exert similar toxicity as silver through dermal and oral exposure but to be more toxic through inhalation. For the aquatic environment, the available information indicates that dissolved silver (ions) is more toxic than silver in the form of nano-particles.** This statement however, still need more research and data to provide the conclusive evidence.

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# Appendix 1 –Silver-containing products

The questions concern exclusively textile products

Question	Falling	Increasing	Unchanged
Was the last 5 years' sale of silver-containing textiles =>			
Remarks, if any:			
Question	Falling	Increasing	Unchanged
What are your expectations for the future sale of silver-containing textile products - will it be =>			
Remarks, if any:			

Question	Yes	No
Does your company market silver-containing textiles for children?		
If yes, please list the type of products:		
What is the approximate % share of the nanosilver products of the overall product line within each product group (e.g. sportswear, footwear, mattresses, shower curtains, etc.)?		

Question	Yes	No	Don't know
Is the silver content in your products in nanoform?			
Remarks, if any:			
If not nanosilver, please inform in which form the silver is present?			

## Appendix 2 – Products with antibacterial properties

The questions concern exclusively textile products

Question	Yes	No	Don't know
Is the antibacterial effect of your textile products based on silver?			
If no, please specify the cause of the antibacterial effect:			
Question	Yes	No	Don't know
Is the silver content of your textile products in nanoform?			
If not nanosilver – please inform in which form the silver is present?			

If your textile products are antibacterial due to silver, silver salt/ion, nanosilver or other forms of silver, please fill in the below questionnaire.

Question	Falling	Increasing	Unchanged
Was your last 5 years' sale of silver-containing textiles =>			
Remarks, if any:			
Question	Falling	Increasing	Unchanged
What is your expectations for the future sale of silver-containing textile products – will it be =>			
Remarks, if any:			

Question	Yes	No
Does your company market silver-containing textiles for children?		
If yes, please list the type of products:		
What is the approximate % share of the nanosilver products of the overall product line within each product group (e.g. sportswear, footwear, mattresses, shower curtains, etc.)?		



## Appendix 3 - Silver-containing products, general

The questions concern exclusively textile products

Question	Yes	No
Does your company market silver-bearing textiles for adults??		
If yes, please list the type of products:		
	Yes	No
Does your company market silver-containing textiles for children??		
If yes, please list the type of products:		
What is the approximate % share of the nanosilver products of the overall product line within each product group (e.g. sportswear, footwear, mattresses, shower curtains, etc.)?		

If your company sells silver-containing textiles, please fill in below questionnaire

Question	Yes	No
Is the silver content in your products in nanoform?		
Remarks, if any:		
If not nanosilver, please inform in which form the silver is present?		

Question	Falling	Increasing	Unchanged
Was your last 5 years' sale of silver-bearing textiles =>			
Remarks, if any:			
	Falling	Increasing	Unchanged
What is your expectations for the future sale of silver-containing textile products			
Remarks, if any:			

## Appendix 4 – Treatment methods

### NanoGlide®

NanoGlide® does *not* contain nanosilver. NanoGlide® is an inorganic/organic nanocomposite lubricant. It is synthesized of molybdenum disulfide ( $\text{MoS}_2$ ) and is used for lubricating movable parts. It is used in various applications, i.a. for machines for production of e.g. bearings, gears and pumps.

### Ægis Microbe Shield®

Ægis is a chemical treatment, which consists of only one molecule: 3-Trimethoxy silyl propyl dimethyl octadecyl ammonium chloride. The molecule consists of an anchor of Si, O and H, which reacts with the fibres and makes the treatment stick to the textile. The molecule further has two segments which kill the bacteria they get in touch with. One of these is a positive charge, which will short-circuit negatively charged microorganisms. The other segment is a chain of 18 C, which is hydrophobic and has strong bonding to the fibres. At the same time each chain acts like 25,000 swords penetrating and puncturing the microorganisms. In this way the killing of the microorganisms is double secured: by 'short-circuiting' combined with 'stabbing'.

### Polygiene®

Fra [www.polygiene.com](http://www.polygiene.com):



**Polygiene\***

#### **Polygiene AB**

Stadiongatan 65

SE-217 62 Malmö

Sweden

Phone: +46 40 530 202

Fax: +46 40 530 210

Email: [info@polygiene.com](mailto:info@polygiene.com)

- Polygiene is based on natural silver salts made from recycled silver.
- Silver salt is naturally present both in water (drinking water as well as sea water) and in the soil.

- Polygiene particles have a large amount of silver ions per surface area. Due to the structure of the particle, very small amounts of silver are required. Treating 5000 garments or approximately 1000 kilos of fabric requires the same amount of silver as one or two silver rings.
- Polygiene is applied to the fabric during the finishing stage at the same time as other treatments. This helps minimizing the impact on the environment because additional energy or water is not required.

### **Permanent odor control that treks far and wide**

Independent tests demonstrate the high efficacy of Polygiene technology against microbes, such as bacteria, viruses and fungi, which makes textiles stay fresh and odor free.

#### **Stay fresh and hygienic**

Polygiene neutralizes odor by stopping the growth of odor-causing bacteria and fungi.

#### **Effective and permanent**

The odor-free effect is guaranteed for the normal lifetime of the product and has been verified through field tests by leading brands as well as independent laboratory tests.

#### **Safe**

Thanks to the company's heritage in the healthcare sector, Polygiene meets the highest standards for skin safety and hygiene. It does not interfere with the skin's natural bacterial flora and is approved for use on bandages for open wounds.

#### **Climate smart**

Polygiene makes clothing, footwear and gear last longer because bacteria and excessive washing do not break down fibres as quickly as they do on untreated material. Because Polygiene-treated apparel and gear stay fresh longer, they require less washing at lower temperatures. Plus you can pack less luggage when you travel, which is also good for the environment.

#### **Approvals**

Polygiene is [bluesign](#)® approved, which is the most demanding environmental certification of textiles, and is on the [Oeko-Tex](#)® list of approved products. Based on natural silver salt made from recycled silver, Polygiene is registered under the EU Biocidal Product Directive and the US Environmental Protection Agency.

#### **How it works**

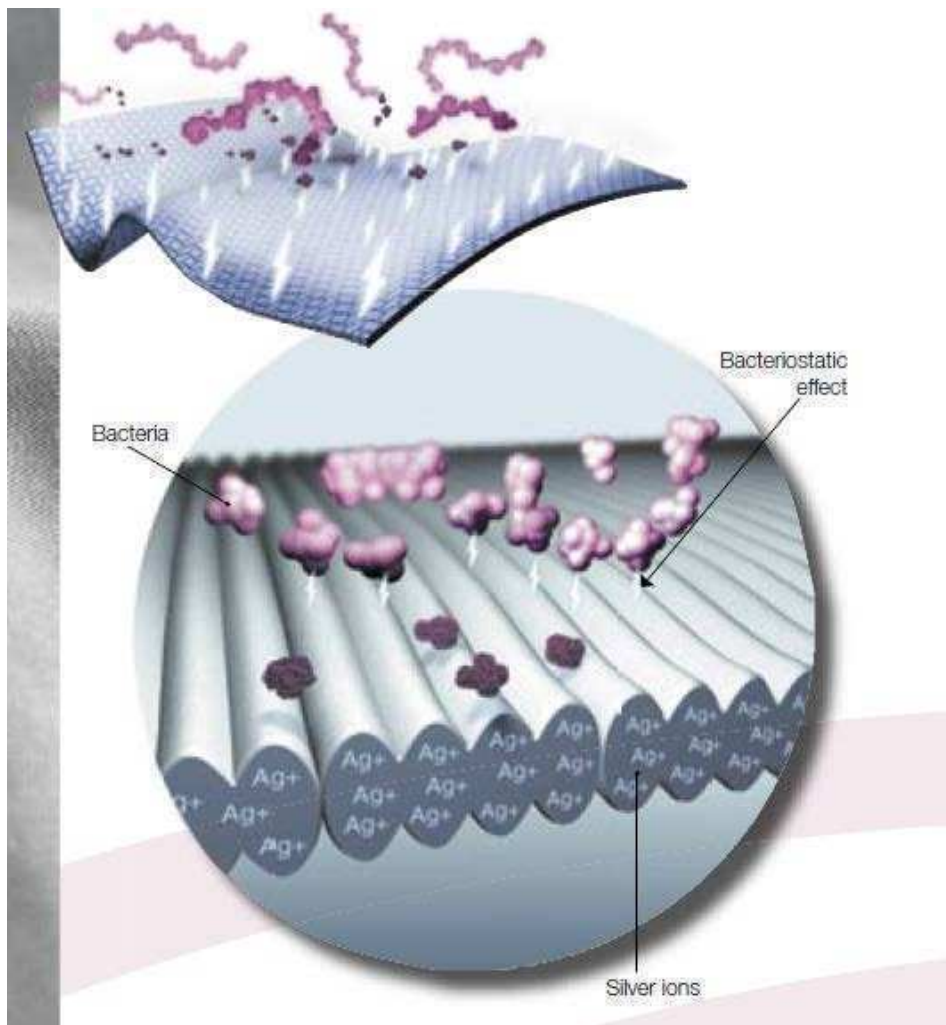
Sweat is odorless but helps create the perfect environment for odor-causing bacteria to multiply. When sweat mixes with odor-causing bacteria on fabrics, odor results.

Polygiene uses natural silver salt, a highly effective antimicrobial agent, to safely inhibit the growth of odor-causing bacteria and fungi. However, Polygiene does not affect the natural bacterial flora of your skin. And without odor-causing bacteria, you stay fresh.

#### **Coolmax® freshFX™**

The active ingredient in Coolmax® freshFX™ qualifying fibres is a durable, non-migratory silver-based antimicrobial additive. This additive has been proven by the

supplier to be highly effective in the laboratory against a wide range of microorganisms, including bacteria, fungi and algae. When incorporated into approved materials, such as polyester staple and filament, it has been shown to impact bacteriostatic, fungistatic and algistatic properties to the material, and to articles, such as garments, containing the approved material.



The mechanism of action involves the slow release of silver ions from its inorganic cage matrix through ion-exchange. The silver ions can then interact with microbes to disrupt their cellular functions, thereby inhibiting the growth of the microbial colonies. Microbes can feed off of components in human sweat and body oil, resulting in odorous by-products. The silver-based additive effectively suppresses the generation of the odorous by-products by inhibiting microbial growth on the fabric.

The active ingredient is spun directly into the yarn, rather than being topically applied, and an inorganic cage matrix protects it. Therefore it can be expected to remain effective for the life of the garment even after repeated laundering.

## **Sanitized<sup>®</sup> Silver**

Fra [www.sanitized.com](http://www.sanitized.com):

### **Is the Sanitized<sup>®</sup> Silver nanotechnology?**

No, it is not nanotechnology. Sanitized<sup>®</sup> Silver is neither produced by nanotechnology nor does the particle size of Sanitized<sup>®</sup> Silver fit into the nano-range.

### **Who ensures that Sanitized<sup>®</sup> Silver is not nano-silver?**

An independent Institute has certified that Sanitized<sup>®</sup> Silver is not a nanosilver-based product. Particle size and partition of Sanitized<sup>®</sup> Silver has been measured by "Generic Latex Measurement". The result confirmed clearly that the particle size of Sanitized<sup>®</sup> Silver is out of the nano-range.

### **Are articles with Sanitized<sup>®</sup> Silver technology safe?**

Yes, they are safe. Sanitized<sup>®</sup> Silver fulfils strict requirements proven by stringent quality tests. This makes Sanitized<sup>®</sup> Silver safe for man and environment. It is accepted by Oeko-Tex 100 - Class I (safe for babies) as well as bluesign<sup>®</sup>.

### **Is Sanitized<sup>®</sup> Silver safe for the environment?**

Yes. Sanitized<sup>®</sup> Silver is safe for man and environment. Sanitized<sup>®</sup> Silver has been designed to durably bond on the fibre. Therefore, Sanitized<sup>®</sup> Silver does not leak into the waste water. This has been proven by stringent quality tests following strict ecological requirements and certified by independent institutes. An awareness concerning health, safety and the environment is an integral component of the SANITIZED business policy.

### **Is Sanitized<sup>®</sup> Silver safe for the skin?**

Yes, it is safe. Sanitized<sup>®</sup> Silver does not have a negative effect on skin; it is durably bonded to the fibre. This has been proven by strict international quality tests and confirmed by independent organisations: Test report OECD 404 confirms no skin irritation and test report OECD 406 proves that there is no sensitization of the skin. These results are also confirmed by Oeko-Tex 100 - Class I (safe for babies).

### **Is Sanitized<sup>®</sup> Silver dangerous for the respiratory system?**

Definitely not. Sanitized<sup>®</sup> Silver is durably bonded to the fibre and cannot be inhaled.

### **Can the silver finish leak into the washing machine and cause water contamination?**

Sanitized<sup>®</sup> Silver does not cause water contamination. Sanitized<sup>®</sup> Silver does not transfer onto other clothing articles during home laundering because it is bonded to the fibre. It does not leak into the waste water. This is proven by stringent tests that meet highest requirements.

### **How can SANITIZED prove that there is no risk for man and environment?**

The safety of Sanitized<sup>®</sup> Silver is proven by strict tests that meet highest requirements and it is certified by Oeko-Tex 100 – Class I (safe for babies) as well as bluesign<sup>®</sup>.

## Appendix 5 – SEM images

The surface of the samples is examined in scanning electronic microscope (SEM) with facilities for X-ray microanalysis (EDX). The SEM and EDX technique is for surface investigation. It was attempted to measure diameters of approx. 20 particles or clusters of particles on the surface of each of the examined samples.

