KEMI
Swedish Chemicals Agency

PM 8/15

Antibacterial treatment of clothes – does it really have an effect?

–Measurement of antibacterial effects of treated apparel before and after washing

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KEMI
Swedish Chemicals Agency

The Swedish Chemicals Agency is a supervisory authority under the Government. We work in Sweden, the EU and internationally to develop legislation and other incentives to promote good health and improved environment. We monitor compliance of applicable rules on chemical products, pesticides and substances in articles and carry out inspections. We review and authorise pesticides before they can be used. Our environmental quality objective is A Non-toxic Environment.

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Foreword

On behalf of the Swedish Government, the Swedish Chemicals Agency has developed an action plan for a non-toxic everyday environment. Reducing the chemical risks in the everyday environment is a step along the way to attaining the Swedish Parliament's environmental quality objective "A Non-Toxic Environment". As part of our work on the action plan, the Swedish Chemicals Agency works to reduce unnecessary use of antibacterial substances in everyday goods. Antibacterial substances can be harmful to health and the environment, and are suspected to contribute to the development of resistance. The purpose of the antibacterial treatment is often unclear, so its benefits are difficult to assess.

Fabrics are sometimes marketed with anti-bacterial labels, which suggest the textile to be more hygienic and thus imply some type of health advantage. In other cases, claims are made to suggest anti-odour treatment or "freshness". Whether or not such claims hold what they promise is impossible to decide for the consumer. By this study, we wanted to test whether the claims made can be proven right.

The overarching purpose of this study is to increase knowledge in the area and to highlight the problems that may be associated with the use of antibacterial substances in clothing. With this memorandum, the Swedish Chemicals Agency wishes to draw the attention of manufacturers, importers, authorities and the general public to the use of antibacterial substances in textiles.

The study was carried out by a consultant: Peter Askew from Industrial Microbiological Services LTD was our contractor. Ulrike Frank, at the Unit for Substance Evaluation, was responsible for the implementation of the project.

Content

Summary

Antibacterial treatment of textiles is usually marketed with the function of preventing odours. But sometimes it is suggested that textiles treated in this way even display some sort of hygienic advantage. In a previous study, the Swedish Chemicals Agency analysed treated textiles with a primary focus on sports and leisure wear before and after washing. We investigated the content of the antimicrobial substances triclosan, triclocarban and silver *(1)*. The study showed that on average 60 % of the antibacterial substances were washed out after 10 washes. We wanted to find out whether the leaching of the antibacterial substances also lead to a lesser functionality, or if the remaining substance in the textile was nevertheless sufficient to ensure an antibacterial or an anti-odour effect.

Thus in the present study, the same textile articles from the washing study were examined with respect to their antibacterial effects. Unwashed and washed samples of the textiles were tested for their antibacterial activity. To examine whether bacterial growth was inhibited in a treated textile we compared growth between a treated and an untreated textile. We tested with two different standard bacteria. Any inhibition of growth was regarded as antibacterial activity. It is shown that two thirds of the tested samples (68 %) did not exhibit any antibacterial effect after 10 washing cycles. Almost a third of the samples (28 %) did not even show an effect before washing.

In parallel, we studied if odour-reduction claims could be measured by a laboratory test instead of by costly odour-trials with test persons. The study showed that the applied laboratory test does not work to confirm such claims. It is suspected that other chemicals from the treatment of textiles during manufacture interfere with the test-system.

This document describes the purpose of the study, provides background to antibacterial treatment of textiles, presents results and discusses the conclusions to be drawn from this study.

Sammanfattning

Antibakteriell behandling av textilier marknadsförs vanligtvis med argument om att det förhindrar dåligt lukt. Ibland påstås det att antibakteriellt behandlade textilier även har en slags hygienisk fördel. I en tidigare studie har Kemikalieinspektionen analyserat textilier som sportkläder, strumpor och disktrasor före och efter tvätt. Vi undersökte halten av de antimikrobiella ämnena Triklosan, Triklocarban och Silver *(1)*. Studien visade att i genomsnitt 60% av de antimikrobiella ämnena tvättades ut efter 10 tvättar. Vi ville veta om förlusten av ämnena även ledde till förminskad funktionalitet, eller om den resterande halten av ämnet var tillräcklig för att säkerställa en antibakteriell effekt.

Därför undersökte vi textilierna från tvättstudien igen, denna gång för att undersöka deras antibakteriella effekt. Vi testade otvättade och tvättade textilprov för att se om de visade antibakteriell aktivitet. För att testa om tillväxten av bakterier var mindre i en behandlad textilie jämförde vi den med en obehandlad textilie. Vi testade med två olika standardbakterier. Varje minskning av bakterietillväxt jämfört med en obehandlad textilie tolkades som antibakteriell aktivitet. Två tredjedelar (68 %) av textilierna visade ingen antibakteriell effekt efter 10 tvätt. En knapp tredjedel (28 %) av textilierna visade inte någon aktivitet även innan tvätt.

I en parallell studie ville vi testa om luktminskning kunde mätas i ett laboratorietest istället för att använda dyra lukttester med testpersoner. Testerna visade dock att laboratorietesten inte fungerade för detta ändamål, förmodligen för att textilierna behandlats under tillverkning med ämnen som störde testsystemet.

Det här dokumentet beskriver syftet med studien, ger bakgrunden till antibakteriell behandling av textilier, presenterar resultat och diskuterar slutsatser som kan dras från denna studie.

