Table 3. Recoveries (%) and Coefficients of Variation (CV: %) of Deutrated Organotin Compounds Used as Surrogate Standard Measured in Each Laboratory Resluts ($n$=3)[a]

| Sample | Setting concentration | | TBT | | | | | | TPT | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | A | B | C | D | E | F | A | B | C | D | E | F |
| Textile | 0.1 μg/g | Ave | 92 | 85 | 86 | 82 | 91 | 105 | 92 | 77 | 99 | 68 | 75 | 76 |
| | | CV | 2.2 | 16 | 4.9 | 12 | 7.4 | 4.4 | 5.3 | 26 | 42 | 24 | 14 | 8.5 |
| | 1.0 μg/g | Ave | 86 | 80 | 84 | 89 | 93 | 93 | 74 | 72 | 122 | 82 | 63 | 80 |
| | | CV | 1.0 | 19 | 2.7 | 1.7 | 2.2 | 6.7 | 9.3 | 15 | 8.2 | 5.0 | 25 | 6.0 |
| | 10 μg/g | Ave | 93 | 100 | 70 | 72 | 95 | 94 | 100 | 91 | 67 | 53 | 64 | 76 |
| | | CV | 4.0 | 2.9 | 43 | 6.4 | 3.4 | 15 | 4.1 | 6.7 | 83 | 11 | 13 | 6.6 |
| Water-based adhesive | 0.1 μg/g | Ave | 92 | 99 | 66 | 91 | 100 | 96 | 92 | 100 | 102 | 84 | 98 | 97 |
| | | CV | 2.2 | 0.53 | 4.1 | 2.7 | 2.6 | 18 | 5.3 | 0.015 | 10 | 2.5 | 11 | 4.2 |
| | 1.0 μg/g | Ave | 86 | 99 | 75 | 90 | 100 | 95 | 74 | 100 | 106 | 84 | 97 | 97 |
| | | CV | 1.0 | 0.12 | 6.2 | 1.4 | 4.1 | 11 | 9.3 | 0.0058 | 9.5 | 2.2 | 9.5 | 6.5 |
| | 10 μg/g | Ave | 93 | 99 | 68 | 90 | 100 | 106 | 100 | 100 | 96 | 85 | 102 | 90 |
| | | CV | 4.0 | 0.13 | 1.4 | 1.3 | 3.3 | 9.4 | 4.1 | 0.042 | 6.9 | 1.2 | 3.9 | 12 |
| Oil-based paint[b] | 0.1 μg/g | Ave | 158 | 43 | 39 | 39 | 44 | 49 | 225 | 43 | 80 | 47 | 53 | 55 |
| | | CV | 4.0 | 0.62 | 3.5 | 0.42 | 2.4 | 3.7 | 3.0 | 5.4 | 1.4 | 1.9 | 2.9 | 12 |
| | 1.0 μg/g | Ave | 63 | 39 | 5.6 | 40 | 45 | 51 | 90 | 39 | 20 | 49 | 54 | 60 |
| | | CV | 18 | 17 | 40 | 1.7 | 3.5 | 0.68 | 12 | 18 | 45 | 0.83 | 0.63 | 3.9 |
| | 10 μg/g | Ave | 55 | 42 | 11 | 40 | 44 | 46 | 80 | 45 | 30 | 50 | 53 | 54 |
| | | CV | 21 | 4.3 | 57 | 1.1 | 1.3 | 1.6 | 21 | 2.4 | 56 | 1.7 | 3.1 | 9.7 |

[a] Recovery of surrogate standard (%) = 100 × (B/A). A = (area of surrogate compound obtained from standard curve/area of TeBT-$d_{36}$ obtained from standard curve), B = (area of surrogate compound obtained from sample/area of TeBT-$d_{36}$ obtained from sample), [b] Theoretical recovery of oil-based paint sample is 50% because half of oil paint samples was used for analysis.