The resolution for the SEM technique is approximately 5 nm for sample surfaces with an optimal electronic conductivity. No nanoparticles under 5 nm can be shown. The resolution for the EDX technique is approximately 1  $\mu\text{m}$  and allows detection of silver down to 0.1 w/w % (1 mg/g). A single silver nanoparticle down to 5 nm cannot be detected by EDX, but if silver nanoparticles are clustered together or are present on the surface in concentrations larger than 0.1 w/w % EDX can detect the silver nanoparticles.

Exhibit A1 to A12 show SEM images collected from the textile surfaces of 12 samples. 4 SEM images are shown for each sample. The SEM images are recorded with the backscattered electron detector (RBSD) at 100, 500, 1000 and 5000 times magnification. Details down to 50 nm will easily show up in the images recorded at 5000 times magnification. The samples were examined at magnifications well over 5000 times. The magnifications which give the most representable SEM image of the samples are included in this appendix 5.

The backscattered electron signals coming from the samples will depend on the element composition of the different parts in the image fields. Backscattered electron signals coming from ex. silver particles will appear brighter in the SEM images than backscattered electron signals coming from organic material eg., cotton or polymer. In the SEM images silver particles and silver threads will appear with a very light grey tone (almost white) compared with the textile fibres, which appear with a darker grey tone.

In some of the SEM images light spots appear, which is due to the electron charging. During the SEM examination it was not possible to derive the electrons from the surface of the fibres. They are accumulating and appear as light spots on the surface of the fibres. That the bright spots observed on the fibres is due to this phenomenon is verified during the SEM examination, where it is registered that the light spots change places on the sample surfaces after each scan with the electron beam.

Exhibit A1 (36492-1) shows four SEM images collected from the running T-shirt. The EDX investigation shows no silver on the surface of the sample. The SEM images show that the fibres in the sample appear almost without particles.

Exhibit A2 (36492-2) shows four SEM images collected from the microfibre cloth. The SEM and EDX investigation shows silver particles on the surface of the textile sample. The silver particles have diameters from approximately 0.2 to 1.0  $\mu\text{m}$ . The silver particles are located as single particles on the fibres. The particles are placed with a relative long distance to each other. The silver particles appear white and are pointed out in the SEM images with a red circle. From the results of the investigation it is assessed that the silver particles on the fibres were produced using a deposition process.

Exhibit A3 (36492-5) shows four SEM images collected from the insole. The SEM and EDX investigation shows silver particles on the surface of the textile sample. The silver particles have diameters from approximately 0.2 to 1.0  $\mu\text{m}$ . The silver particles are located as single particles on the fibres. The particles are placed with a relatively long distance to each other. The silver particles appear white and are pointed

out in the SEM images with a red circle. From the results of the investigation it is assessed that the silver particles on the fibres were produced using a deposition process.

Exhibit A4 (36942-6) shows four SEM images collected from the insole of the sandal. The SEM and EDX investigation could not detect nanoparticles or silver on the surface of the sample. The SEM images show that the fibres in the sample appear without coating. The fibres in the sample are imbedded in the rubber part of the sample. The rubber part contains a lot of fillers. The fillers are made of silicates. The silicates appear with a light grey tone in the SEM images. The rubber material of the sandal contains a lot of very small particles, but it is not possible to determine whether they consist of silver.

Exhibit A5 (36942-7) shows four SEM images collected from the ski underwear/pulley. The EDX investigation could not detect silver on the surface of the sample. The SEM images show that the fibres in the sample are partly coated with particles. The particles on the fibres are not made of silver. The particles appear with a grey tone similar to the grey tone of the fibres in the SEM images and are therefore not believed to be made of silver.

Exhibit A6 (36942-8b) shows four SEM images collected from the hat/scarf/headband. The SEM and EDX investigation shows no silver nanoparticles or silver threads on the surface of the sample. The SEM images show that the fibres in the sample appear without coating.

Exhibit A7 (36942-10) shows four SEM images collected from the cuddly toy. The SEM and EDX investigation shows silver threads in the sample. The silver threads are made of a collection of silver fibres. The silver threads have a diameter of approximately 150  $\mu\text{m}$ . The silver threads are interwoven with a distance of approximately 1 mm. The silver threads appear white and are pointed out in the SEM images with a red circle. From the results of the investigation it is assessed that the silver threads were produced using a coating process.

Exhibit A8 (36942-12) shows four SEM images collected from the body stocking. The SEM and EDX investigation shows no silver nanoparticles or silver threads on the surface of the sample. The SEM images show that the fibres in the sample appear without coating.

Exhibit A9 (36942-13) shows four SEM images collected from the tank top for children. The SEM and EDX investigation show no silver nanoparticles or silver threads on the surface of the sample. The SEM images show that the fibres in the sample appear without coating.

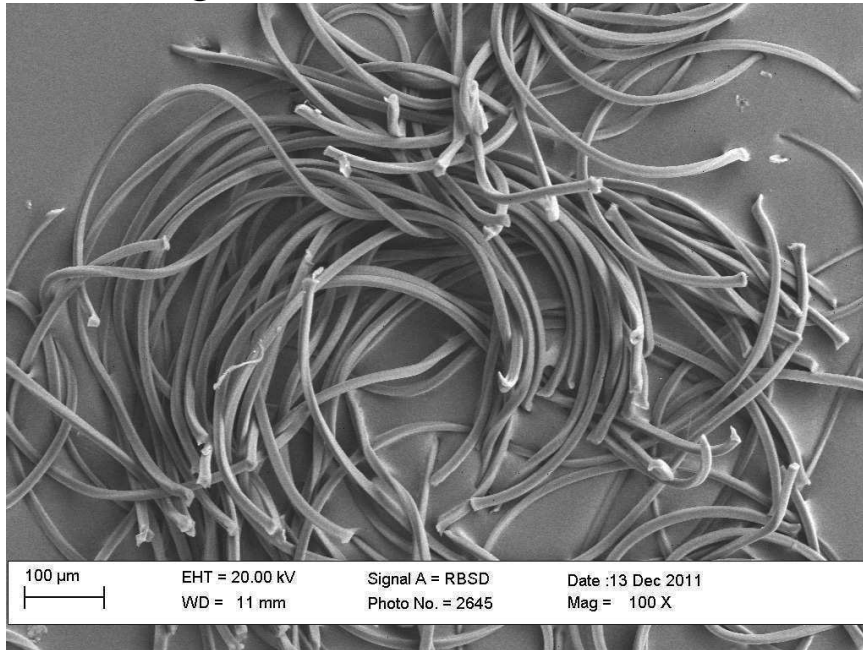
Exhibit A10 (36942-15) shows four SEM images collected from the top mattresses. The SEM and EDX investigation shows silver fibres as one of many fibres in some of the textiles threads. The silver fibres have a diameter of approximately 10  $\mu\text{m}$ . The threads containing silver fibres are interwoven with a distance of approximately 0.1 to 1 mm between the threads. The silver fibres appear white and are pointed out in the SEM images with a red circle. From the results of the investigation it is assessed that the silver threads were produced using a coating process.

Exhibit A11 (36942-16) show four SEM images collected from the nursing pad. The SEM and EDX investigation shows no silver particles or silver threads on the surface of the sample. The SEM images show that the fibres in the sample appear without coating.

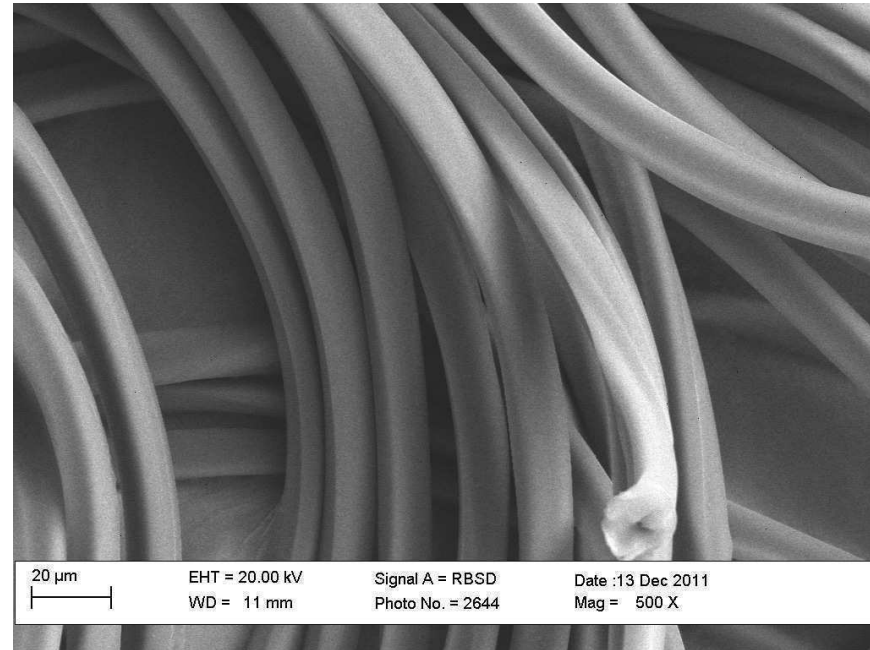


Exhibit A12 (36942-17) shows four SEM images collected from the running sock. The SEM and EDX investigation shows no silver nanoparticles or silver threads on the surface of the sample. The SEM-images show that the fibres in the sample appear without coating.

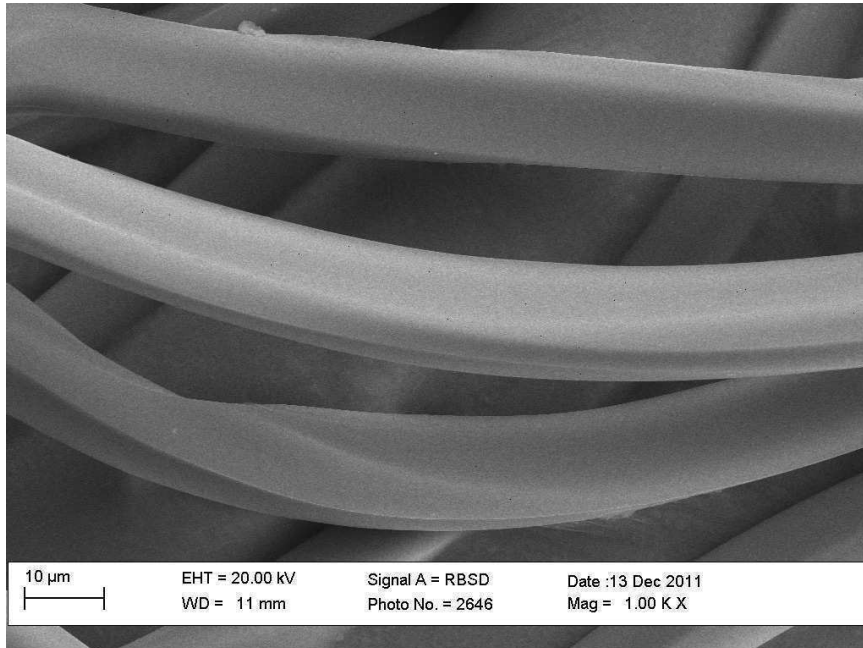
# Running T-shirt



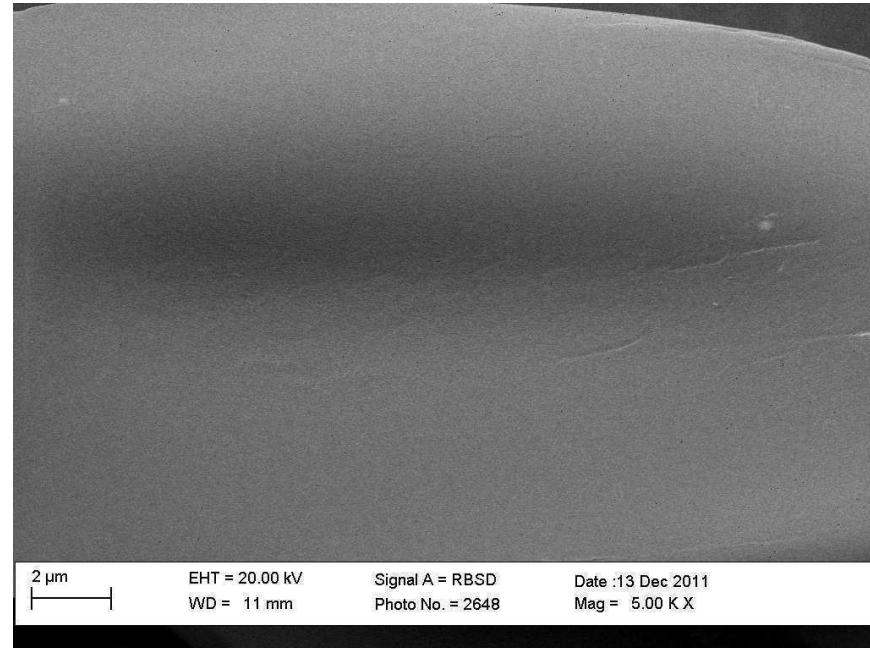
(Scanning Electron Microscope image)



(Scanning Electron Microscope image)

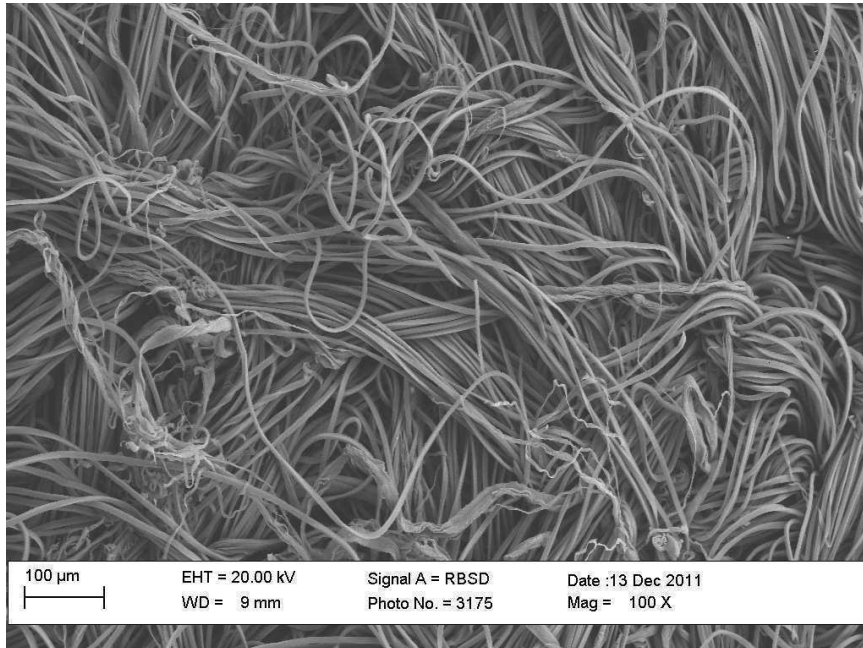


(Scanning Electron Microscope image)

