曝露防止対策等を含めた管理方法を考慮していく必要がある。

欧州バイオサイド指令では抗菌剤としての使用を継続する物質については届け出が必要となり、本検討に用いた物質のうち、木材防腐剤の用途ではNジメチル-N'-フェニル-N"-(フルオロジクロロメチルチオ)スルファミド (DMPFS)、TPN、IPBCが、殺虫剤及び殺ダニ剤としてIPBCが、防汚剤としてDMPFS、TPN、TCMTBTが既存活性物質としての再審査を受ける対象となって2006年までにデータを提出することとなっている。イソボルニルチオシアノアセテート(IBTA)、FPI、TCMSPは既存活性物質の再調査計画への届け出がされなかった。

以下に、分担研究者によって提出された上記文献で不足しているデータと再評価結果をまとめた。

変異原性試験としては、細菌を用いる突然変異試験、哺乳類細胞を用いる染色体異常試験、及びマウスリンフォーマTK試験があるが、細菌の試験と細胞の試験では違う結果も得られており、抗菌剤についてはその性質上、哺乳類細胞を用いる遺伝子突然変異を検出する試験の方が正確な評価ができると思われた。バイオサイド指令の申請既存活性物質については、いずれも陽性と判定されている。

GPMT法では18種の抗菌剤に皮膚感作性が認められている。また、非放射性マウスリンパ節増殖法(non-RI LLNA法)ではGPMT陽性の17種中14種の感作性を検出した。GPMT法ではBNPDの感作率は低いものの、他はいずれも感作率が高かった。最低誘導濃度から皮膚感作性の強度を順位づけた。最低誘導濃度が0.1%未満と低いものはTPN、メチレンビス(チオシアネート)(MBTC)、IPBC、TCMSP、CPIP及びTCMTBTであり、極めて強い皮膚感作性を有すると判定されている。

9種の抗菌剤について生殖発生毒性に関して催奇形性を評価した結果、ZPTにのみラット胎児に骨格奇形を誘発する可能性が示唆された。妊娠動物及び胎児に対する無毒性量(NOAEL)はZPTが最も低く2.7 mg/kgで、PCMAは最も高く300 mg/kgであった。内分泌かく乱物質のスクリーニング法であるヒトエストロゲンレセプター(ER)結合試験と組換え酵母を使用したYES試験を行った試験データでは、ER結合試験で20種のうち12種が、YES試験で1種が陽性となった。特に、YES試験で陽性となったp-クロロ-m-クレゾール(PCMC)には更なる評価が必要としている。

抗菌加工製品の市場調査の結果、著しく抗菌化率が拡大している製品分野は、肌着、下着であり、包丁、冷蔵庫、便器及びふきんも伸びている。繊維製品には有機系抗菌剤としてはヒノキチールやヒバ油、無機系では銀、ゼオライトでの加工が確認されている。壁紙やじゅうたん、及びそれらに使用される塗料や接着剤等にはFPI、DMPFS、TPN、IPBC、CPIP及びBECDIPが、木材防腐剤にはBECDIP、IPBC、CPIP等が検出されている。特に欧州では、DMPFS及びTPNは木材防腐剤及び防汚剤として、IPBCは木材防腐剤、殺虫剤、殺ダニ剤として、TCMTBTは防汚剤として継続使用するための申請がされ、評価のために早急な審査書類の提出が求められている。こうした抗菌剤については、我が国でも安全性評価の優先順位を高くする必要があると考えられる。

今回実施した消費者でのアンケート調査、市販製品における製品表示、MSDSの実態調査の結果から、抗菌加工製品では、ACD等の慢性的な健康被害に関して、製品表示、MSDSが消費者への製品情報の伝達手段として十分に生かされていない現状が確認できた。

抗菌剤について、具体的な健康被害の事

例が報告されていないとしても、要注意である。すなわち、抗菌・防臭、防ダニ、防虫、防カビ等の加工剤として使用されている化学物質には、農業、工業、医療等の他の分野において農薬や殺菌剤として使用されているものや、それらと構造的に非常に類似しているものがある。したがって、皮膚感作性とともに、変異原性、生殖・発生毒性、内分泌かく乱性、神経毒性等についても十分注目していく必要がある。

消費者が抗菌加工製品を安全に使用できるかどうかを評価するためには、抗菌加工製品に使用されている抗菌剤が、どのような毒性（ハザード）を有しているか、どのような経路で、どのくらいの量が体内に取り込まれる可能性があるか等を明らかにする必要がある。すなわち、抗菌加工製品の安全性評価のためには、抗菌剤の毒性（ハザード）だけでなく、抗菌剤への曝露の実態に即したリスクの程度を予測する必要がある。