1 Scope

This study was conducted on 30 textiles (training garments, socks, dish-cloths, see Annex III) analysed in a previous study for their content of 3 different antibacterial substances (triclosan, triclocarban and silver) before and after washing. About 60 % of the analysed substance did on average leach out after 10 washes. We wanted to know whether the leaching of the antibacterial substances also lead to a lesser functionality, or if the substance left in the textile was nevertheless sufficient to ensure the antibacterial effect claimed. Therefore we conducted a study to test the antibacterial activity of these textiles. In parallel, we studied if odour-reduction claims could be measured by a laboratory test instead of by costly odour-trials with test persons.

2 Background

Antibacterial substances are substances which are intended to kill or inhibit bacteria. Substances which shall prevent harmful effects of organism on human beings, animals or material are called biocides. Usually, these substances are poisonous and can be associated with health or environmental risks. That is why biocides are regulated by EU Law (Biocidal Products Regulation *(3)*). They have to be authorized before use and certain rules apply. One of the rules is that these substances should be effective: it should be demonstrated that the claims made are working. Another principle is that their use should be limited to the minimum necessary.

Eventually, only biocides which show an acceptable risk and sufficient efficacy will be permitted to be used in the EU. Articles treated with substances not authorized in the EU may only be imported until 2017. Triclocarban is meanwhile not allowed to be used any more in the EU as a biocide. Triclosan will be banned as a biocide in the EU in the near future due to lack of efficacy and because of unacceptable risks for the environment. (Both substances are, however, allowed to be used in cosmetics in the EU). Different forms of silver may continue to be used for the time being, as the evaluation of silver is ongoing. But also other substances not examined in this study can be applied to achieve antibacterial effects in textiles and other articles.

What is the purpose of antibacterial treatment?

Textiles, particularly sports textiles, inner soles of shoes or other textiles which are prone to bad smell, are treated with antibacterial substances to avoid unpleasant odours. Smells are built up when sweat and other excretions are metabolised by bacteria. When such odour-causing bacteria are killed or inhibited, they cannot metabolise anymore and the garment or other article does not smell. Producers of anti-odour treated articles sometimes even claim an advantage for the environment, because such textiles need less washing, according to them. Experience shows, however, that the washing behaviour of users is not dependent on whether their textile is treated or not *(4)*.

Usually, anti-odour treatment is not tested directly for whether and how well it works. There is no laboratory test available at the moment which gives an indication of the effectiveness of the anti-odour treatment. To properly test such treatment, odour test-panels with test-persons would have to be applied. This is costly and has also the problem of subjectivity. Therefore, most producers of anti-odour treated articles rely on tests which measure the anti-microbial activity instead. How durable such effects are under normal conditions of use (i.e. regular washing) is usually not taken into consideration. Fear of complaints is low – odour is such a

subjective factor that customers will only in very rare cases complain if their anti-odour treated garment starts smelling.

In an earlier study *(1)*, the Swedish Chemicals Agency could show that the antibacterial substances the garments are treated with are washed out rather quickly. Although only 10 washing cycles were carried out, which is in many cases not even simulating normal use over a year, on average 60 % of the substance had been washed out.

Apart from the purpose of reducing smells, many articles are marketed with much more unclear claims. Labels like “antimicrobial” or “hygienic” imply some kind of health advantage in using these articles. Such claims are not limited to textiles, but all sorts of products can be found with these types of claims. Such unclear claims focus on a general fear of bacteria which is easily generated in the average customer. The less clear the claim, the more difficult it is to test. Usually, it is difficult to judge whether these types of articles have any effect; bacteria can’t be seen and efficacy studies are usually not available. There are indications though that the excessive use of antibacterial agents leads to health problems, such as an increase in allergic reactions to food or substances in the air, instead of fighting them *(7)*. Even the benefits of using antibacterial products in the household do not seem to be evident. In a study where 300 households in Pakistan were encouraged to regular handwashing with soap with and without Triclocarban, no significant difference was found between the two groups in the incidence of different diseases such as pneumonia and diarrhoea (8). In a similar study with 238 households in New York, no significant differences were found for symptoms of viral infectious diseases between groups using antibacterial or non-antibacterial products for general cleaning, laundry and handwashing *(9)*.

When the textiles tested were bought in 2011, hardly any rules existed for making antibacterial or anti-odour claims. Manufacturers of articles could make claims and rely on the fact that there was no control of the properties promised, and that neither the customers would complain. Meanwhile certain requirements have been set up by the EU biocides legislation. Since 1 September 2013, articles which are sold with a biocidal claim (e.g. an antibacterial claim or an anti-odour claim) have to be labelled and information has to be given which biocidal substance they contain and possibly which risks are connected with their use. In this way, manufacturers of treated articles are required to consider their claims and the possible risks with a treatment more carefully. Since then, articles with an anti-bacterial claim have become rarer. But unfortunately, many articles manufacturers and retailers are still unaware of the labelling requirements *(5)*.

But even when an article is properly labelled and information about the treatment is given, requirements for efficacy are still lenient. Only biocidal products like mosquito repellents or wood preservatives have to prove efficacy during the authorisation process. For treated articles, this is not the case. Thus, many claims for anti-bacterial effects or anti-odour effects can never be proven right or wrong because testing is not obligatory.

The present study makes an attempt to show how efficacious antimicrobial treatment of different treated textiles is.

3 Materials and Methods

A total of 30 textile articles were purchased at retailers, with a primary focus on sports and leisure wear. For nearly all articles, some sort of claim such as “anti-odour”, “hygienic” or “antibacterial” were made (cf list of articles in Annex III). In a previous study, the articles had been washed and analysed, before and after washing, for the antibacterial substances triclosan, triclocarban and silver (for details of the analytics see *(1)*). With the exemption of some of the articles containing silver, it was not revealed which substance the textiles were treated with. The analysis for triclosan and triclocarban was an educated guess, as these substances are often used for treating textiles. In 18 of the samples, one of the above mentioned substances were found, in 12 samples none of the three was found; it is unclear, whether they were treated with different substances, or if the claims made were based on other aspects (e.g. “naturally antimicrobial” fibres).