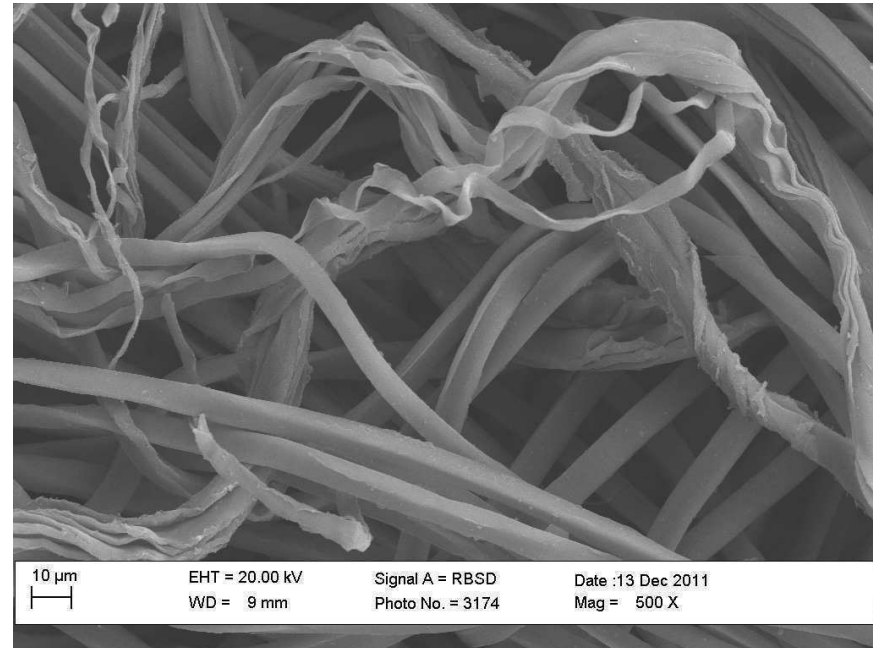


(Scanning Electron Microscope image)

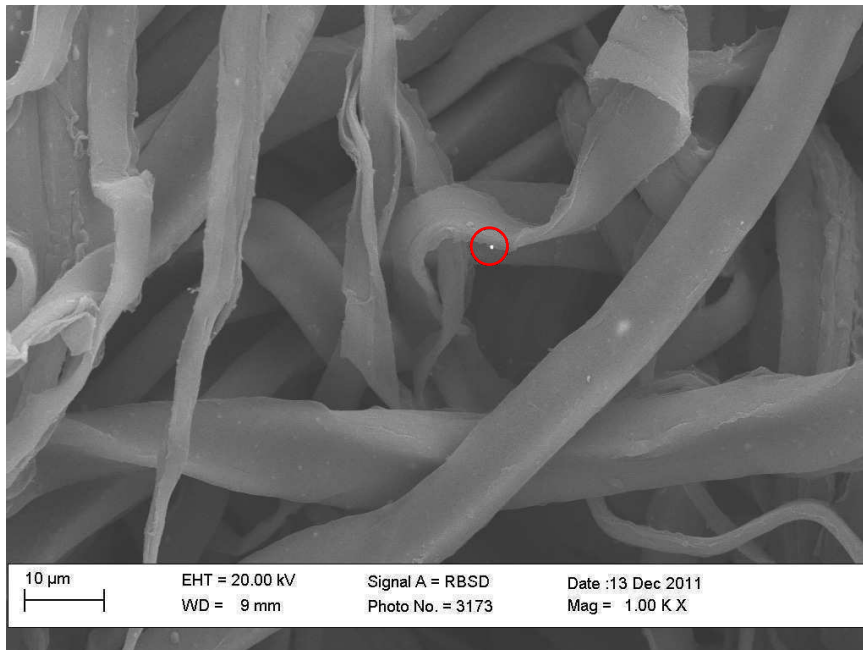
# Micro fiber cloth



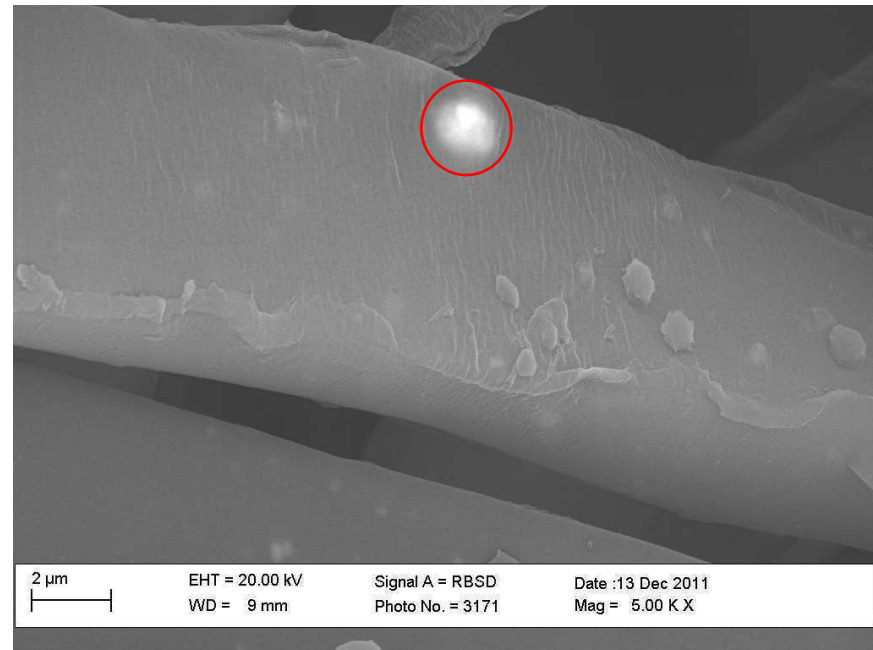
(Scanning Electron Microscope image)



(Scanning Electron Microscope image)

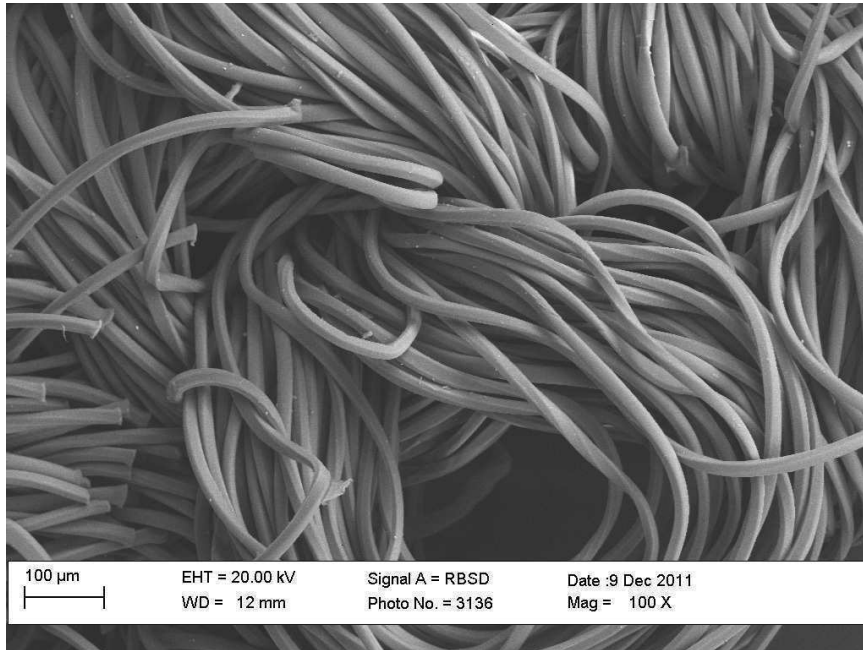


(Scanning Electron Microscope image)

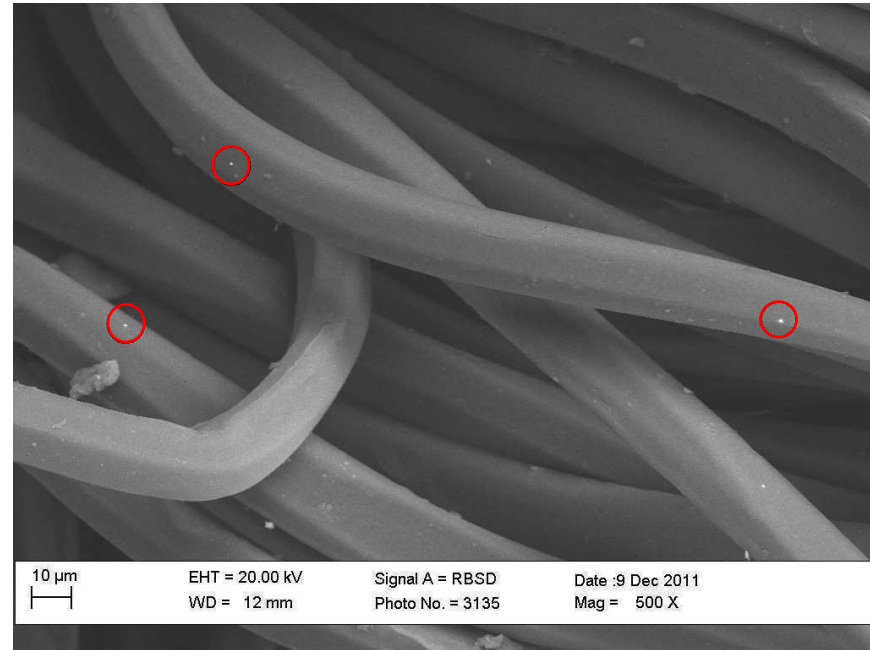


(Scanning Electron Microscope image)

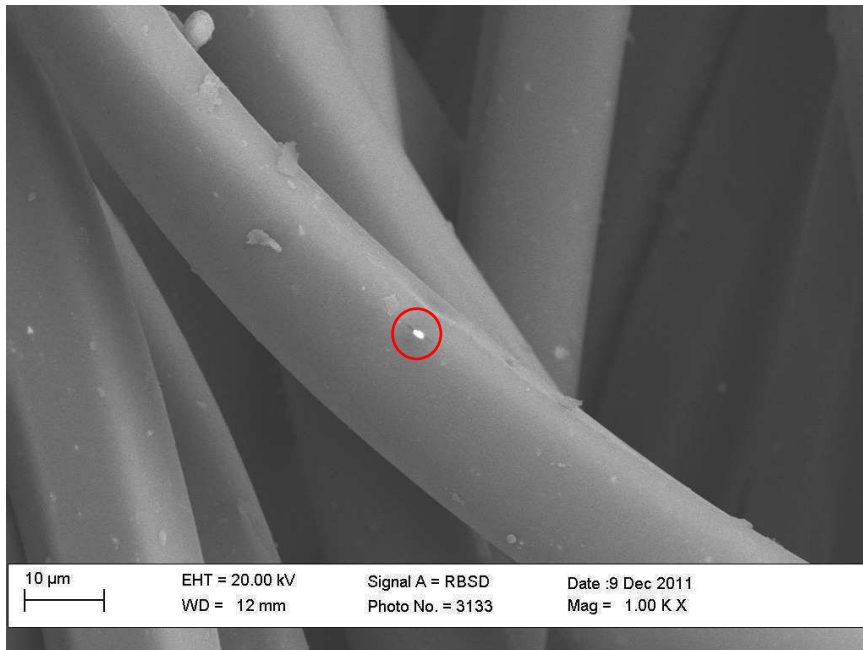
# Insole



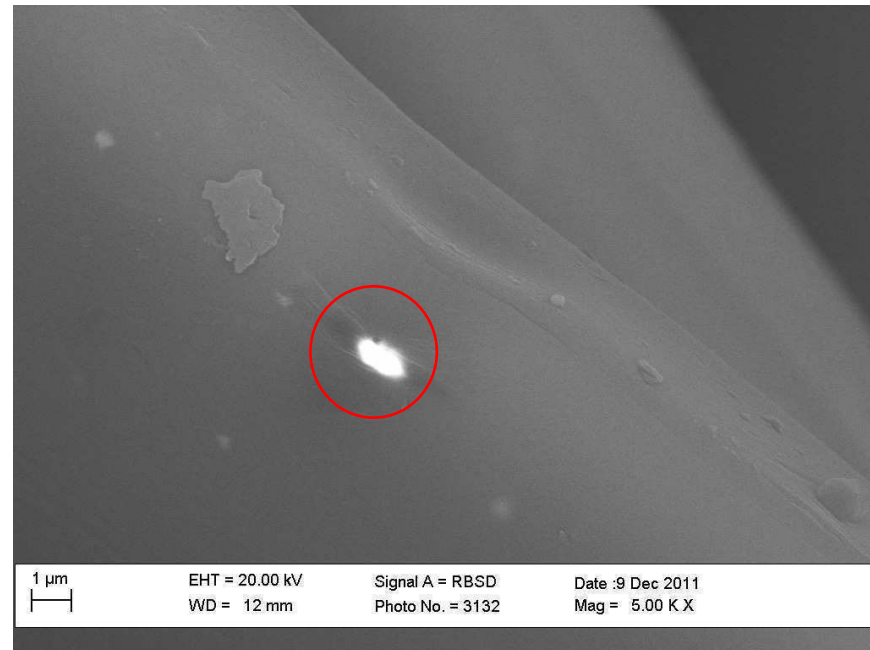
(Scanning Electron Microscope image)