すなわち、①抗菌剤の毒性試験データをもとに、抗菌剤の毒性の強度を確認する、②抗菌剤の加工濃度、抗菌加工製品を用いた溶出試験（溶出溶媒としてヘキサン、20%エタノール、人工汗、人工唾液等を使用する）の結果をもとに、どのくらいの量の抗菌剤が抗菌加工製品から、汗・唾液等を介して経皮ルート、経口ルート、あるいは室内空気中に揮散して経呼吸器ルートを通じて体内に移行していくかを予測する、③抗菌加工製品の用途、製品のサイズ、使用時間、使用頻度を考慮しながら、経皮ルート、経口ルート、経呼吸器ルートを通じたヒトへの曝露量を予測し、抗菌剤への曝露に伴うリスクの大きさを算出する。

抗菌加工製品の有害性情報に関して、①健康被害の原因究明（原因製品と原因化学物質の関連性を明らかにすること）、②MSDSの充実（労働衛生上の健康被害の発生防止のために、抗菌剤メーカーから中間・最終製品メーカーへ、用途、曝露ルート・曝露レベルを考慮したリスク評価も含めた有害性情報等の製品情報を伝達できること）、③健康被害の事例情報（種類、重症度）を具体的に記載する等、消費者にも理解しやすい製品表示を通じて、製品情報の伝達機能を質量ともに高めていくとともに、製品表示、業界・メーカーのホームページ等を通じて幅広く製品情報を公開して、消費者の理解度を高めていくことが重要である。

特に、皮膚バリア・代謝機能等が完成していない乳幼児、皮膚バリア・代謝機能等が低下してくる高齢者、特に化学物質への感受性が特に高くなっているアレルギー患者・化学物質過敏症患者等への影響を考慮しつつ、抗菌剤・抗菌加工製品の安全性評価を厳密に実施する必要がある。

今後、平成15-17年度における調査結果をもとに、抗菌剤の毒性（ハザード）評価とともに、抗菌加工製品からの抗菌剤の溶出に伴う曝露評価について、汎用される抗菌剤のタイプ、製品の材質、用途に沿って抗菌加工製品を用いた溶出試験法をさらに改良し、評価法としての精度を向上させることによって、「抗菌加工製品に関する曝露評価ガイドライン」、「抗菌加工製品に関する安全確保マニュアル作成の手引き」の作成に向けた取り組みを効率的に進めていくことに資することができる。

最後に、(独)製品評価技術基盤機構・化学物質管理センターによるバイオサイド検討委員会における資料等の参照・引用許可申し入れを快諾いただいた、(独)製品評価技術基盤機構・化学物質管理センター・重倉光彦所長及び資料作成者の鈴木裕氏に深謝致します。

E　健康危害情報

なし

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G　知的所有権の取得状況

なし

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Photolysis and Antimicrobial Activity of Hinokitiol in Antimicrobial/ Deodorant Processed Textiles

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Abstract: The authors have developed an analytical method for hinokitiol using HPLC to determine its content in various textiles treated with it on the market and reported that hinokitiol was not detected in all of the textiles tested. Aiming to clarify the cause for the absence of hinokitiol, hiba oil, synthetic hinokitiol and hinoki leaf oil were microcapsulated and the sample fabrics were prepared by treating with each microcapsulated preparation. Then, the survival rate of hinokitiol in the treated fabric was determined after a light fastness test using a xenon-ark lamp. The results indicated that hinokitiol was much liable to photolysis. The antimicrobial activity of the treated fabrics with synthetic hinokitiol on *S. aureus* was higher after the light exposure during the test than before, suggesting the photolysis products of hinokitiol have higher antimicrobial activity than hinokitiol itself.

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1. Introduction

Hinokitiol (beta-thujaplicin, 2-hydoxy-4-isopropyl-2, 4, 6-cycloheptatrien-1-one) is a natural antimicrobial substance discovered in acid oil extract from *Japanese cypress* such as *Taiwan Cupressus, Aomori Thujapsis, Western Redcedar* [1, 2]. Since hinokitiol has antimicrobial effects, it has been synthesized for industrial uses and utilized as a compounding agent for toothpaste, hair lotion, etc [3]. Hinokitiol has been also used as an approved food preservative [4]. Moreover, fabrics treated with hinokitiol, which is a natural antimicrobial ingredient friendly to the human body, are increasing recently [5, 6]. However, its contents in these fabrics on the market are unknown. We have developed a method using HPLC to analyze hinokitiol in fabrics [7], and analyzed of commercially available textiles marked "treated with hinokitiol or hiba oil". However, hinokitiol was not detectable in all tested fabrics [7]. This suggests that hinokitiol might have already disappeared in the textiles put on the market because of its properties; liable to photolysis, sublimating, complex forming activity with metals, etc. In order to clarify the cause of its disappearance, the JIS light fastness test with a xenon-ark lamp was performed for each standard fabric treated with microcapsulated hiba oil, synthetic hinokitiol or hinoki leaf extract, and time-course changes in the amount of hinokitiol remaining in those fabrics were investigated. In addition, an antimicrobial activity test was conducted with these fabrics to investigate the correlation between the antimicrobial activity and the survival rate of hinokitiol.