化前にシリカゲルカラムによる精製を行っており，DPT の方が TPT 及び TBT よりも極性が強く，カラムに保持され溶出されていないと推測され，油性塗料試料についても TPT の脱フェニル分解とそれに伴う DPT 生成の可能性は否定できない．

分析操作に関して，油性塗料試料のシリカゲルカラムによる精製では 80 mL の溶出溶媒を用いるため，ミニカラムではなくオープンカラムの方が操作し易いとの指摘があった．また，衛生試験法における器具・容器包装及び玩具中の DBT 分析[29]では，DBT のガラス壁面への吸着を防ぐために，標準溶液をアセトンで調製する際に塩酸を数滴加えており，本試験法におけるアセトンでの標準品調製時にも同様の操作を行う方がよいとの指摘があった．その他，同一機関でメーカーの異なる 2 台の GC-MS を比較したところ，片方の機器では検量線の一番低濃度（注入時：0.01 μg/mL）の測定が困難であった．そのため，分析機器の状態などによっては安定して測定できる濃度まで試料溶液を濃縮する必要があると考えられる．

**3. 公定法の改正について**　有害物質含有家庭用品規制法で TPT 及び TBT が規制されている家庭用品は，おしめ，おしめカバー，下着などの繊維製品，家庭用接着剤及び塗料，家庭用ワックス，くつ墨及びくつクリームと多岐にわたる．そのため，繊維，水性及び油性製品の 3 つに大きく分類して試験法が規定されている．[1] 今回，試験法の改正のために検討した方法も，現行法と同様に抽出方法を 3 つに分けて行った．これらの家庭用品では，夾雑物質が製品毎に大きく異なり，それらの影響によって TPT 及び TBT の回収率に差が生じるため，サロゲート物質として TPT 及び TBT の重水素化体を用いた定量法が検討され，その有効性が報告されている．[7,22,23] 本試験法でもそれらと同様に，サロゲート物質を分析操作の第一段階から添加することで夾雑物質の影響が補正され，各機関の分析値の CV 値が小さく抑えられて良好な精度（再現性）が得られ，サロゲート物質が非常に有効であることが示された．

現行法では 2 次元 TLC 分析時の検出限界値が試験法全体の検出限界値として採用されている．[27] これは錫として 1.0 μg/g であり，TPT 及び TBT としてそれぞれ 3.25 及び 2.75 μg/g となる．また，フレームレス AAS の検出限界値は錫として 0.2 μg/g とされ，[27] TPT 及び TBT としてはそれぞれ 0.65 及び 0.55 μg/g である．今回の試験法はこれらの値を下回る試料でも定量可能であった．特に，過去に油性塗料中で TBT が高濃度（1240–1380 μg/g）で検出されている[17]にもかかわらず，現行法では油性塗料中の TPT 及び TBT の定量・定性は困難である[27]とされてきたが，今回の試験法では定量分析が可能であった．また，有機錫化合物の誘導体化に関していくつかの方法が存在するが，$NaBEt_4$ を用いる方法は比較的簡易であり有効であると言える．以上から，$NaBEt_4$ で対象化合物を誘導体化し，サロゲート物質を用いて GC-MS で測定する本法は改正試験法として有効であると考えられた．

ただし，今回の試験では一部で夾雑物質などの影響や分析操作中の損失によると推測されるサロゲート物質の回収率のばらつきが認められた．そのため，基準値超過を判定するためには，定量試験ではなく比較試験が望ましいと考えられた．すなわち，あらかじめ対象化合物を含まないことが確認された対照試料に，基準値と同じ濃度の対象化合物を添加し，所定の操作で分析して各対象化合物とサロゲート物質とのピーク面積比を求めておき，試料中の各対象化合物とサロゲート物質とのピーク面積比を比較し基準値超過の有無を判定する方法である．また，繊維試料及び油性塗料では試料保存中に TPT が分解する可能性があることから，試料入手後速やかに分析することが望ましいと思われる．