(Scanning Electron Microscope image)



(Scanning Electron Microscope image)



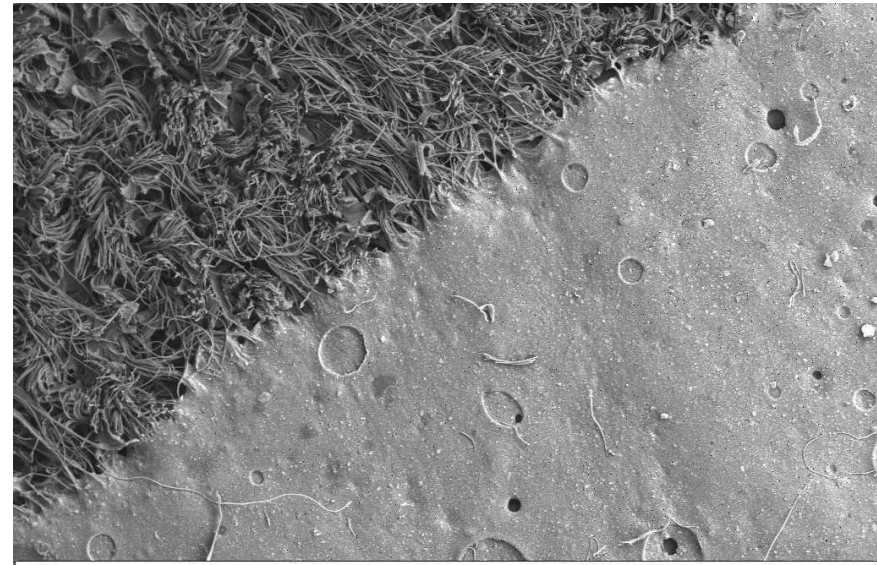
(Scanning Electron Microscope image)

# Sandal



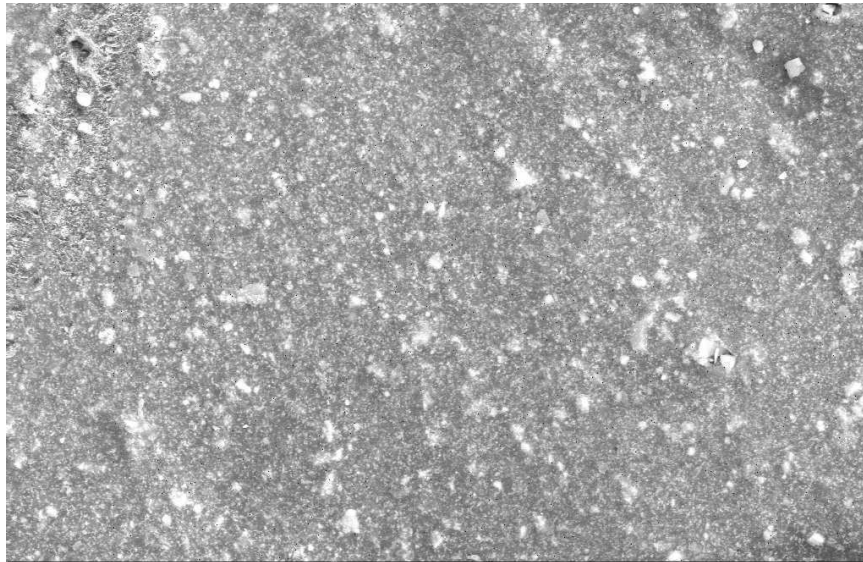
100  $\mu$ m      EHT = 20.00 kV      Signal A = RBSD      Date :9 Dec 2011  
WD = 12 mm      Photo No. = 3130      Mag = 100 X

(Scanning Electron Microscope image)



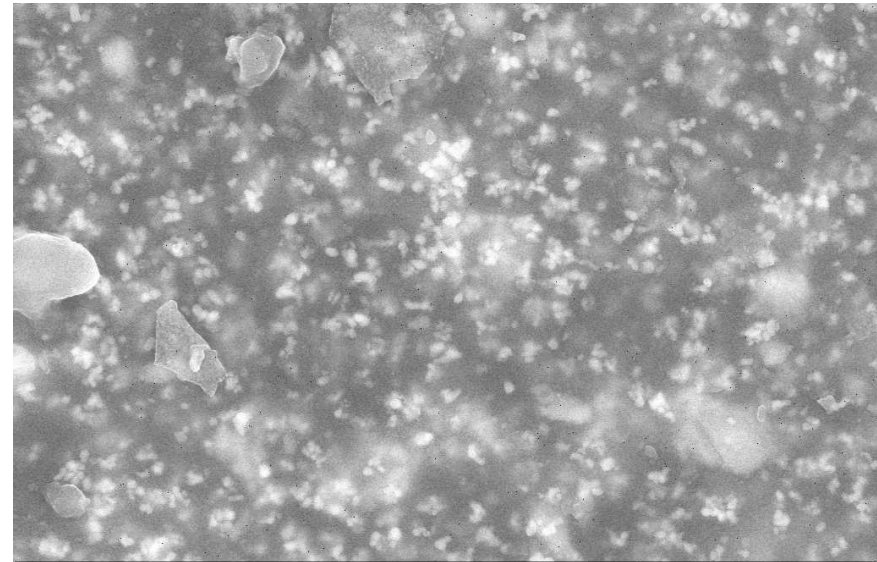
100  $\mu$ m      EHT = 20.00 kV      Signal A = RBSD      Date :9 Dec 2011  
WD = 12 mm      Photo No. = 3131      Mag = 50 X

(Scanning Electron Microscope image)



10  $\mu$ m      EHT = 20.00 kV      Signal A = RBSD      Date :9 Dec 2011  
WD = 12 mm      Photo No. = 3129      Mag = 500 X

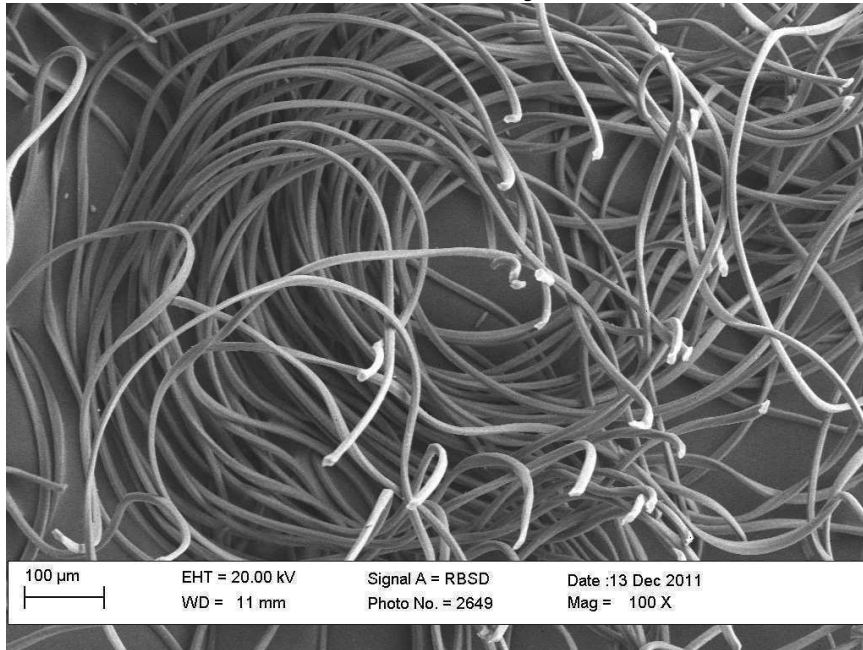
(Scanning Electron Microscope image)



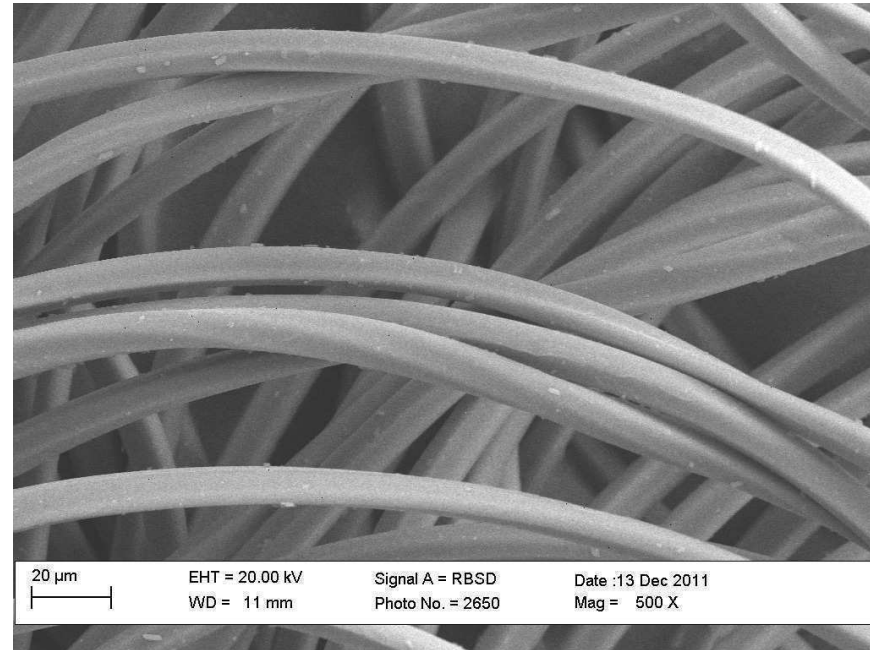
2  $\mu$ m      EHT = 20.00 kV      Signal A = RBSD      Date :9 Dec 2011  
WD = 12 mm      Photo No. = 3128      Mag = 5.00 K X

(Scanning Electron Microscope image)

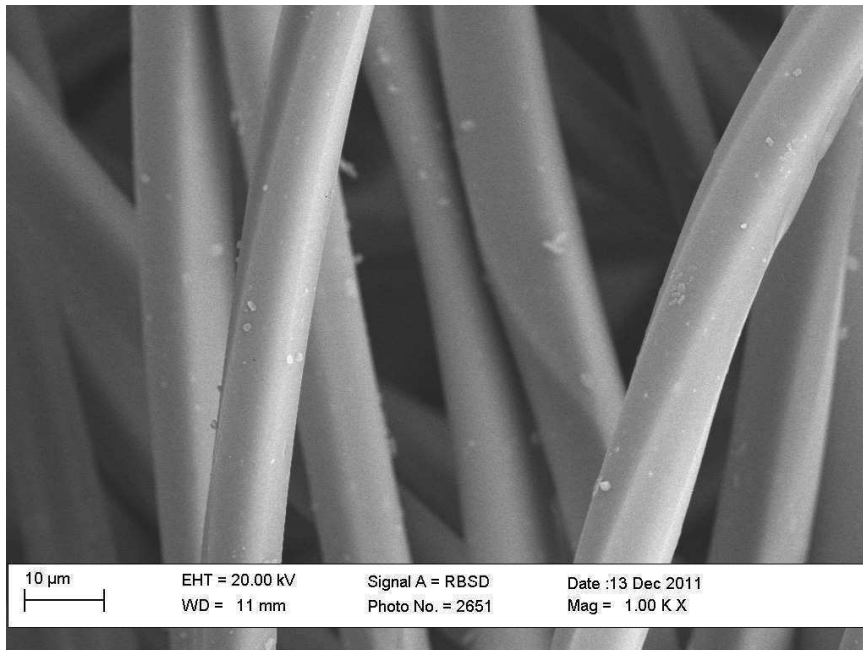
# Ski underwear / Pulley



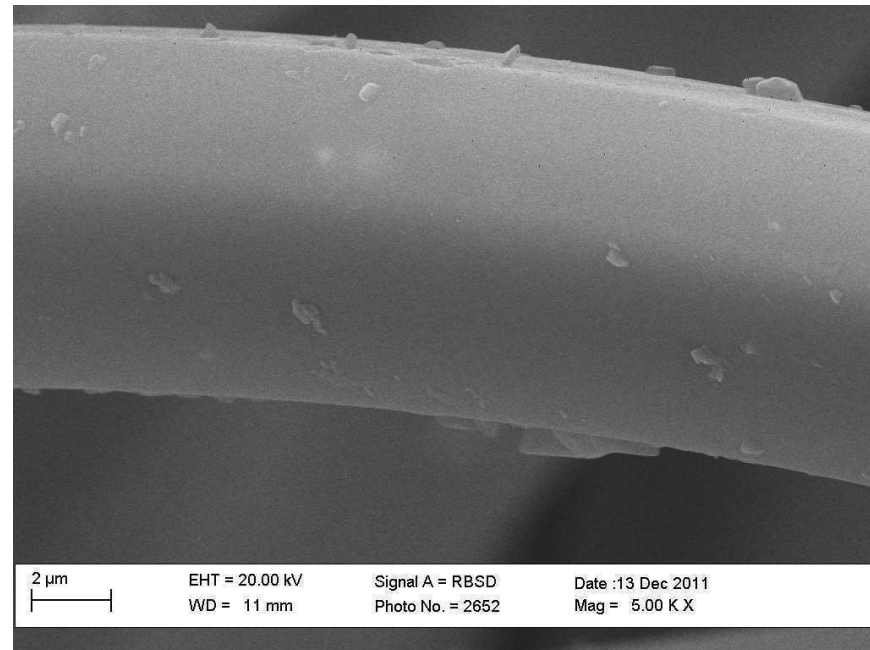
(Scanning Electron Microscope image)



(Scanning Electron Microscope image)