For the present study, articles which did have unclear claims or were none of the three biocides were found, were removed from the study, resulting in 25 samples remaining. The antibacterial activity of these textile samples, unwashed and washed 3 or 10 times, was determined using the OECD Textiles Method *(2)*. Effects against two species, *Staphylococcus aureus* and *Escherichia* coli, which are model organisms for Gram-positive and Gram-negative species, were examined. Growth reduction of a bacterial inoculum placed on the textile samples and incubated for 24 hours was measured. (For a detailed description how the antibacterial activity was determined, see Annex I.)

In a parallel study, the suitability of a laboratory method to test odour reduction was tested with the same samples. For the odour testing, a method under development was employed, the IBRG test for modelling odour-inhibition *(6)*. In this test, the bio-conversion of urea to ammonia by *Proteus vulgaris* was used as the odour model. The less ammonia could be detected, the better the odour-inhibition works according to the model. (For details of the test, see Annex II.)

4 Results

A relevant amount of the samples did not show any activity against one of the species even before washing. Nine out of 25 unwashed samples did not show any antimicrobial activity against *Escherichia coli*, 7 of the unwashed samples (28 %) did not show any activity against both species. After 10 washes however, only 4 of the samples still showed activity against both species, 4 more samples showed activity against *S. aureus* only. (Note: not all samples were tested after 10 washes, samples which already showed poor results after 3 washes were not tested further). In conclusion, altogether only 4 samples (16 %) exhibited a durable effect against both of the test species after washing, 8 samples (32 %) did show an antibacterial effect against one species after washing.

Looking at the different types of treatments, it can be seen that no significant activity was detected against either species in the samples that contained triclosan, but a wide range of effects were observed in the samples that contained silver. Activity was also seen in some samples where none of the three antimicrobial agents were detected. This might be due to the use of other antibacterial substances which were not analysed.

The results of the odour-test were rather confusing. In many of the samples, huge amounts of ammonia were detected, much more than in the reference material, and more than could have been formed from the amount of urea added. It is suspected that this might be attributed to the

presence of nitrogen-containing process auxiliaries and other surface treatments on the finished textiles. These would not have been present on the reference material.

5 Conclusions

The results of the test for antibacterial activity show that these types of treatments for textiles are insufficiently effective. Even though a washing regime of only 10 washing cycles was employed, more than two thirds (68 %) of the tested samples did not show any activity after washing. It is difficult to judge for a consumer, when choosing a textile with an anti-bacterial or anti-odour treatment, whether it will hold what it promises when used and washed normally. The chances are high that it will not.

The results of the odour-testing show that this test cannot be applied at the moment as a model for odour inhibition. Obviously, chemicals present in the textiles interfere with the ammonia production in this test. There does not seem to be an option at present to test these types of claims in the laboratory. Odour-panels would have to be employed to verify claims for odour-reduction or odour-inhibition.

Consequently, it can't be decided at present, whether antibacterial activity is a good replacement for odour testing as a suitable odour-test is not available. Most manufacturers only assume that antibacterial activity is an indication for odour-prevention. But even if the inhibition of bacteria can be demonstrated, does this really prevent clothes from stinking? And is this a more efficient method than washing or weathering?

Antibacterial treatment of textiles which are washed regularly pose a risk to the environment as the antibacterial substances are leached out and reach the environment with the washing water, as was shown in our previous study. But there might even be concerns for the health of people who often come into contact with antibacterial substances. In the U.S., where the use of antibacterial substances is even more common than in Europe, such substances can be regularly found in the blood of the majority of the population (*10*, see e.g. tables for Triclosan and disinfection bi-products). Antibacterial substances can trigger allergies *(7)* and can be toxic for the environment *(11)*.

Considering the doubtful efficacy of such a treatment, is it really worth it to take the chances of possible negative effects for health and the environment? We think it is not. If the risks connected with antibacterial treatment are taken, it should be shown that the desired effect is reached and is lasting even under normal conditions of use, that is regular washing. It should even be demonstrated that a problem exists in the first place and that antibacterial treatment solves the problem. Otherwise, treatment should be avoided.

6 Recommendations for Manufacturers of Articles who consider anti-bacterial treatment

- Define your problem. Is unpleasant smell occurring? Is your article “unhygienic”? In which way? (Normally bacteria do not grow on dry surfaces).
- Ask you biocide provider (provider of a masterbatch) to demonstrate the effect of the treatment *under normal conditions of use* (that is e.g. washing for textiles or occasional wiping for floors). The untreated sample should demonstrate the problem that the treated sample is promising to solve (i.e. the untreated sample smells unpleasant after a certain wearing-time, there is significantly more bacteria to be found on the untreated floor than on the treated floor). The treated sample should show that the problem is solved.
- Consider were your article will be used. Normal household goods do not need any anti-bacterial treatment. Bacteria do not grow on dry surfaces. Even in hospitals, it is often unnecessary to use treated material. It is more important to prevent human-to-human contamination. If you consider treating material for a surrounding which has to be kept cleaner than normal (e.g. a hospital), it should be demonstrated that the treatment has a positive effect on a relevant parameter (eg. infections with MRSA) in a field study. The simple statement that a material is “hygienic” is not sufficient!