2. Materials and Methods

2.1 Preparation of treated fabric samples

Hiba oil extracted from the trunk and root of the hiba tree (1), synthetic hinokitiol dissolved in mineral oil (2), hinoki leaf oil obtained through steam distillation of hinoki leaves (3) and mineral oil alone as the control (4) were used for the treatment of fabric. Four kinds of fabrics treated with antimicrobial preparations were thus prepared. Hiba oil microcapsulated was obtained from Environmental Science Development Co., Ltd., and (2), (3) and (4) were used after microcapsulation in our laboratory.

2.1.1 Hiba oil treatment for cotton fabric

A stock solution for treatment of fabric was prepared by dispersing microcapsules filled with hiba oil and a resin binder, and diluting with water. After soaking a white cotton fabric into the solution, the mangle expression was adjusted to 100% (weight ratio of fabric to solution was 1.0). The

fabric was dried for one hour using a hot blast baking machine set to 100℃ and each microcapsulated preparation was fixed to the fabric surface. In the present study, 2%-treated fabric was made using a 50-fold diluted solution. The fabric was stored in shade immediately after treating. The following reagents and equipment were used :

Hiba oil : Environmental Science Development Co., Ltd.

Microcapsules filled with Hiba oil: Environmental Science Development Co., Ltd.

Stock solution for fabric treatment (coating agent) : suspension of microcapsules filled with hiba oil and resin binder in water at a ratio of 2 : 8 : 90 (produced by Environmental Science Development Co., Ltd.)

White cotton fabric for treatment: Kanakin No.3, Japanese Standards Association for JIS TEST methods for color fastness to xenon arc lamp light (according to JIS L 0803 : 1998)

Mangle : electric, compressor type mangle (Tsuji Senki Kogyo Co., Ltd.)

Baking test instrument (Daiei Kagakuseiki Mfg. Co., Ltd.)

2.1.2 Synthetic hinokitiol treatment for cotton fabric

Hinokitiol 1. 6 g was dissolved in 80 g of mineral oil and 46 g of a dispersing agent was added. A mixture of 400 g of 13% wall material reagent and 0.5 g of NaOH was further added to the hinokitiol solution and emulsification was conducted by stirring at 1,200 rpm for one hour using a homomixer (Tokushu Kika Co., Ltd.) at a temperature ranging from 20 to 30℃. Granular size was macroscopically estimated by microscopy. Then, 3.0 g of a hardener and NaOH solution (1.5 g of NaOH dissolved in 40 ml of water) was added to the emulsion and allowed to stand for 1～2 hours after raising the temperature to 40℃ to stimulate the hardening of wall-constructing materials. This emulsion was 5-fold diluted with a spreader containing an adsorbent at 5% and a fixing agent at 1% and used as a test solution. White cotton fabric was immersed in the test solution as described in 2.1.1 and the mangle expression was adjusted to 100%. The fabric was dried up to fix the microcapsules on it. The reagents used were as follows :

Hinokitiol : hinokitiol SP (Takasago Perfume Co., Ltd.)

Mineral oil : Nisseki Hisol SAS 296 (Petroleum Group 3) produced by Nippon Petrochemicals Co., Ltd.

Capsular wall constructing material: Gohsenol GL-05R (The Nippon Synthetic Chemical Industry Co., Ltd.)

Sodium hydroxide : 1st grade reagent of Wako Pure Chemical. Co., Ltd.

Dispersing agent: Sumijule N-75S (Sumitomo Bayer Urethane Co., Ltd.)

Hardener: Epikure U, denatured lipopolyamine and epoxy curing agent (Japan Epoxy Resins Co., Ltd.)

Adsorbent : Lozenon 102

Fixing agent : Nikko Softener CW (Nikko Giken Co., Ltd.)

2.1.3 Hinoki leaf oil treatment for cotton fabric

Emulsification of hinoki leaf oil (Biocol oil produced by Biobicol Co., Ltd) was conducted as mentioned in 2. 1. 2. After adding the spreader consisting of an adsorbent and a fixer to the emulsion, a strip of cotton fabric was treated with the emulsion.

2.1.4 Mineral oil treatment (as the control treatment) for cotton fabric

The control fabric was made in order to examine the background of the antimicrobial effect by applied reagents except for the hinokitiol, etc. Mineral oil without the addition of hinokitiol was emulsified and a strip of cotton fabric was treated with the emulsion as mentioned above.