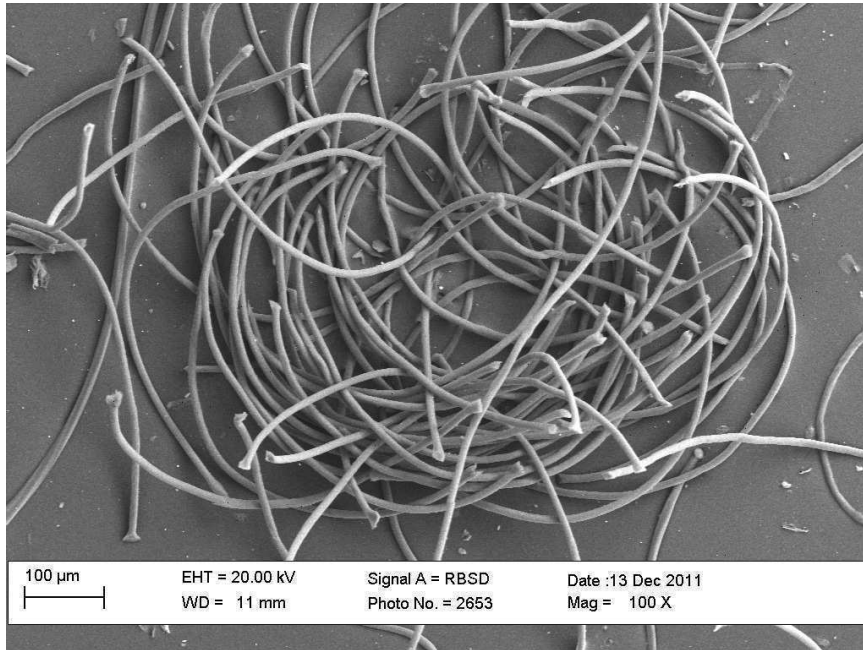


(Scanning Electron Microscope image)

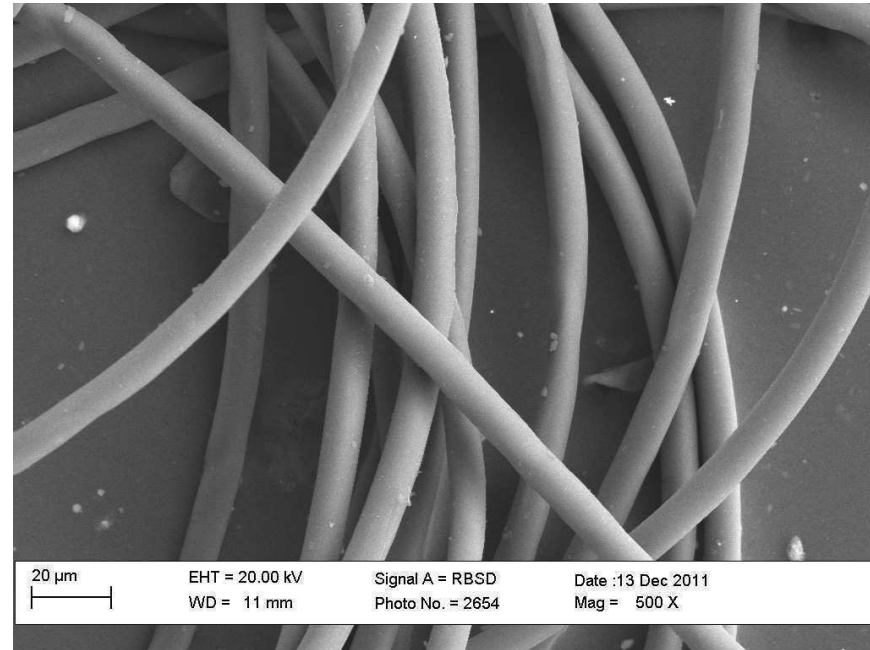


(Scanning Electron Microscope image)

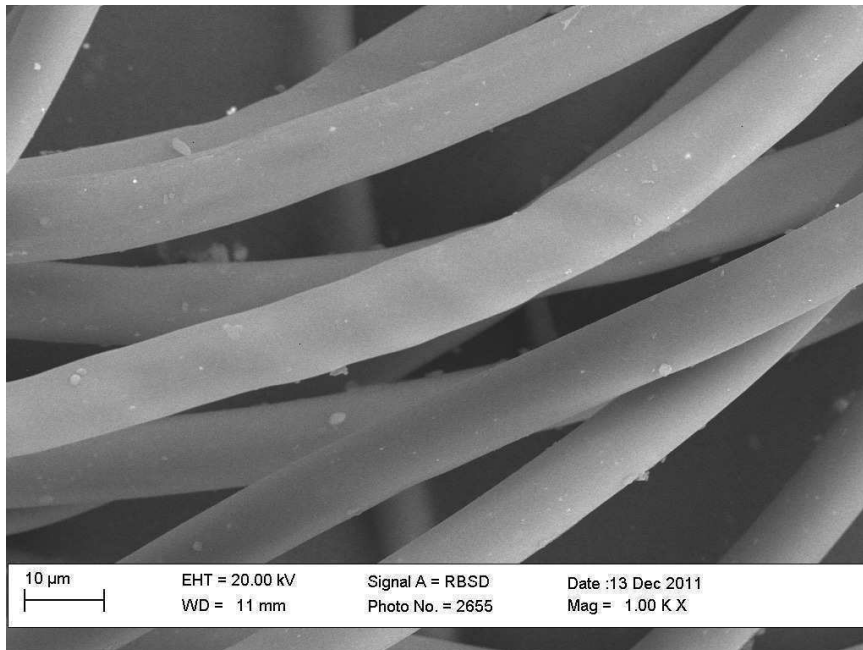
# Hat / scarf / headband



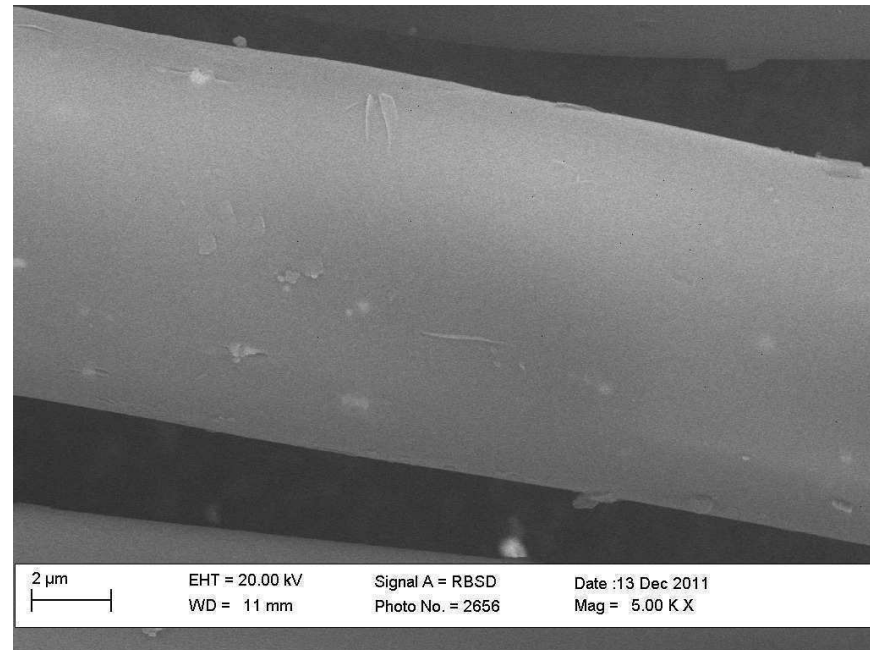
(Scanning Electron Microscope image)



(Scanning Electron Microscope image)

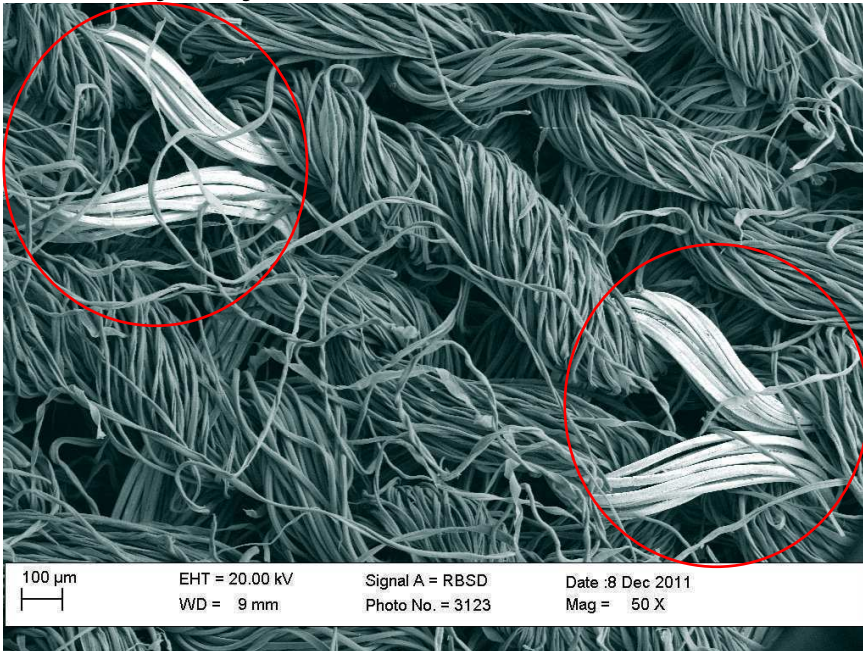


(Scanning Electron Microscope image)

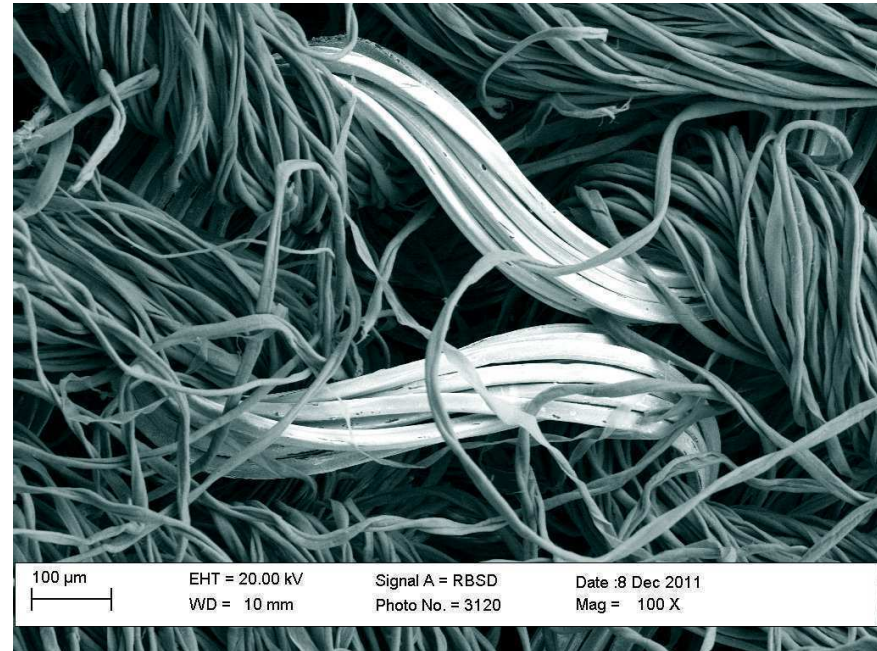


(Scanning Electron Microscope image)

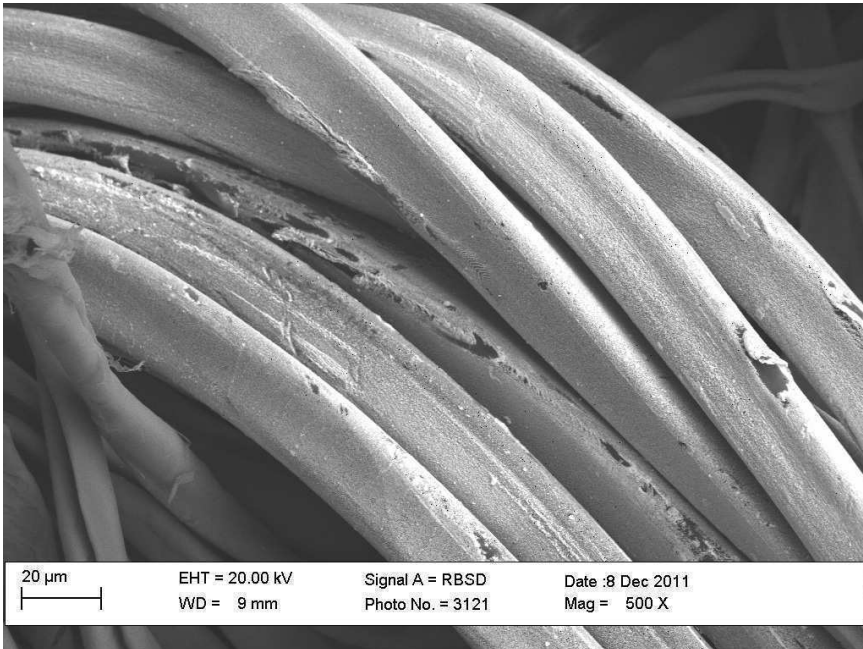
# Cuddly toy



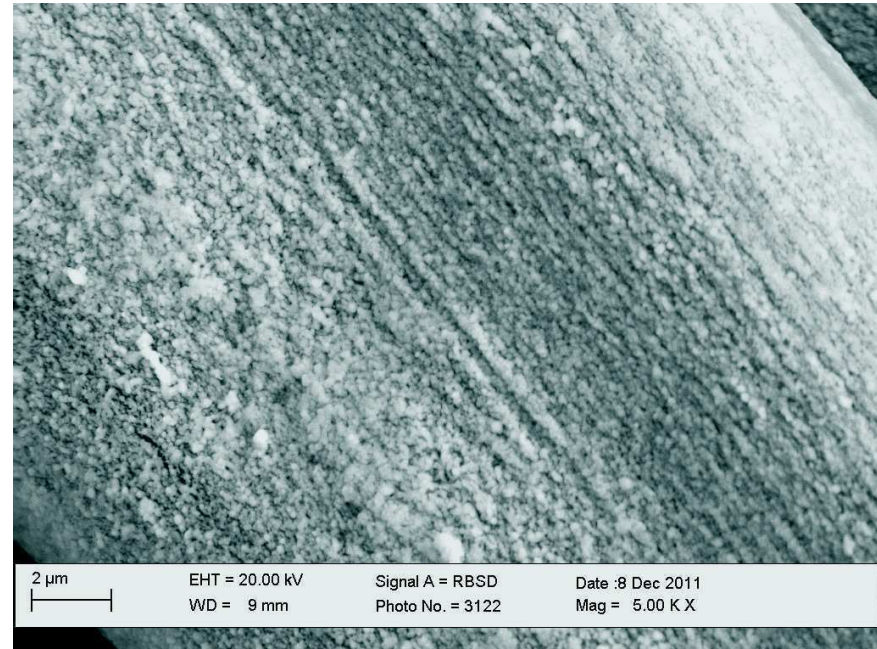
(Scanning Electron Microscope image)