7 References

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8 Glossary

Biocidal product

According to the Biocidal Products Regulation ((EU) No 528/2012) biocidal products are " Any substance or mixture, ….. consisting of, containing or generating one or more active substances, with the intention of destroying, deterring, rendering harmless or otherwise exerting a controlling effect on, any harmful organism by any means other than mere physical or mechanical action. …."

Examples include disinfectants, preservatives, antiseptics, pesticides, herbicides, fungicides and insecticides.

Treated article

According to the Biocidal Products Regulation ((EU) No 528/2012) a treated articles is "Any substance, mixture or article which has been treated with, or intentionally incorporates, one or more biocidal products."

and

"A treated article that has a primary biocidal function shall be considered a biocidal product."

Claim

A claim describes roughly what type of organism shall be controlled in a certain situation or use area. Basically, a claim is a promise to solve a problem which occurs through an organism or a consortium of organisms. A claim should be proven by efficacy tests.

Authorisation

Biocidal products have to be authorised before they can be made available on the market. The authorisation is given by the national competent authorities of the different Member States or by the European Chemicals Agency (ECHA). Even the active substances contained in biocidal products have to be approved at EU level. Only active substances and biocidal products which fulfil the requirement of being sufficiently effective, having no unacceptable effects on the target organism, and having no unacceptable effects on the health of humans or on the environment, can be authorised.

Efficacy

The ability of a biocidal product or active substance to exert the desired effect (that is to destroy or control a harmful organism) as it is described in the claim within a given time.

Anti-bacterial treatment

Treatment of an article or a material with a substance which inhibits the growth of or reduces the number of bacteria.

Anti-microbial

Anti-microbial is a more general term and means that a substance inhibits the growth of or reduces the number of microbes such as fungi, bacteria, spores, viruses. However, it does not

mean that a substance necessarily has an effect on all these groups. Usually, it is described in the claim which are the target organisms of a product.

Biocidal products regulation

European legislation which regulates the use and making available on the market of biocidal products.

9 Annex I

Determination of Antibacterial Activity against Staphylococcus aureus and Escherichia coli using the OECD Textile Method

Author: IMSL, Industrial Microbiological Services LTD, Report No IMSL 2013/10/021.3A-1

Test materials

Samples of textile either unwashed, washed 3 times or washed 10 times in detergent were supplied by the Swedish Chemicals Agency. A sample of unfortified fabric (35 % polyester / 65 % cotton) was supplied by IMSL to act as a reference material. All samples were held in the dark at 20°C prior to initial testing. Replicate (6) sub-samples (each weighing 0.4g) were cut from randomly selected areas of each textile for use in this study.

Test Method

Antibacterial activity was determined using the OECD Test Method *(2).* Replicate (3) sub-samples (each 0.4 g) were inoculated with an aliquot (0.2 ml) of a suspension of either *Staphylococcus aureus* or *Escherichia coli* suspended in the 1/500 nutrient broth described in ISO 22196[1]. The samples were then incubated at high humidity (> 95% RH) for 24 hours at 37°C. After this contact interval, the individual sub-samples were transferred to an aliquot of a neutraliser solution validated for silver-based biocides (SCDLP – see[1]). These were then agitated vigorously and the resulting suspension was analysed for the number of colony forming units present by dilution plate count using Trypticase Soya Agar. Plates were incubated for 24 hours at 37°C and then the colonies were counted.

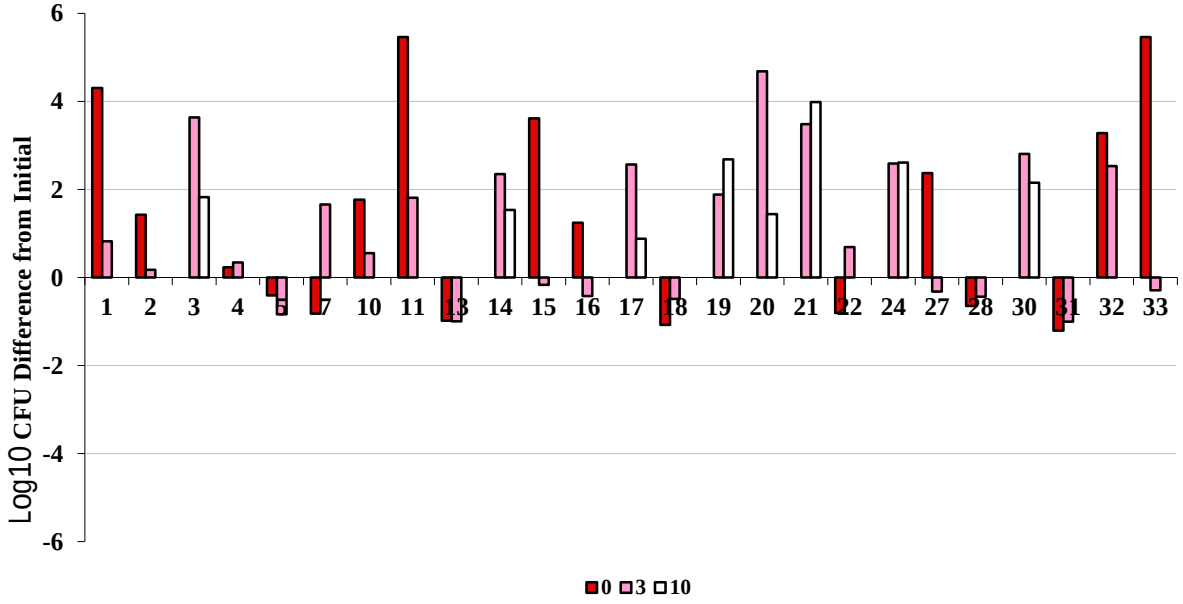
Statistical analysis of data

All data were converted to colony forming units (CFU) cm^{-2} and then transformed (log10) to provide a data set that conformed to a Gaussian distribution. Potential outliers were tested using Dixon's Q-test (P = 0.05). Statistical significance of any effects in the residual data set were detected was tested by analysis of variance (ANOVA, P = 0.05).