## 結　　論

有害物質含有家庭用品規制法における TPT 及び TBT 試験法の改定に向けて，これまでに開発してきた分析法を基に改良を加えた試験法を考案し，6 機関で繊維製品，水性接着剤及び油性塗料について既知濃度（0.1, 1.0, 10 μg/g）の同一試料を用いて妥当性を検討した．その結果，TPT について繊維及び油性塗料試料では，試料保管時に脱フェニル分解したと考えられ，試料作製時に比べて濃度が大幅に低下していた．しかしながら，分析値の CV 値は概ね 10% 以下であり分析法の精度には問題ないと考えられた．TBT の測定値については，配付試料と比べていくつかの 0.1 μg/g 試料で 120% を超えたり 70% 未満となったりしたが，それ以外の試料で

は70–120%の範囲内となり，CV値についてもそのほとんどが10%以下と精度も問題ないと考えられた．今回，試験に用いた試料中のTPT及びTBTの濃度範囲は，現行法の基準値を下回っており，本試験法は現行法よりも低濃度まで測定できた．一方，改正試験法としては，夾雑物質や測定機器の状態などが分析値に影響すると考えられたことから，サロゲート標準物質を使用し対照試料を用いた比較試験とすることが望ましいと考えられた．また，TPTについては繊維及び油性塗料試料中での分解が考えられたことから，実際の検査では試料入手後に速やかに分析することが望ましいと思われる．

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# Survey of Primary Aromatic Amines Originating from Azo Dyes in Commercial Textile Products in Direct Contact with Skin and in Commercial Leather Products in Japan

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**Summary**

Twenty-six carcinogenic primary aromatic amines (PAAs) originating from azo dyes in commercial textile products that can potentially come into direct contact with human skin (31 products; 41 samples) and in leather products (23 products; 23 samples) in Japan were investigated. Twelve and 11 PAAs were detected in the textile and leather products, respectively, nearly all at low concentrations (below 1.0 μg/g). However, the concentrations of benzidine (45-593 μg/g) in one shawl and six sheets and covers (seven samples) exceeded European Union (EU) regulatory limits (below 30 μg/g). Concentrations of o-toluidine (430 μg/g), benzidine (31 μg/g), and 3,3'-dimethylbenzidine (40 μg/g) in leather products (hand-crafted leather) also exceeded EU regulatory limits. Shawls, sheets, and covers can come into direct contact with human skin. Thus, an exposure evaluation should be performed for benzidine in these products.

**Key words:** primary aromatic amine, azo dye, textile product, leather product

## INTRODUCTION

Since the second half of the 19th century, azo dyes have been widely used in various products such as textiles, leather, paper, etc. because of their low cost and variety of available colors. At present, more than 3000 azo dyes are used globally, constituting 65% of the global commercial dye market[1)]. However, carcinogenic primary aromatic amines (PAAs) are generated through reductive decomposition of certain azo dyes by microorganisms on the skin, intestinal bacteria, and the liver[2-4)]. These azo dyes include more than quarter of all of azo dyes that have been synthesized globally; 250 azo dyes contain the benzidine structure[5)]. Thus, in 1994, the German government banned the use of certain azo dyes in consumer goods that can potentially come into direct, long-term contact with human skin[6)]. According to this regulation, azo dyes degraded through reduction into 20 different PAAs were banned for the first time. Subsequently, European Union (EU) also banned 22 PAAs in 2002 (EU Directive/2002/61/EC)[7)]. These azo dyes have been restricted by the regulation Registration, Evaluation, Authorization and Restriction of Chemicals (REACH)[8)] since 2009. Additional countries have also prohibited 22-24 PAAs, including China, Korea, and Vietnam[9)]. Furthermore, voluntary regulations for textile products also contain limits on 24 PAAs (Oeko-Tex® Standard 100)[10)].

The Rapid Alert System for non-food consumer products (RAPEX) reports weekly violations of EU regulations in the EU market[11)]. Most contravention cases concerning PAAs in 2010-2011 were reported for clothing, fashion, and interior products. In addition, the majority of violations were reported for products manufactured in China and India. Surveys of PAAs in consumer products in the EU have been performed and the present situation regarding PAAs is clear although only a few studies of PAAs in actual commercial products have been carried out in Japan. Therefore, we previously investigated the concentrations of PAAs in 86 commercial textile products (121 samples) in Japan in Jan-Mar 2009[12)]. This previous study detected high concentrations of benzidine, 3,3'-dimethoxybenzidine, and 2,4-diaminotoluene, exceeding the EU regulatory value in several cotton placemats manufactured in India.