(Scanning Electron Microscope image)



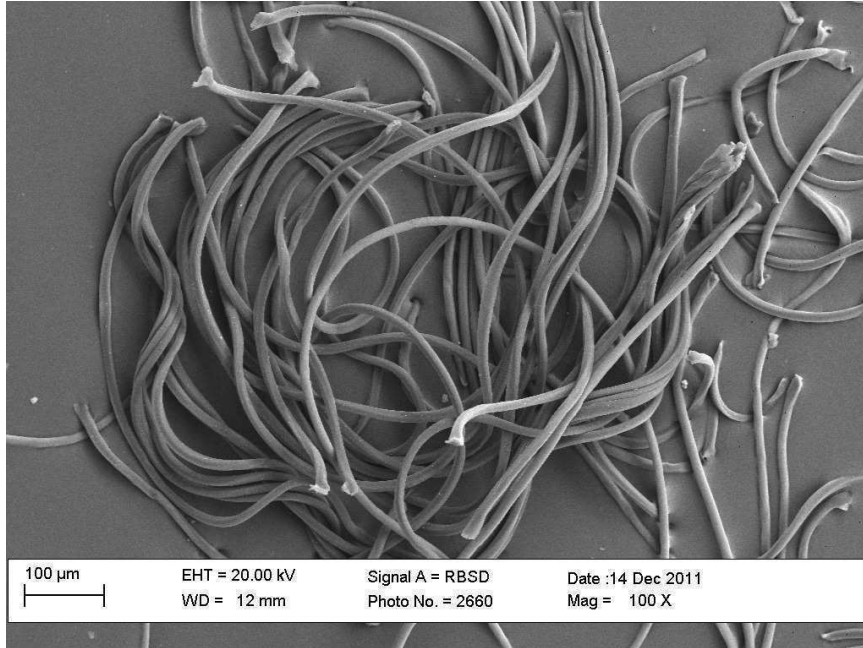
(Scanning Electron Microscope image)



(Scanning Electron Microscope image)

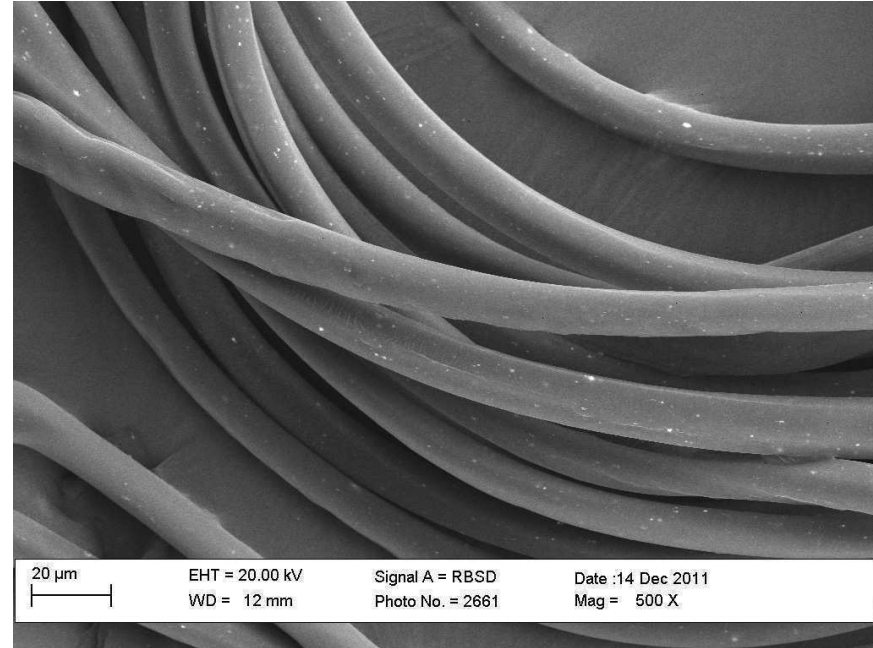


# Bodystocking long-sleeved



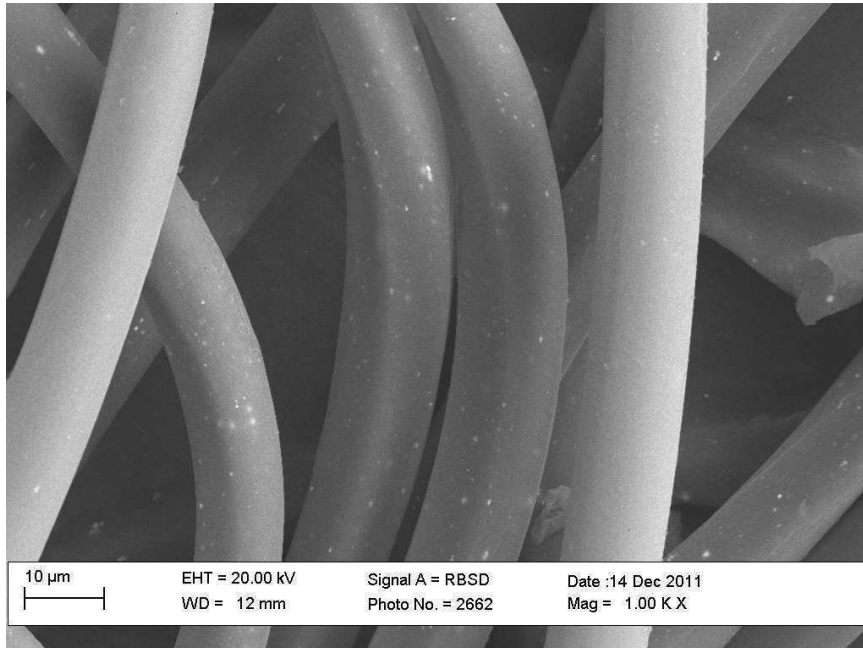
100  $\mu$ m      EHT = 20.00 kV      Signal A = RBSD      Date :14 Dec 2011  
WD = 12 mm      Photo No. = 2660      Mag = 100 X

(Scanning Electron Microscope image)



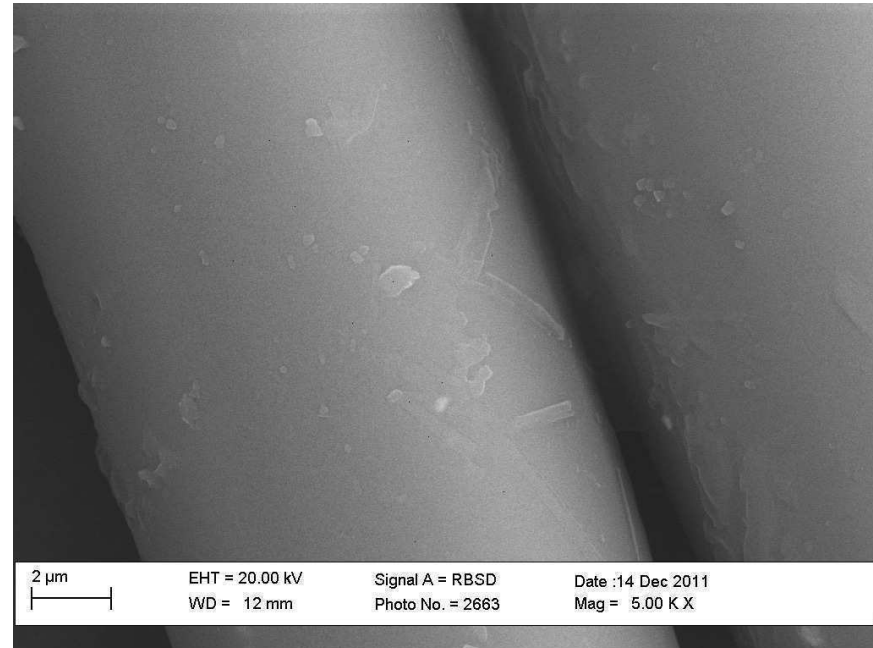
20  $\mu$ m      EHT = 20.00 kV      Signal A = RBSD      Date :14 Dec 2011  
WD = 12 mm      Photo No. = 2661      Mag = 500 X

(Scanning Electron Microscope image)



10  $\mu$ m      EHT = 20.00 kV      Signal A = RBSD      Date :14 Dec 2011  
WD = 12 mm      Photo No. = 2662      Mag = 1.00 K X

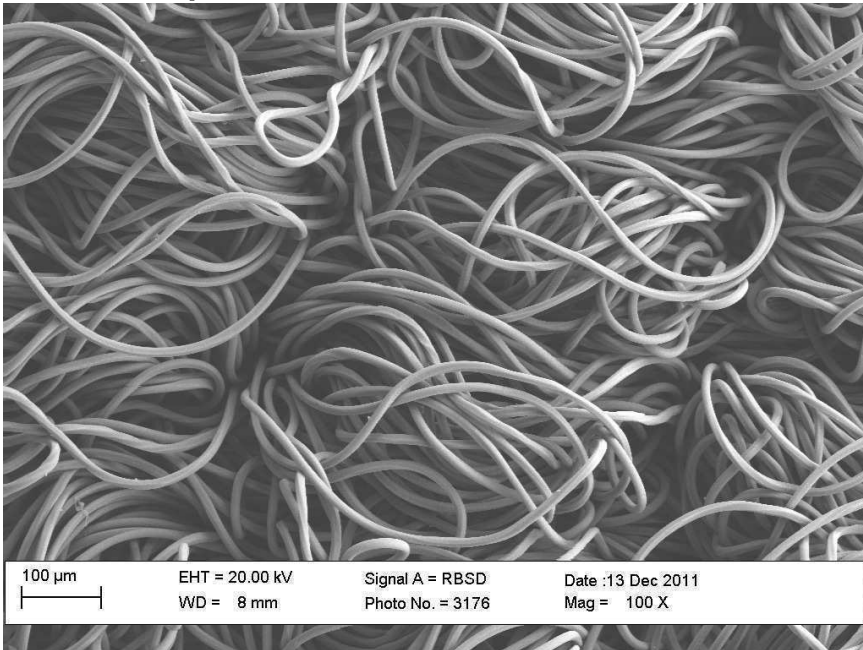
(Scanning Electron Microscope image)



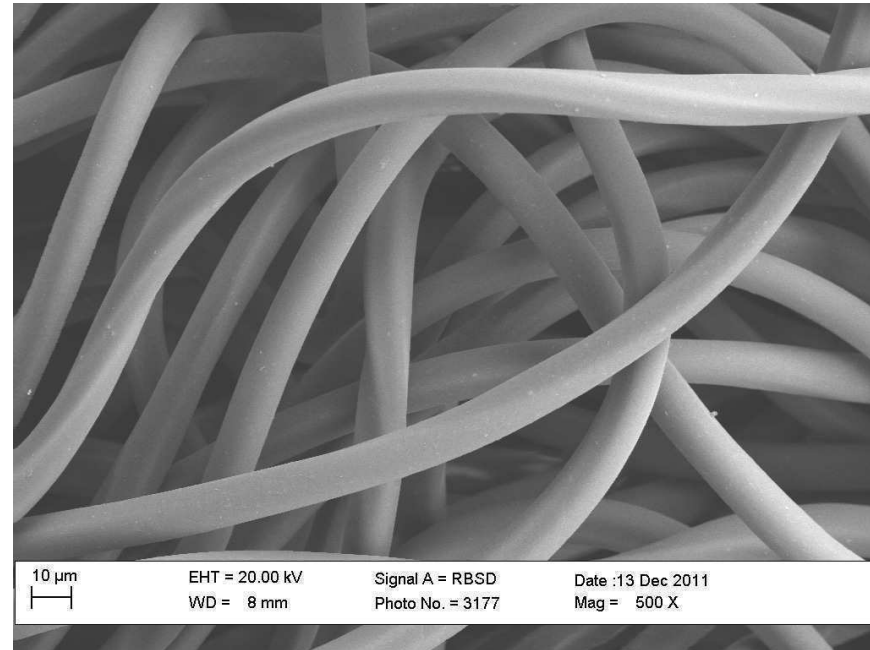
2  $\mu$ m      EHT = 20.00 kV      Signal A = RBSD      Date :14 Dec 2011  
WD = 12 mm      Photo No. = 2663      Mag = 5.00 K X

(Scanning Electron Microscope image)