[1] ISO 22196:2011 - Measurement of antibacterial activity on plastics and other non-porous surfaces.

Figure 1: Reduction of Populations on Treated Textiles after 0, 3 or 10 washes (Results as Log_{10} CFU cm^{-2} Reduction from the IMSL Fabric Control).

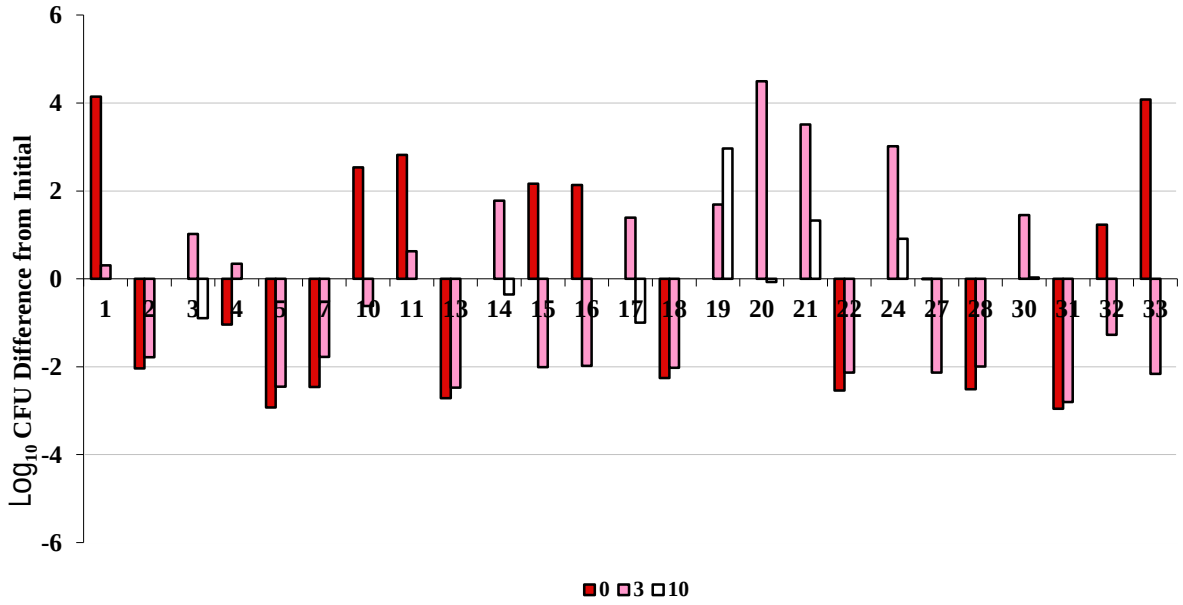
Staphylococcus aureus



Positive values show the presence of antibacterial activity.

Figure 2: Reduction of Populations on Treated Textiles after 0, 3 or 10 washes (Results as Log_{10} CFU cm^{-2} Reduction from the IMSL Fabric Control).

Escherichia coli



Positive values show the presence of antibacterial activity.

Table 1: Results of testing with Staphylococcus aureus and Escherichia coli (Log_{10} difference between the Geometric Mean of 3 Replicates as Colony Forming Units cm^{-2} and the Initial Population).

Sample	Chemical analysis	Claim	Number of Washes Staphyloococcus aureaus			Number of Washes Escherichia coli		
			0	3	10	0	3	10
1	Silver	SilverNODOR (silver yarn)	4.3	0.8		4.1	0.3	
2	Silver	Antibacterial yarn	1.4	0.2		-2.0	-1.8	
3	Silver	Silver		3.6	1.8		1.0	-0.9
4	Triclosan/-Triclocarban	Sanitized	0.2	0.3		-1.0	0.3	
5	--	Antibakterial, anti-odour	-0.4	-0.8		-2.9	-2.5	
7	--	Prevents growth of odour-causing bacteria	-0.8	1.7		-2.5	-1.8	
10	Silver	Odour-resistant	1.8	0.6		2.5	-0.6	
11	Silver	Coolmax Fresh FX, Prevents bad smell	5.5	1.8		2.8	0.6	
13	Silver	Odour-resistant	-1.0	-1.0		-2.7	-2.5	
14	--	HyActive Fabric, Highly odour resistant		2.4	1.5		1.8	-0.4
15	--	Gladiodour, Odour control	3.6	-0.2		2.2	-2.0	
16	Silver	Polygiene active odour control	1.2	-0.4		2.1	-2.0	
17	Silver	Polygiene permanent odour control	0.0	2.6	0.9		1.4	-1.0
18	--	Bamboo charcoal, bacterial resistant, anti-odourization, smell resistant	-1.1	-0.5		-2.3	-2.0	
19	--	Inhibit odour-formation		1.9	2.7		1.7	3.0
20	Silver	Active odour-control, Polygien		4.7	1.4		4.5	0.1
21	Silver	Active odour-control, Polygiene		3.5	4.0		3.5	1.3
22	Silver	Dri-fit	-0.8	0.7		-2.5	-2.1	
24	Silver	Rudolf Silver+Protection		2.6	2.6		3.0	0.9
27	--	Antimicrobial	2.4	-0.3		0	-2.1	