Textile products that could potentially come into direct contact with human skin are regulated in the EU, and placemats do not always come into contact with human skin. Thus, the concentrations of PAAs needed to be investigated in textile products that do come into directly contact with human skin. Furthermore, contravention cases for leather products have also been reported by RAPEX[11)]. However, the concentrations of PAAs in commercial leather products in Japan are unknown. Thus, the aim of this study was to investigate the concentrations of PAAs in commercial textile products that can potentially come into direct contact with skin and in commercial leather products. Twenty-six PAAs were investigated in this study (Table 1).

Table 1 PAAs investigated

| Compounds | CAS No. | REACH Annex XVII[a] | IARC Group[b] | Supplier[c] |
|---|---|---|---|---|
| Aniline | 62-52-3 | − | 3 | D |
| o-Toluidine | 95-53-4 | + | 1 | D |
| 2,4-Xylidine | 95-68-1 | − | 3 | F |
| 2,6-Xylidine | 87-62-7 | − | 2B | F |
| o-Anisidine | 90-04-0 | + | 2B | D |
| 4-Chloroaniline | 106-47-8 | + | 2B | C |
| p-Cresidine | 120-71-8 | + | 2B | D |
| 2,4,5-Trimethylaniline | 137-17-7 | + | 3 | C |
| 1,4-Phenylenediamine | 106-50-3 | − | 3 | F |
| 4-Chloro-o-toluidine | 95-69-2 | + | 2A | B |
| 2,4-Diaminotoluene | 95-80-7 | + | 2B | C |
| 2,4-Diaminoanisole | 615-05-4 | + | 2B | D |
| 2-Naphthylamine | 91-59-8 | + | 1 | A |
| 5-Nitro-o-toluidine | 99-55-8 | + | 3 | B |
| 4-Aminobiphenyl | 92-67-1 | + | 1 | A |
| 4-Aminoazobenzene | 60-09-3 | + | 2B | E |
| 4,4'-Oxydianiline | 101-80-4 | + | 2B | B |
| 4,4'-Methylenedianiline | 101-77-9 | + | 2B | D |
| Benzidine | 92-87-5 | + | 1 | A |
| o-Aminoazotoluene | 97-56-3 | + | 2B | B |
| 4,4'-Methylenedi-o-toluidine | 838-88-0 | + | 2B | B |
| 3,3'-Dimethylbenzidine | 119-93-7 | + | 2B | C |
| 4,4'-Thiodianiline | 139-65-1 | + | 2B | D |
| 4,4'-Methylene-bis-(2-chloro-aniline) | 101-14-4 | + | 1 | B |
| 3,3'-Dichlorobenzidine | 91-94-1 | + | 2B | C |
| 3,3'-Dimethoxybenzidine | 119-90-4 | + | 2B | D |

[a] +: Listed PAAs in the REACH Annex XVII[8)]

[b] IARC clasification groups: 1 = Carcinogenic to humans, 2A = Probably carcinogenic to humans, 2B = Possibly carcinogenic to humans, 3 = Not classifiable as to carcinogenic to humans[13)]

[c] A: SUPELCO, B: Sigma-Aldrich, C: AccuStandard Inc., D: Wako Pure Chemical Industiries, Ltd., E: Fulka, F: Tokyo Kasei Kogyo Co., Ltd.

## MATERIALS AND METHODS

### *Samples*

Commercial textile products that come into direct contact with skin and leather products were purchased from several retail stores in Japan in December 2011 and January 2012. The details (use, color, etc.) of the samples are listed in Table 2. Textile products were separated by color as much as possible (Table 2). A total of 31 textile products (41 samples) and 23 leather products (23 samples) were analyzed. To express the color of the products exactly, samples in which PAAs were detected at levels greater than the EU regulatory limit were color-classified according to the Munsell color system.