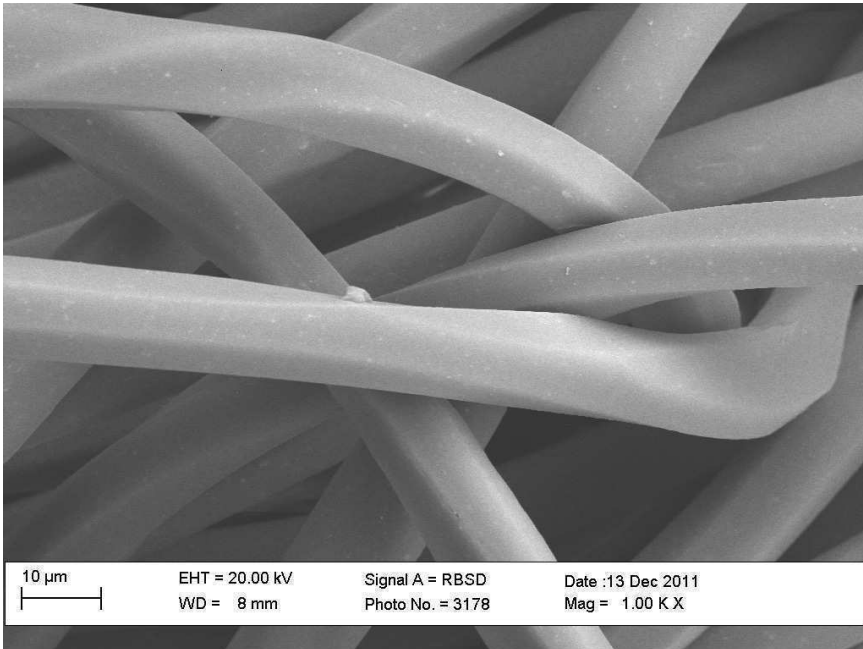
# Tank top for children



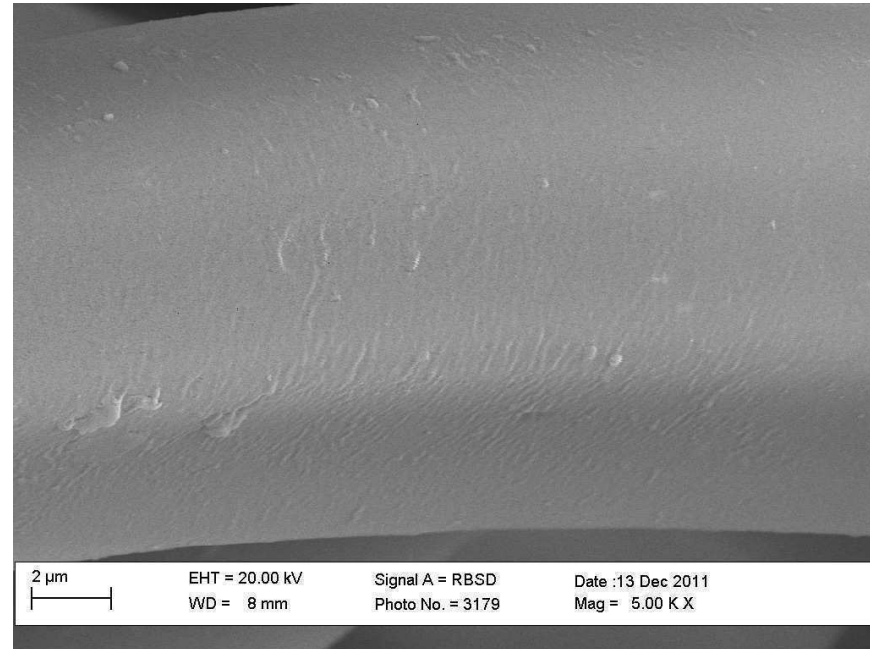
(Scanning Electron Microscope image)



(Scanning Electron Microscope image)

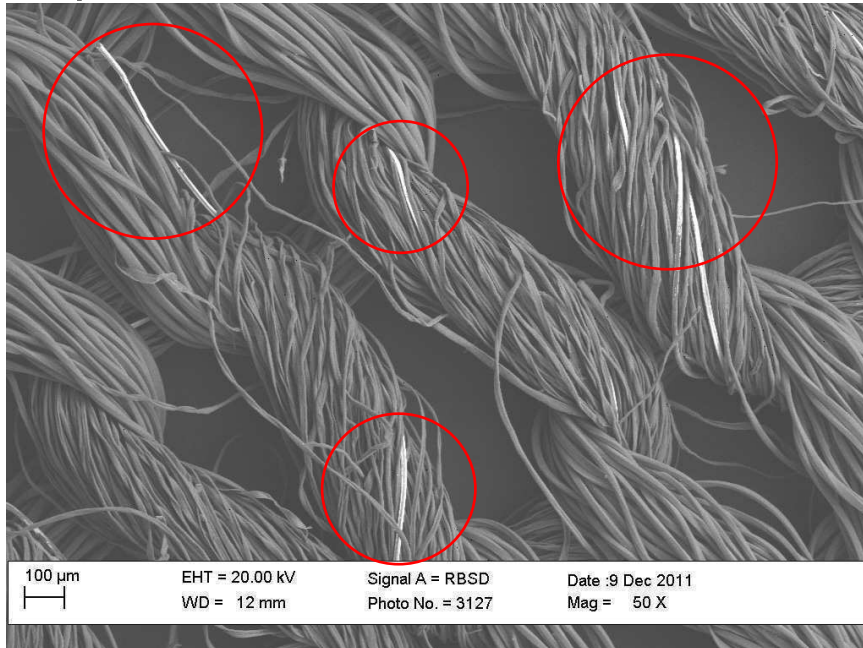


(Scanning Electron Microscope image)

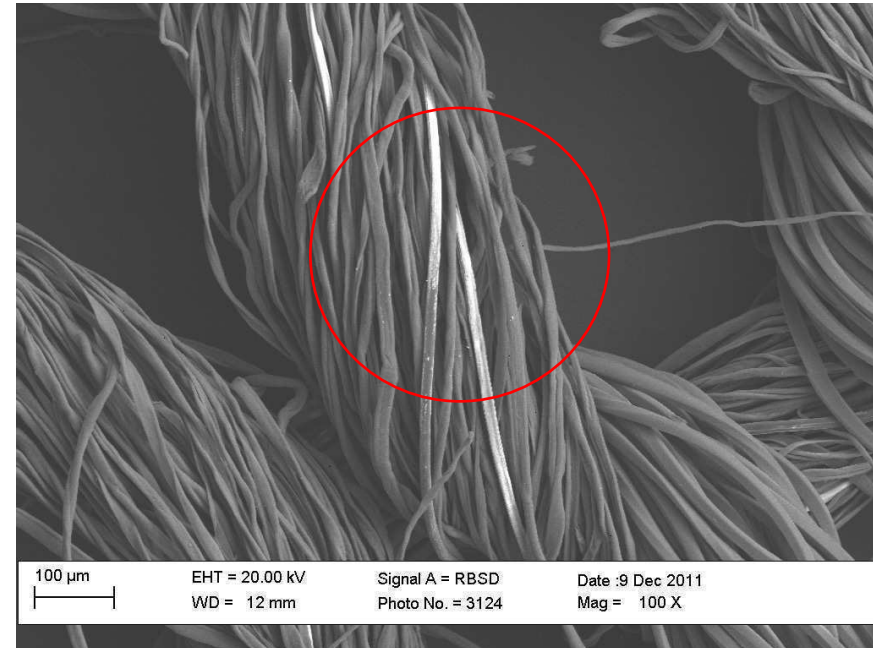


(Scanning Electron Microscope image)

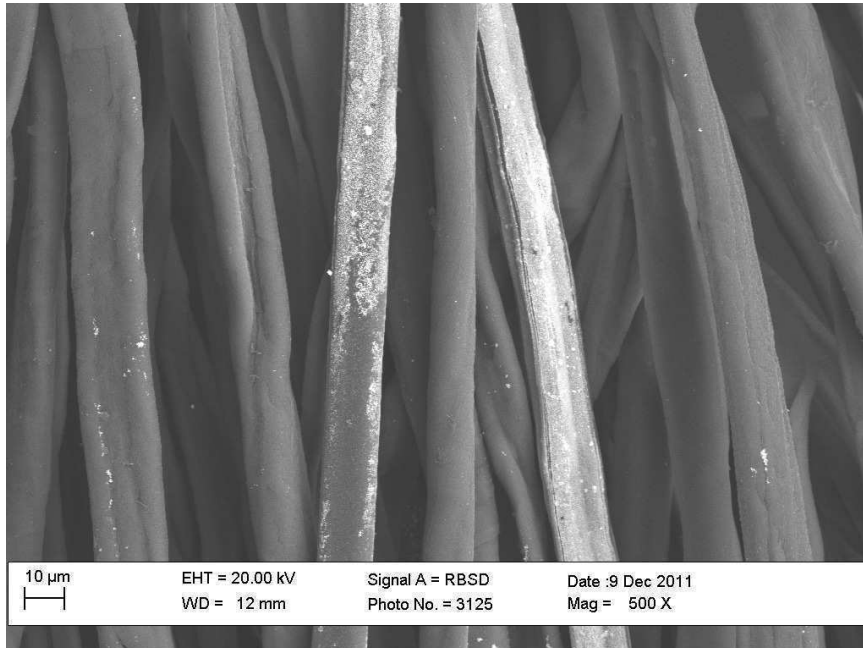
# Top mattresses, 36492-15



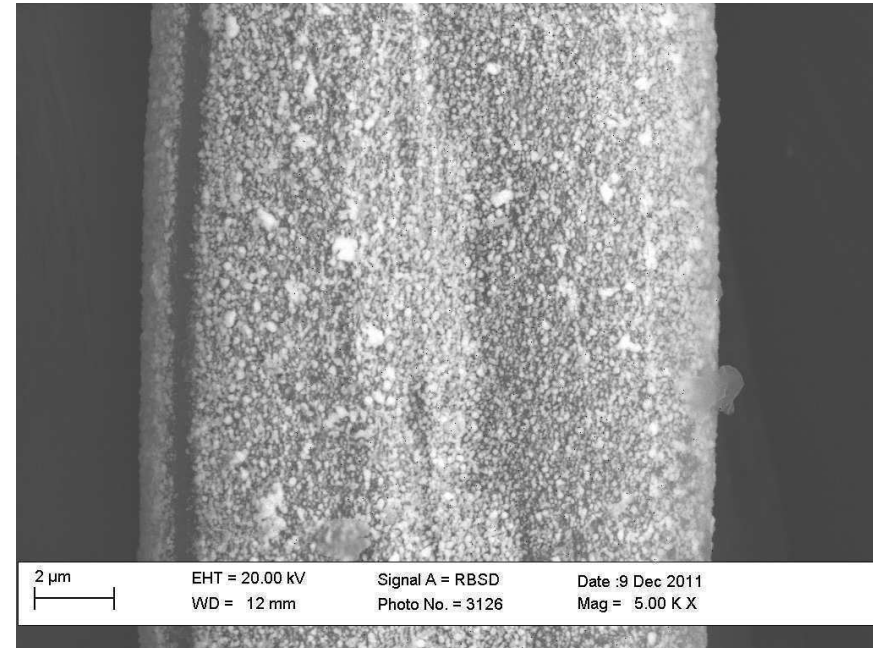
(Scanning Electron Microscope image)



(Scanning Electron Microscope image)

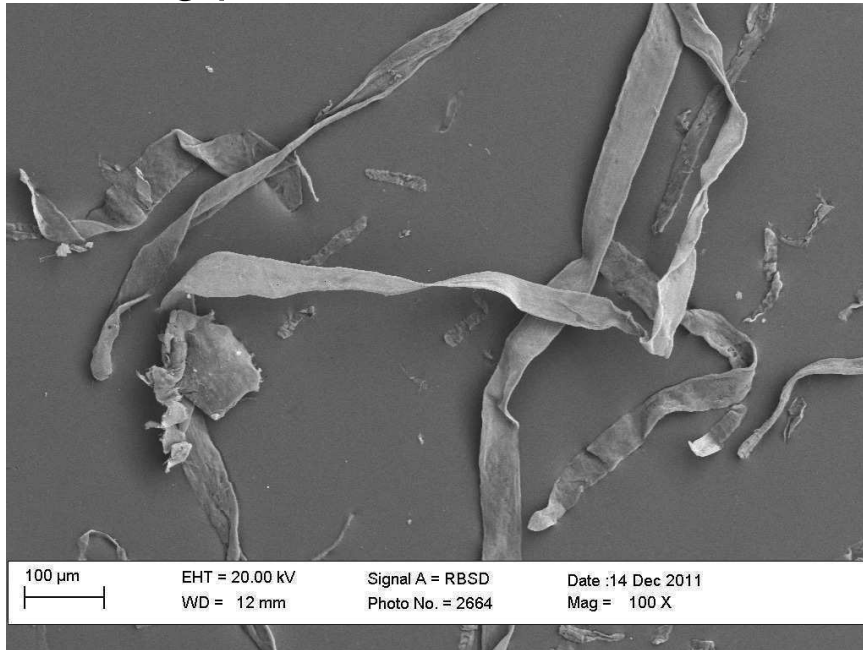


(Scanning Electron Microscope image)