2.2 JIS light fastness test using xenon arc lamp (7.5kW, water-cooling, direct irradiation method)

A high energy, xenon long-life weather meter SC-750 (Suga Test Instruments Co., Ltd.) was used according to test methods for color fastness using a xenon arc lamp light JIS L 0843-1998 (the same standardized procedures as ISO 105-B02). Each processed fabric was exposed to the lamp for 30 min, 1 hour or 10 hours under the following conditions : black panel temperature 63℃, humidity 50%, no raining and low energy density.

2.3 Procedures for hinokitiol analysis

A 5-gram strip of treated fabric was put into a 500 ml round-bottomed flask and 250 ml of methanol was poured in. Then, extraction was conducted at 70℃ for one hour by refluxing. Glassware was washed with 100 ml of methanol and the washings were joined to the extract. The mixed solution was concentrated to 5 ml and transferred to a graduated 10 ml test tube. The solution was further concentrated to 2 ml by blowing highly pure argon gas into the tube and used as the test sample after filtration. When its concentration was too high, the solution was injected to HPLC after dilution. Glassware was washed out with EDTA solution and all experimental runs were carried out under light-shielded conditions. The instruments used and experimental conditions were as follows :

HLPC: Hewlett Packard Instrument series HP1100 (pump, low-pressure gradient system, column incubator,

auto injector and photodiode array detector)

Data processor : Hewlett Packard Chemstation

Column : L-column ODS (ϕ4.6 mm×150 mm) produced by Chemicals Evaluation & Research Institute, Japan

Injection volume : 10 μl

Column temperature : 40℃

Flow rate of mobile phase : 1 ml/min

Mobile phase : acetonitrile/20 mM potassium dihydrogenphosphate (30 : 70),

Wave length for measurement : 240 nm and 320 nm

2. 4 Antimicrobial activity test

Antimicrobial activity was assayed by the new agar plate method (NAP method). *Staphylococcus aureus* IFO 12732, a gram-positive bacterium, and *Klebsiella pneumoniae* ATCC 4352, a gram-negative bacterium, were used for testing. Of each sample fabric, three strips of 0.1 g were put into a bottle and sterilized at 120℃ for 15 min. An aliquot of overnight-incubated culture with nutrient broth (Difco) was 100-fold diluted followed shaking incubation for 3 hours. The cells were harvested from the culture by refrigerated centrifuge. After washing the cells with cooled buffered saline, the cells were suspended with 1% trypton solution to a concentration of 10^5 cells/ml.

Agar powder Nakarai TESDQ (Nakarai Pharmaceutical Co., Ltd.) was added to the buffered saline to the concentration of 1.5% and dissolved by heating. Agar plate was prepared with the agar solution sterilized using autoclave. A strip of test fabric was put on the agar plate and 0.1 ml of the cell suspension was spread on it, followed by incubation at 37℃ for 20 hours. Sterilized buffered saline containing 0.2% Tween 80 (polyoxyethylene sorbitan monooleate), a nonionic detergent was used for washing out the cells from the test fabric. A test fabric was immersed into 10 ml of Tween 80 containing buffered saline and stirred twice for 5 sec by flash mixer. After serial dilution of the washing with the buffered saline, each diluted solution was used for determination of viable cell number on the agar plate.

Each three strips of treated and untreated fabrics were used to determine the mean number of cells for assessment of antimicrobial activity. The mean number of cells recovered from the two strips of untreated fabric immediately after spreading the cell suspension (0.1 ml) on the agar plate was used as the number of inoculated cells. The antimicrobial activities were estimated based on the following formula :

$$SA\ (\%) = \{(b-c)/(b-a)\} \times 100 \quad \cdots\cdots [1]$$

$$CA\ (\%) = \{(a-c)/a\} \times 100 \quad \cdots\cdots [2]$$

a : the number of inoculated cells

b : the number of cells recovered from untreated fabric after incubation

c : the number of cells recovered from treated fabric after incubation

When there exist relations of a<c<b and a>c, the activities were defined as bacteriostatic activity (SA) and bactericidal activity (CA), respectively.

3. Results and Discussion

3.1 Hinokitiol analysis for treated fabric

The content of hinokitiol was determined based on UV absorption at 240 nm and 320 nm using the HPLC equipped with photodiode array detector [7]. Four objects were prepared (10 μg and 100 μg of hinokitiol and hiba oil containing 10 μg and 100 μg of hinokitiol), and each was added to a 500 ml flask containing 250 ml of methanol for recovery. When analysis was made as described above, the recovery of hinokitiol was 100% in either case and the reproducibility was also good. But the recovery by our analytical method for hinokitiol in antimicrobial/deodorant processed textiles [7]was 10～14% for addition of 10 μg and 40～55% for that of 100 μg and the reproducibility was also not satisfactory. However, the hinokitiol lost during the analysis was found to be very little. So, we conducted quantitative analysis of hinokitiol according to this method.