Sample	Chemical analysis	Claim	Number of Washes Staphyloococcus aureaus			Number of Washes Escherichia coli		
			0	3	10	0	3	10
28	Triclosan/-Triclocarban	Antibacterial	-0.6	-0.4		-2.5	-2.0	
30	Silver	Silver		2.8	2.2		1.5	0.0
31	Silver	Naturally antibacterial	-1.2	-1.0		-3.0	-2.8	
32	Silver	47th Element (silver)	3.3	2.5		1.2	-1.3	
33	Silver	Silver	5.5	-0.3		4.1	-2.2	

10 Annex II

Determination of the Anti-Odour Activity of Textiles

Author: IMSL, Industrial Microbiological Services LTD, Report No IMSL 2013/10/021.3B-1

Test Materials

Samples of textile either unwashed, washed 3 times or washed 10 times in detergent were supplied by the Swedish Chemicals Agency. A sample of unfortified polyester-cotton textile (35 % polyester / 65 % cotton) was supplied by IMSL to act as a reference material. Additional samples, used to calibrate the method, were also supplied by IMSL (these had been made under controlled conditions for use in international ring tests) and consisted of treated (silver ion donor) and untreated 100 % polyester textile and treated (silane quaternary ammonium compound) and untreated polyester-cotton textile (35 % polyester / 65 % cotton). All samples were held in the dark at 20°C prior to initial testing. Samples for testing in this study were selected based on the results of the previous study on antibacterial properties *(1)*. Samples presenting a range of effects were selected. Prior to testing, the moisture holding capacity of the textiles was determined. A sample (1 g) of each textile was moistened with distilled water and then allowed to drain. The moisture holding capacity was recorded as the increase in weight expressed as a percentage of the dry weight.

Method

The bio-conversion of urea to ammonia by *Proteus vulgaris* was used as the odour model in this study. Samples of textile were placed into individual, sealable chambers and then inoculated with cells of *Proteus vulgaris* suspended in a solution of urea. The chambers were then sealed and incubated for 24 hours at 28°C. The bio-conversion of urea to ammonia was measured by capturing any ammonia released and then determining its concentration using a colorimetric analytical method.

Preparation of Test Samples

Replicate (3) sub-samples (each weighing 1 g) were cut from randomly selected areas of each of the textiles. Each sub-sample was placed into an air-tight chamber along with an open vial containing sterilised distilled water (5 ml) to act as a ”trap” to capture any ammonia liberated.

Test Microorganism and Inoculum

The microorganism used was *Proteus vulgaris* (NCIMB 4175). It was held as a primary stock culture at 4°C for the duration of the study. One day prior to testing, a secondary subculture was prepared on Trypticase Soya Agar and incubated at 37°C. Immediately prior to use, a cell suspension was prepared by dispersing a number of colonies in sterilised distilled water to achieve a cell density of 1.5 - 5.0 x 10^8 cells ml^{-1} in the final inoculum (extreme care was taken to ensure no media was transferred with the colonies to the diluent). All cell densities were measured using a counting chamber (Thoma $^{1}/_{400}$ mm^2 x 0.02 mm depth) under 400X magnification and phase contrast illumination. Where too low a cell count was achieved, further cells were added and the suspension was re-counted. Where too high a cell count was achieved, the cell suspension was diluted with sterilised distilled water as appropriate to achieve the cell densities specified above. This cell suspension was then diluted to give a target of ca 5 x 10^6 cells ml^{-1} in $^{1}/_{500}$ nutrient broth (see[2]) containing 1% (w/v) urea.

[2] ISO 22196: 2011. Measurement of antibacterial activity on plastics and other nonporous surfaces.

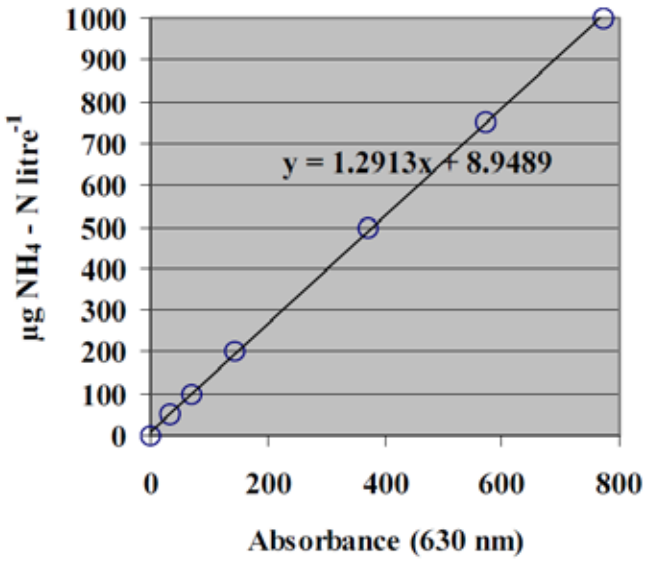
Inoculation and Incubation

An aliquot (equal to 25 % of the moisture holding capacity of the textile under test) of the final cell suspension described above was used to inoculate each of sub-samples. The test samples were then incubated at for 24 hours at 28°C.