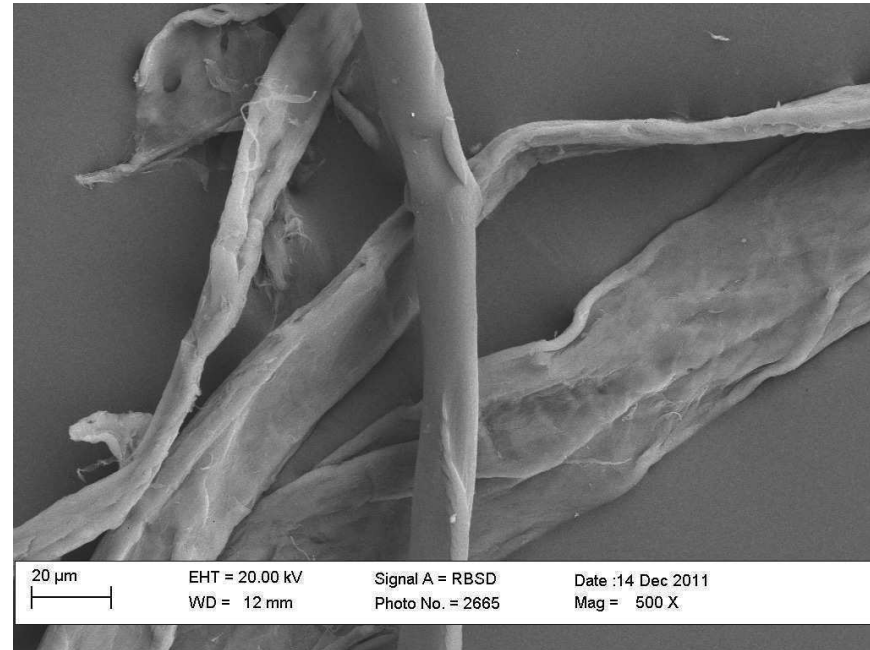


(Scanning Electron Microscope image)

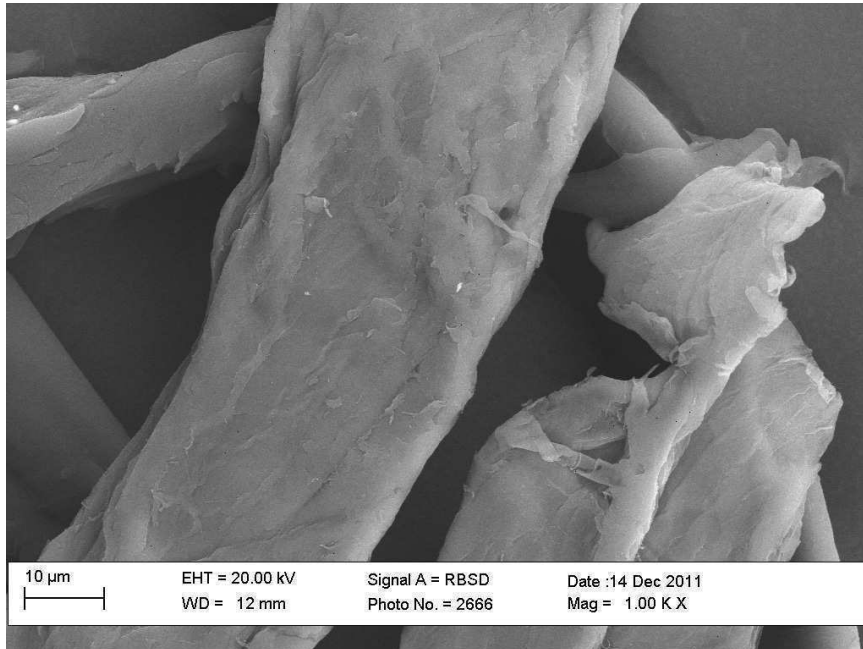
# Nursing pad



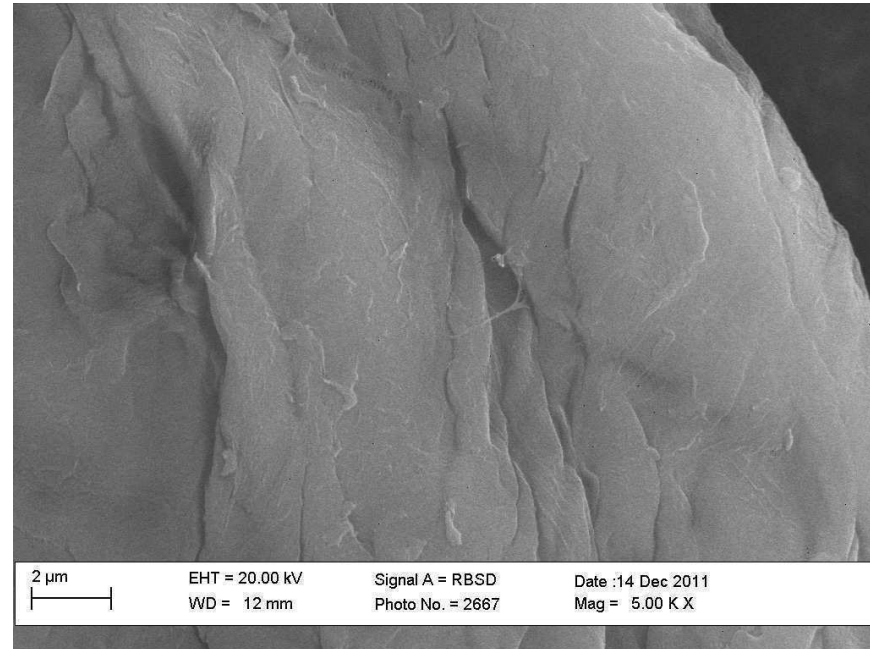
(Scanning Electron Microscope image)



(Scanning Electron Microscope image)

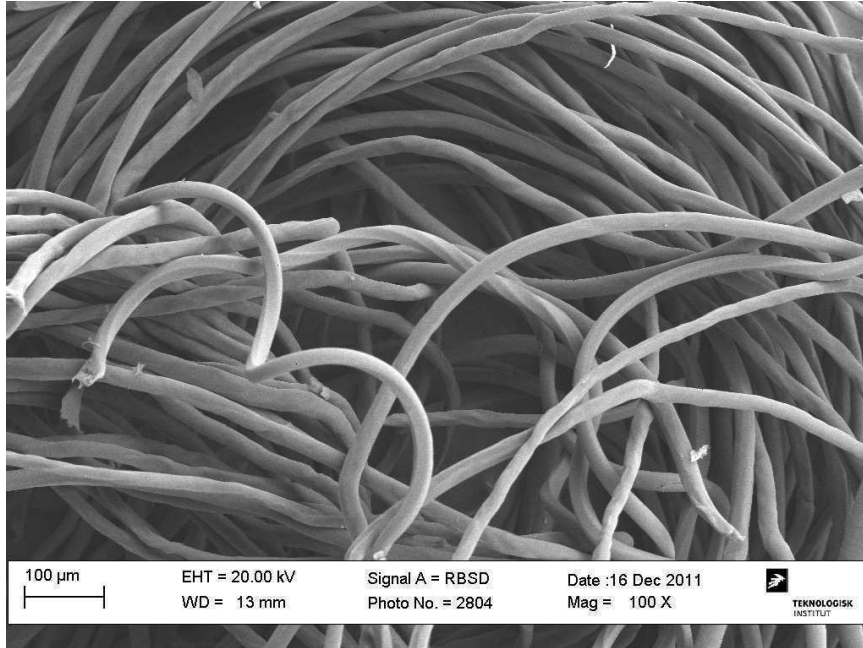


(Scanning Electron Microscope image)

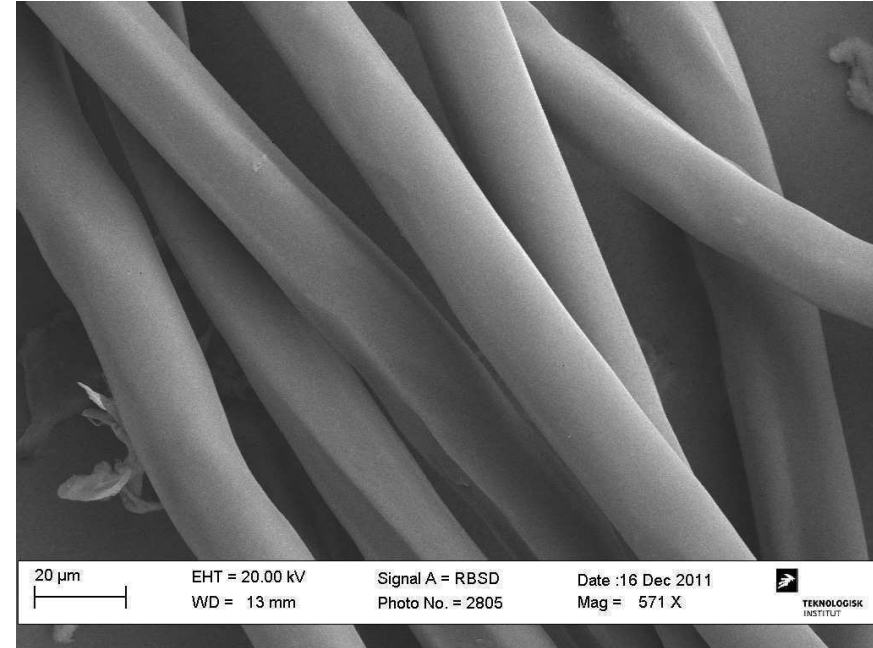


(Scanning Electron Microscope image)

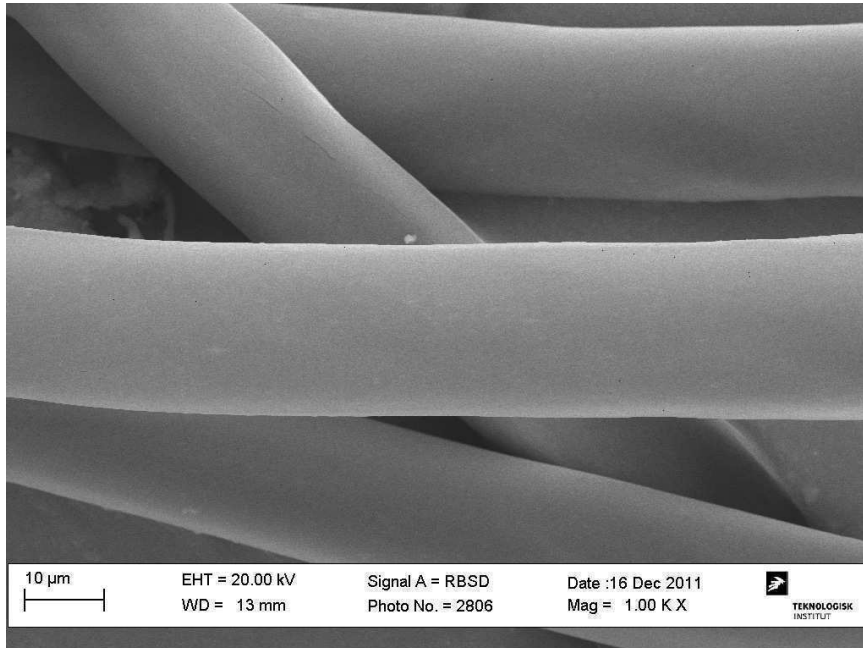
# Running sock



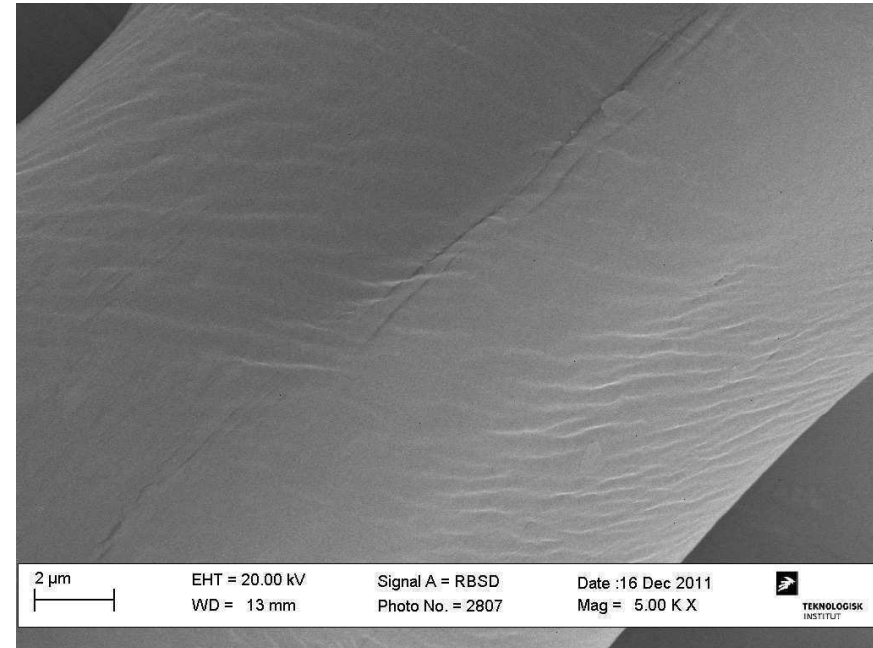
(Scanning Electron Microscope image)



(Scanning Electron Microscope image)



(Scanning Electron Microscope image)



(Scanning Electron Microscope image)



## Summary

The survey identified 94 textile products marketed in Denmark via retail, internet shops or other means where there could be suspicion of content of nanosilver. Since no nanosilver was found in any of the products, the results of this survey did not support the hypothesis that nanosilver treated textiles are widely used in Denmark. Further analyses of 16 selected products revealed silver content in 15 of the products. Studies of migration of silver to artificial saliva or sweat and during washing revealed a varying release of silver that could be substantial (up to 84% during washing). Based on the existing data and the survey results, there appear to be no risk of health effects or environmental effects.

Undersøgelsen identificerede 94 tekstilprodukter hvor der kunne være mistanke om indhold af nanosølv og som der markedsføres i Danmark via detailhandel, internetbutikker eller andet. Da der ikke blev fundet nanosølv i nogen produkterne, understøtter resultaterne af undersøgelsen ikke hypotesen om, at nanosølv behandlede tekstiler er almindeligt anvendt i Danmark. Kemiske analyser af 16 udvalgte produkter viste sølvindhold i 15 af produkterne. Undersøgelser af migration af sølv til kunstigt spyt eller sved og under vask afslørede en varierende frigivelse af sølv, der kan være betydelige (op til 84% i løbet af vask). Baseret på de eksisterende data og undersøgelsen resultater, synes der ikke at være nogen risiko for miljø- eller sundhedsmæssige effekter.



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