First, determination of hinokitiol was made with fabrics stored with shielding from immediately after three different treatments : 1)hiba-oil treatment, 2)synthetic hinokitiol treatment and 3) hinoki-leaf oil treatment. Determination was made with each three strips. Hinokitiol 5.4 μg/g and 45.7 μg/g were detected in the hiba oil treated fabric and synthetic hinokitiol treated fabric, respectively, whereas it was not detected in the fabric treated with hiba leaf oil. These results would be attributed to the fact that hinokitiol is included in the root and trunk but not in the leaves.

The hinokitiol contents were determined with each three strips of fabric after color fastness test using the xenon arc lamp for 30 min. Hinokitiol was not detected in hiba-oil treated fabric and its content in the synthetic hinokitiol treated fabric was markedly reduced from 45.7 to 0.4 μg/g after the light fastness test. Moreover, hinokitiol was not detected in all samples exposed with the xenon arc lamp for 1 hour and 10 hours in the light fastness test. The hinokitiol contents in these fabrics are shown in Table 1.

Previously, we reported that five kinds of textiles on the market indicating hinokitiol treatment were analyzed, but hinokitiol was not detectable in any of them. So, we thought

Table 1 Analytical Results of Hinokitiol Residual Amounts in Processed Fabric after Photoirradiation

Photoirradiation time (Xenon lamp)	0 min	30 min	1 hour	10 hours
Hiba Oil	5.4 μg/g	N.D.	N.D.	N.D.
Synthetic Hinokitiol	45.7 μg/g	0.4 μg/g	N.D.	N.D.
Hinoki Leaf Oil	N.D.	N.D.	N.D.	N.D.

N.D. : Not detected

that hinokitiol had disappeared from those textiles through sublimation, photolysis, complex formation, etc. Therefore, we had some doubt about the indication that the product has antimicrobial activity because of hinokitiol treatment [7]. The present study demonstrated that hinokitiol was easily lost from the treated fabrics through photolysis and sublimation. Since the temperature in the instrument for the light fastness test (high energy xenon long-life weather meter) was 29℃, which was much lower than the temperature (100℃) for adhering the fabric with those microcapsules, loss of hinokitiol due to sublimation was not considered. The light exposure for 20 hours under the present conditions for the light fastness test seemed to be corresponding to natural exposure due to sunlight in the daytime (ca. 10 hours). So, light exposure with the xenon arc lamp for 10 hours seemed to correspond to sunlight exposure for nearly half of daytime (5 hours). The fact that most hinokitiol in the treated fabrics disappeared after light exposure for 30 min shows the degree of fastness was very low for hinokitiol. The present results agree well with the previous data about hinokitiol-treated textiles on the market [7].

3. 2 Evaluation of antimicrobial activity

3.2.1 Evaluation of antimicrobial activities on *Staphylococcus aureus* (Table 2)

Bacteriostatic activity (SA) was 99% only for the synthetic hinokitiol treated fabric among the test fabrics after storage with shielding (not exposed to the xenon arc lamp) and antimicrobial activity of the others was not significant. Then, an antimicrobial test was conducted with fabrics after the light fastness test for 30 min and 10 hours with the xenon arc lamp. The antimicrobial activity of the fabrics treated with synthetic hinokitiol after the test for 30 min was similar to that of the untested one. The activity of the treated fabrics was increased after the test for 10 hours to bactericidal activity (CA 91%). Although there remained little hinokitiol in the fabric after exposing to the lamp, antimicrobial activity was rather increased after the exposure, whereas for the control fabric treated with mineral oil alone, antimicrobial activity was not detectable even after the exposure, indicating that the increase of activity was not due to the used agents except for hinokitiol.

Table 2 Antimicrobial Activity Evaluation for Processed Fabrics to *S. aureus* after Photoirradiation

Photoirradiation time (Xenon lamp)	0 min	30 min	10 hours
Hiba Oil	0	0	0
Synthetic Hinokitiol	SA 99%	SA 99%	CA 91%
Hinoki Leaf Oil	0	SA 17%	0
The Mineral Oil (Control)	0	0	0

CA : bactericidal activity SA : bacteriostatic activity

There were no changes in antimicrobial effects of the fabrics treated with the other two preparations, i.e., those fabrics had no antimicrobial activity. Therefore, it was thought that the antimicrobial activities of ingredients other than hinokitiol in hiba oil and hinoki leaf oil would be low.