Determination of Ammonia

Ammonium-N contained in the water in the trap was determined using the phenol-nitroprusside method[3]. Immediately prior to analysis a range of concentrations 50 - 10,000 µg ammonium-N litre^{-1}) of ammonium sulfate in water were prepared to provide a standard curve for the determination. To a sample (5 ml) from each standard (in duplicate) or "trap", an aliquot (0.2 ml) of phenol-alcohol reagent (10 g phenol in 100 ml ethanol), an aliquot (0.2 ml) of sodium nitroprusside (1 g in 200 ml distilled water) and an aliquot (1 ml) of oxidising reagent (22.79 g trisodium citrate dihyrate and 1 g sodium hydroxide in 100 ml distilled water to which 25 ml 10- 15 % sodium hypochlorite had been added immediately prior to use) was added. The samples were then allowed to stand, in the dark, at room temperature (20-22°C) for 1 hour. The absorbance at 630 nm was then measured using a spectrophotometer. A standard curve was then plotted of absorbance against concentration of ammonium-N and the concentration in the traps was determined by interpolation using the line equation (see Figure 1). All data was converted to mean Fg ammonium-N and then transformed (log_{10}) to provide a data set that conformed better to a Gaussian distribution. The confidence intervals of the means was then calculated.

Figure 1: Example Standard Curve



[3] Solòrzano L (1969), Determination of Ammonia in Natural Waters by the Phenol Hypochlorite Method, J Limnol. Oceanog, 14 (5), 799-801.

Validation of the Method

The method was validated using samples of textile prepared for use in international ring tests of the International Biodeterioration Research Group (IBRG). The textiles were prepared under controlled, non-production conditions to ensure that the correct amount of antimicrobial agent was present and that no interfering auxiliaries were present. The results of the determination of antibacterial activity using the IBRG textile method/draft OECD Tier 1 method as described in Annex I and the results from using the odour inhibition test are shown in Tables 1 and 2.

Table 1: Validation of Method

Sample	Antibacterial Activity (log_{10} CFU* difference between 0 and 24 hours)		Odour[G] [NH_4-N] µg/l
	E. coli	*S. aureus*	
Polyester textile, Untreated	-2.1	0.2	191
Polyester textile, Ag^+ Treated	2.0	3.5	3
Polyester-cotton textile, Untreated	0.6	-0.3	107
Polyester-cotton textile, S-QAC Treated	4.3	4.3	16

Keys: *= Colony forming Units g-1
G= Geometric mean of 3 replicates

Table 2: Confidence Intervals

Log_{10} µg-NH_4-N l^{-1} by sample	n	Mean	95% CI	SE	SD
Polyester textile, Untreated	3	2.228	1.887 to 2.568	0.0792	0.1372
Polyester textile, Ag^+ Treated	3	0.523	-1.177 to 2.224	0.3951	0.6844
Polyester-cotton textile, Untreated	3	2.030	1.860 to 2.200	0.0396	0.0685
Polyester-cotton textile, S-QAC Treated	3	1.190	0.912 to 1.468	0.0646	0.1119

It can be seen from the results above that no activity was detected against the Gram negative species for the Ag+ untreated (the most relevant species to *P vulgaris*) but a very small effect was detected against the Gram Positive one. A small amount of activity was detected against the Gram Negative species for the S-QAC untreated (no activity was detected against the Gram Positive species). This might account for the lower concentration of ammonia evolved from the S-QAC untreated than the other untreated material. The presence of both treatments resulted in a significant reduction in the amount of ammonia evolved.

Results

Sample	Chemical analysis	Claim	Number of Washes		
			0	3	10
IMSL control			355		
1	Silver	SilverNODOR (silver yarn)	**16**	627	
2	Silver	Antibacterial yarn		37	
3	Silver	Silver	**46**		
4	Triclosan/-Triclocarban	Sanitized		279	
5	--	Antibakterial, anti-odour	209		
7	--	Prevents growth of odour-causing bacteria	7795	1061	
10	Silver	Odour-resistant		3060	
11	Silver	Coolmax Fresh FX, Prevents bad smell	105	9734	
13	Silver	Odour-resistant	2657		
14	--	HyActive Fabric, Highly odour resistant		10673	
15	--	Gladiodour, Odour control	7429	5025	
16	Silver	Polygiene active odour control	195	11089	
17	Silver	Polygiene permanent odour control	**25**		2595
18	--	Bamboo charcoal, bacterial resistant, anti-odourization, smell resistant		5956	
20	Silver	Active odour-control, Polygien	131		
22	Silver	Dri-fit	2188	2607	
24	Silver	Rudolf Silver+Protection	1479	2239	14791
27	--	Antimicrobial		6057	
28	Triclosan/-Triclocarban	Antibacterial		900	
30	Silver	Silver	603	23988	7762
31	Silver	Naturally antibacterial		13335	
32	Silver	47th Element (silver)	98		
33	Silver	Silver	**58**	257	1380

Bold figures show a significant effect.

Annex III

Detailed Description of Samples

Picture	Sample No.	Article	Trade mark	Name	Material	Claim/Marketing	Price (skr)	Retailer
	1	Socks	X-socks.	Run sky run	31/14/18/14/12/11 % SkinNODOR/SilverNODOR/ Nylon/Robur/Mythlan/ Elastodiene	SilverNODOR/ (silver yarn)	250	Runners Store
	2	Socks	Asics	Kayano sock. Women's running	57/20/15/5/2/1 % Polyamid/Polyamid Skinlife/Polypropylen/ Polyamid Nanoglide/Elastan/ Polyester Lumen	"antibacterial yarn"	150	Runners Store

Picture	Sample No.	Article	Trade mark	Name	Material	Claim/Marketing	Price (skr)	Retailer
	3	Socks	Falke	Running RU 4 Cushion Women	37/31/24/8 % Polypropylen/Polyamid Silver integrated/Cotton/Wool	Falke silver	190	Runners store
	4	Socks	Funq wear	Stödstrumpor	55/30/15 % Cotton/Polyamid/Elastan	Santized ”ett ÖkoTex-certiferat medel som motverkar odör”	185	Läns-försäkringar

Picture	Sample No.	Article	Trade mark	Name	Material	Claim/Marketing	Price (skr)	Retailer
	5	Socks	BlueWear	Arbetssocka ”antibakteriell” 39/42 4-pk	71/14/10/3/2 % Cotton/Polyester/Nylon/ Elastodien/Elastan	“Purista är en antibakteriell behandling som motverkar dålig lukt”	99	Jula
	7	Socks	Under Armour	Allsport	88/10/1/1 % Olefin/Nylon/Polyester/ Spandex	ArmourBlock –prevent growth of odor-causing bacteria in the sock	99	Stadium

Picture	Sample No.	Article	Trade mark	Name	Material	Claim/Marketing	Price (skr)	Retailer
	10	Underwear	Craft	Craft Layer 1 Zero. Zero Extreme wmn zip turtleneck	100 % Polyester	“luktresistent”	500	Intersport
	11	Underwear	Craft	Pro Zero. Women PZ Extreme long underpant	100 % Polyester	Coolmax Fresh FX i plaggen förhindrar dålig lukt	400	Intersport

Picture	Sample No.	Article	Trade mark	Name	Material	Claim/Marketing	Price (skr)	Retailer
	13	Underwear (children)	Isbjörn of Sweden	Ulltröja Thin Kiddie Roundneck	40/57/3 % Wool/Polyester/Elastan	”Odour resistance” [4]	349	Uteungar.se
	14	Underwear (children)	The North Face	Tekniskt underställ	100 % Polypropylen	HyActive fabric. “Highly odor resistant”	599	Uteungar.se

[4] Isbjörn of Sweden has communicated that they never marketed their apparel with a claim of odour-resistance, and that the claim made by their distributor (uteungar.se) was not agreed with them. They don’t work together with the distributor any more. They claim that they do not either treat their apparel with anti-bacterial substances and that the amount of silver found is due to contamination of another textile production. The results of the analysis of silver of our earlier study show indeed only very small amounts which could be due to contamination.

Picture	Sample No.	Article	Trade mark	Name	Material	Claim/Marketing	Price (skr)	Retailer
	15	Underwear	Patagonia	M's Cap 2 LW Zip Neck	46/54 % Polyester/ Recycled Polyester	Gladiodor odor control	569	Addnature.com
	16	Underwear (children)	Peak Performance	Jr base LS T	86/14 % Polyester/Elastan	Polygiene Active Odor Control	400	NK

Picture	Sample No.	Article	Trade mark	Name	Material	Claim/Marketing	Price (skr)	Retailer
	17	T-shirt	Haglöfs	Actives Cool Roundneck	100 % Recycled Polyester	Polygiene. Permanent odor control	475	Addnature.com
	18	T-shirt	Newline	Ionic carbon shirt wmns	100 % Polyester	Bamboo charcoal Bacterial resistant, anti-odourization, smell resistent	500	Runners store

Picture	Sample No.	Article	Trade mark	Name	Material	Claim/Marketing	Price (skr)	Retailer
	19	Underwear	Kari Traa	W's Butterfly hipster + top	95/5 % Polyamid/Spandex	"inhibit odour formation"	499	Addnature.com
	20	Buff	Buff	Kukuxumusu. Junior Size (fåret Shaun)	100 % Polyester Microfiber	"Active odor Control" Polygiene (silver)	179	Team Sportia

Picture	Sample No.	Article	Trade mark	Name	Material	Claim/Marketing	Price (skr)	Retailer
	21	Buff	Disney Baby Buff	”Winnie the Pooh”	100 % Polyester Microfiber	”Active odor Control” Polygiene (silver)	119	XXL Sport & Vildmark
	22	Trainings T-shirt (children)	Nike	Nike Miler Running, Boys 152-158 cm	100 % Polyester	Dri-fit	199	XXL Sport & Vildmark

Picture	Sample No.	Article	Trade mark	Name	Material	Claim/Marketing	Price (skr)	Retailer
	24	Trainings T-shirt	Crivit sports	Running top/löpartröja	48/47/5 % Polyester/Polyester 4 Chanel/Elastan	Rudolf Silver+ Protection (silverplus-material)	79.90	Liedl
	27	Tennis headband	Adidas	Adidas CR Tennis HB, headwear	37/36/17/6/4 % Wool/Acryl/Polyamid/ Polyester/Elastodien	“Antimicrobial”	80	Intersport

Picture	Sample No.	Article	Trade mark	Name	Material	Claim/Marketing	Price (skr)	Retailer
	28	Hockey Pants (children)	Bauer	Core compression Jock short. Junior	KROPP: 83/17 % Polyester/Elastan MASKA: 90/10 % Polyester/Spandex	“Antibacterial”	300	Intersport
	30	Dish-cloth	Klin-tec	-	80/20 % Polyester/Polyamid	Silver		Prov från tillverkare

Picture	Sample No.	Article	Trade mark	Name	Material	Claim/Marketing	Price (skr)	Retailer
	31	Underwear	Houdini	Womens's Airborn Crew	75/25 % Wool/Silke	"Naturligt antibakteriell"	899	Naturkompaniet
	32	Body (children)	Eiser trikå	Comfymitt Body with Hood	100 % Cotton	47th Element (silver)	159 kr	Eiser Trikå

Picture	Sample No.	Article	Trade mark	Name	Material	Claim/Marketing	Price (skr)	Retailer
	33	Sleepwear (children)	Eiser trikå	PJ Bottoms + Top Sweetheart	100 % Cotton	Silver	149 kr	Eiser Trikå

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