3.2.2 Evaluation of antimicrobial activities on *Klebsiella pneumoniae* (Table 3)

All of the treated fabrics stored under shielding (not exposed to the xenon arc lamp) showed bacteriostatic activity to *K. pneumoniae* (SA 55, 39, 71 and 23%), while the control fabric (treated with mineral oil containing no hinokitiol) was also bacteriostatic to the bacteria. Though bacteriostatic activity was detected in both fabrics treated with hiba oil and hinoki oil after the light fastness test using the xenon arc lamp, the activity was not detectable in the fabrics treated with synthetic hinokitiol as well as the control one. Since reproducibility of the bacteriostatic activity value which shows lower antimicrobial activity was not so satisfactory, it was difficult to draw a conclusion, but there is a possibility that some ingredients of Hiba oil and Hinoki leaf oil might have antimicrobial effects on *K. pneumoniae*.

Table 3 Antimicrobial Activity Evaluation for Processed Fabrics to *K. pneumoniae* after Photoirradiation

Photoirradiation time (Xenon lamp)	0 min	30 min	10 hours
Hiba Oil	SA 55%	SA 33%	SA 63%
Synthetic Hinokitiol	SA 39%	0	0
Hinoki Leaf Oil	SA 71%	SA 29%	SA 67%
The Mineral Oil (Control)	SA 23%	0	0

SA : bacteriostatic activity

3.2.3 Evaluation of antimicrobial treated textiles on the market

We have reported that antimicrobial efficacies were examined with 20 kinds of antimicrobial/deodorant textiles with labels indicating treatment with natural ingredients such as hinokitiol, kitosan, etc. and antimicrobial effects of those textiles were less than those treated with inorganic and organic chemical agents [8]. In the present study, antimicrobial effects of 5 different textiles on the market in

Table 4 Antimicrobial Activity Evaluation for Marketing Antimicrobial/Deodorant Processed Textile Products (There was a label, when these products were processed using hinokitiol)

Sample	Material	Labelling	SEK mark*	*S. aureus*	*K. pneumoniae*
1 Underwear (for babies)	Cotton	Antimicrobial/insectcidal processing, Hinokitiol	Not indicated	SA 42%	SA 28%
2 Socks	Cotton, Acryl Nylon, Polyurethane	Antimicrobial/deodorant processing, Hinokitiol	Not indicated	SA 99%	SA 60%
3 Toilet seat cover	Acryl 85% Nylon 15%	Antimicrobial/deodorant processing, Hinokitiol	Indicated	CA 100%	SA 35%
4 Dish towel	Cotton	Antimicrobial/deodorant processing, Hinokitiol	Indicated	SA 39%	SA 49%
5 Bedding cover	Cotton	Antimicrobial/insectcidal processing, Hiba oil	Indicated	0	0

CA : bactericidal activity SA : bacteriostatic activity

*SEK mark : A designated label used with permission of Japanese Association for the Function Evaluation of Textiles (JAFET).

which hinokitiol had been reported to be undetectable in spite of the indication [7] were further evaluated by the NAP method. One of those products, a textile for a toilet seat cover, showed a strong antimicrobial activity (CA 100%) to *S. aureus* as shown in Table 4. There was no indication of an antimicrobial agent on its label other than hinokitiol. Although the causal factor of such strong antimicrobial activity was unclear, some agent used or photolysis products of hinokitiol might be involved in the strong activity.

3.3 Conclusions

The present study was aimed to clarify why hinokitiol was not detected in five textiles despite being labeled "treated with hinokitiol" [7], and it was demonstrated that hinokitiol in the treated fabrics rapidly disappears through photolysis. Therefore, it is assumed that by the time they appear on the market, textiles treated with hinokitiol would have lost their hinokitiol. Most hinokitiol-treated textiles on the market are now advertised as valuable products with antimicrobial activity due to the treatment with hinokitiol, but such activity is not always due to hinokitiol.

Antimicrobial activity on *S. aureus* was detected in the fabric containing hinokitiol but the activity was higher after photolysis, suggesting that photolysis products of hinokitiol would have antimicrobial activity higher than that of hinokitiol itself. Antimicrobial activity of hinokitiol treated fabrics was estimated based on the data from 5 repeated assays after light exposure. The treated fabric showed strong antimicrobial activity after exposure to light, and its reproducibility was satisfactorily high. Moreover, it has been reported [9] that when antimicrobial effects of the fabrics treated by the procedures of this experiment were evaluated on three different bacteria, O-157, MRSA and *S. aureus* using the SEK standard test method, an antimicrobial activity evaluation method defined by the Japanese Association for the Function Evaluation of Textiles (JAFET), the antimicrobial effects of the fabrics treated with hinokitiol continued or increased after the light exposure. It seemed that the antimicrobial activity of other ingredients of hiba oil and hinoki leaf oil might be weak on these test bacteria.

Weak antimicrobial activity (bacteriostatic activity, SA) on *K. pneumoniae* was detected even in the fabrics including no hinokitiol. It is assumed that some other ingredients of hiba oil and hinoki leaf oil would have antimicrobial activity. This was also inferred from the fact that antimicrobial activity in fabrics treated with these oil preparations would be little changed after irradiation. In contrast to the case of *S. aureus*, the photolysis products of hinokitiol had no antimicrobial activity to *K. pneumoniae*.

These results indicated that hinokitiol is liable to photolysis and its antimicrobial activity increased depending on the degree of photolysis and the kind of bacteria. There are many reports on antimicrobial activity of hinokitiol [10-14] and its toxicity [15-19]. Hinokitiol has been approved as an antimicrobial food additive [4]. However, there is a report [20] showing that hinokitiol in food is rapidly decomposed by photolysis. There is no report on the correlation between photolysis products and antimicrobial activities, and also on the increase of antimicrobial activity by photolysis as far as we know. The mechanism of the expression of antimicrobial activity and toxicity by hinokitiol should be clarified in detail including the photolysis product as a future problem. Identification of photolysis products and estimation of their antimicrobial effects are underway at present.

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Analysis of Thujopsene in Antimicrobial/Deodorant Processed Textiles as an Index of Hiba Oil

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Abstract: The use of hiba oil (cypress oil) as a safe and natural antimicrobial agent for textiles is on the rise. For investigating the hiba oil content of commercially available textiles, the authors developed an analytical method using thujopsene, the main ingredient of hiba oil, as an index. First, standard fabrics were prepared by fixing microcapsules filled with hiba oil. Using these fabrics, detection of thujopsene by gas chromatography mass spectrometry (GC/MS), extraction of thujopsene from textiles, and purification of the extract were studied, and then an analytical method for finding thujopsene on the level of ng/g-fabric was established. Next, in order to estimate the survivability of thujopsene in marketed products, the JIS color fastness test was performed for the hiba-oil-treated fabrics, and the survival rate of thujopsene in those fabrics was observed. It was proven that thujopsene continued to remain on the level of ng/g-fabric. Furthermore, analysis of thujopsene was carried out for 23 kinds of textile products labeled "processed with hiba oil *or* hinokitiol," which were purchased in the past five years. As a result, thujopsene was detected in 21 products, proving that most commercially available products had been processed with hiba oil. This analytical method is considered effective in determining whether a textile product was actually processed with hiba oil.

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1. Introduction

Hiba (*Thujopsis dolabrata* Siebold et Zuccarini var. *Hondai Makino*), an indigenous tree in Aomori Prefecture and Hokkaido, is a variety of hiba arborvitae (*Thujopsis delabrata* Siebold et Zuccarini) belonging to the hiba arborvitae genus of the hinoki family. Hiba oil (cypress oil) is a light-yellow transparent essential oil extracted from the heartwood of hiba by steam distillation. About 41 compounds have been identified as ingredients of hiba oil [1,2], which are classified into a neutral oil fraction that is used as perfume (thujopsene, terpinolene, 4-terpineol, α-cuprenene, γ-cuprenene, cuparene, cedrol, widdrol, etc.), and an acidic oil fraction that has antimicrobial activities (carvacrol, hinokitiol, γ-thujaplicin, β-dolabrine, etc.). Among the acidic ingredients, hinokitiol [3-5] and carvacrol [6,7] are known to have particularly strong antimicrobial activities and used even as single substances.

Recently, studies on the utilization of hiba oil have been actively promoted [1,8]. In particular, the number of commercially available textile products labeled "processed with hiba oil *or* hinokitiol" has swelled owing to the sales point of their use of safe and natural antimicrobial agents [9,10]. However, the safety and authenticity of hiba oil processing of these products have not yet been verified.

An analytical method was developed for investigating the actual state of textile products processed with hiba oil. Since hiba oil is composed of many ingredients as described above, formulating a method to determine the quantity of hiba oil as a whole is difficult. However, the main ingredient of hiba oil is thujopsene, accounting for between 60-80% of its content [1,2]. If thujopsene in textiles could be analyzed, it would be possible to verify the authenticity of hiba oil processing, and to estimate the content of hiba oil from the analytical value of thujopsene. Hiba-oil-treated fabrics [11-13] were then prepared, and detection of thujopsene by GC/MS, extraction of thujopsene from the fabric and purification of the extract were studied. Moreover, in order to estimate the survivability of thujopsene in commercially available textiles, the JIS color fastness test was applied to the treated fabrics, and the residual rate of thujopsene was observed. Furthermore, analysis of thujopsene was carried out for commercially available textile products labeled "processed with hiba oil *or* hinokitiol," which had been purchased on the market in the past five years.

2. Experimental

2.1 Samples

Textile products labeled "processed with hiba oil *or* processed with hinokitiol," were purchased between fiscal 1996 and fiscal 2000.

2.2 Reagents

Thujopsene of a standard agent (CAS No. 470-40-6, Purity of 97% or higher) made by Fluka Chemie AG (Buchs, Switzerland) and hiba oil (CAS No. 68917-43-1) supplied by Environmental Science Development Co., Ltd. (Osaka, Japan) were used as standard substances. Hiba-oil-filled microcapsules were obtained from Environmental Science Development Co., Ltd. These ceramic microcapsules were filled with hiba oil by the same company. Stock solution for fabric treatment (coating agent) was obtained from Environmental Science Development Co., Ltd. This solution was prepared by dispersing hiba-oil-filled microcapsules and resin binder in water at a weight ratio 2 : 8 : 90. The hiba oil concentration in the stock solution was 1wt%. White cotton fabric for the JIS test for color fastness by xenon arc lamp light (based on JIS L 0803 : 1998) (Kanakin No.3) was purchased from the Japanese Standards Association. Methanol and n-hexane of pesticide residue analysis grade were obtained from Wako Pure Chemicals Co., Ltd. (Osaka, Japan). The octadecyl (C_{18}) mini cartridge column used was Sep-Pack Plus made by Waters Co., Ltd. (Massachusetts, USA).

2.3 GC/MS apparatus and operating conditions

For GC/MS detection, a gas chromatograph GC 6890 Series II (Hewlett Packard, Co., Ltd. [HP], Avondale, PA, USA) equipped with a mass spectrometer HP 5972 MSD was used. A capillary column HP-5 MS (0.25 mmϕ × 30 m, film thickness 0.25 μm, Hewlett Packard) was also used. The column oven temperature was programmed as follows: held at 60℃ for 1.5 min, elevated from 60℃ to 120℃ at 25℃ /min, from 120℃ to 180℃ at 6℃/min and from 180℃ to 280 ℃ at 30℃/min, and then held at 280℃ for 1 min. Helium carrier gas was used at a constant flow-rate of 1 ml/min. Inlet temperature was set at 250℃. The sample was injected using an HP 7673 automatic injector (Hewlett Packard). The injection method was splitless, and the injection volume was 2 μl. The ionization voltage was 70 eV for electron impact ionization. The interface temperature was set at 280℃. The MS spectrum was scanned in the range of m/z 45-450. SIM was performed at m/z 119, 189, 204 for identification of thujopsene and determined at m/z 119.

2.4 Preparation of hiba-oil-treated fabrics

The stock solution, a dispersion of microcapsules filled with hiba oil and resin binder in water, was diluted with water. A white cotton fabric was then soaked in the diluted solution and squeezed with a motor-driven pneumatic test mangle (Tsujii Senki Kogyo Co., Ltd.) adjusted to a 100% pick up (weight ratio of fabric and solution = 1 : 1). The fabric was dried with a hot air circulating baking machine (Daiei Kagakuseiki Mfg. Co., Ltd.) to fix the microcapsules to the fabric. The drying was performed under the following two conditions : at 100℃ for 1 hour and at 80℃ for 20 min. The fabric treated with a 100-fold diluted solution was named a "1%-treated fabric." In the same way, 2% (50-fold diluted solution), 4%, 6% and 8% treated fabrics were prepared by changing the dilution ratio. The treated fabrics were immediately stored in the dark after treating.

2.5 Xenon-arc-light-type color fastness test

The surface of the hiba-oil-treated fabrics was irradiated using a high-energy xenon long-life weather meter SC-750 (7.5 kW, water-cooling type, direct irradiation method, product of Suga Test Instruments Co., Ltd.) according to the JIS L0843-88 xenon-arc-light color fastness testing method (same specifications as ISO 105-B02). The irradiation conditions were as follows : 63℃ of black panel temperature, 50% humidity, rainless, and low energy density. The irradiation times were 9.375 min, 18.75 min, 37.5 min, 75 min, 150 min, 300 min and 600 min, respectively.

2.6 Preparation of test solution

A 2 g portion of chopped sample was taken into a 200 ml round-bottomed flask, to which 100 ml of methanol was added, and refluxed at 70℃ for 1 hour. The extract was filtrated with a glass filter (G-2) into a 200 ml round-bottomed flask. The filtrate was joined with 20 ml of methanol for washing the glass filter, concentrated to 10 ml by a rotary evaporator. The solution was loaded onto a prepared C_{18} cartridge column. Twenty ml of methanol was passed through the column to elute thujopsene. The eluate was concentrated to 10 ml, and taken into a 50 ml centrifugal tube. The concentrate solution to which 10 ml of purified water was added was re-extracted with 10 ml of n-hexane, by shaking for 5 min, and centrifuging at 3,000 rpm for 5 min. The extraction with n-hexane was carried out twice, and the total extract was concentrated to 10 ml, and submitted to GC/MS.