### *Materials*

Information on the 26 PAAs studied in this investigation is provided in Table 1. Anthracene-$d_{10}$ and napthalene-$d_8$ purchased from Kanto Chemical Co., Inc. (Tokyo, Japan) and 2,4,5-trichloroaniline purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) were used as internal standards. Pesticide residue grade methanol, hexane, and methyl-tert-butyl ether (MTBE) were obtained from Kanto Chemical Co., Inc. Citrate acid, sodium dithionite, and sodium hydroxide were purchased form Sigma-Aldrich Japan (Tokyo, Japan). Sodium chloride was obtained from Nacalai Tesque (Kyoto, Japan). The citrate buffer solution was adjusted to pH 6.0 (0. 06 mol/L as citrate acid) and the sodium dithionite solution (200 mg/mL) was prepared immediately before use. Each PAA was dissolved in methanol (1000 or 100 μg/mL) and standard solutions (10 μg/mL) were prepared using MTBE.

### *Sample processing*

Textile samples were processed in accordance with EN 14362-1:2003[14)] (particularly for textiles made of cellulose and protein fibers) with minor modifications. Leather samples were processed in accordance with EN 17234-1:2010[15)] with minor modifications, except

Table 2 Textile and leather products investigated

| | Usage | | Sample name[a] | Colors[a] | Country | Material |
|---|---|---|---|---|---|---|
| Textile | Kerchief | Bandannas | T-A1 | Red | China | Cotton |
| | | | T-A2 | Blue-violet | China | Cotton |
| | | | T-A3 | Navy blue | China | Cotton |
| | | | T-A4 | Red | Indonesia | Cotton |
| | | | T-A5 | Violet | Indonesia | Cotton |
| | | Scarves | T-B1 | Orange | –[b] | Silk |
| | | | T-B2 | Blue-violet | – | Silk |
| | | Shawl | T-B3(SB、YG、O) | Sky blue (SB), Yellow green (YG), Orange (O) | – | Cotton |
| | Clothes | T-shirts | T-C1 | Red | China | Cotton |
| | | | T-C2 | Navy blue | China | Cotton |
| | | Children's T-shirts | T-C3 | Pink | China | Cotton |
| | | | T-C4 | Yellow | China | Cotton |
| | | | T-C5 | Violet | China | Cotton |
| | | | T-C6 | Blue | China | Cotton |
| | | | T-C7 | Orange | China | Cotton |
| | | | T-C8 | Light purple | China | Cotton |
| | | Children's clothes | T-C9 | Navy blue | India | Cotton |
| | | | T-C10 | Pink | India | Cotton |
| | | Children's briefs | T-D1 | Black | China | Cotton |
| | | | T-D2(BL、R) | Black (BL), Red (R) | China | Cotton |
| | | Trunks | T-D3 | Red | Indonesia | Cotton |
| | | | T-D4 | Black | Indonesia | Cotton |
| | Sheets and covers | Multi-covers | T-E1 | Orange | India | Cotton |
| | | | T-E2 | Violet | India | Cotton |
| | | | T-E3 | Yellow | India | Cotton |
| | | | T-E4 | Red | India | Cotton |
| | | Pillow | T-E5(V、O) | Violet (V), Orange (O) | India | Cotton |
| | | | T-E6(G、Y、B、V) | Green (G), Yellow (Y), Blue (B), Violet (V) | India | Cotton |
| | | Sheets | T-E7(DB、B、SB、ST) | Dark brown (DB), Blue (B), Sky blue (SB), Steel blue (ST) | – | Cotton |
| | | | T-E8 | Orange | India | Cotton |
| | | | T-E9 | Navy blue | India | Cotton |
| Leather | Small goods | Gloves | L-A1 | Black | China | Sheepskin |
| | | | L-A2 | Beige | China | Pigskin |
| | | Belts | L-A3 | Red | China | Natural leather |
| | | | L-A4 | Light brown | China | Natural leather |
| | | Wrist bands | L-A5 | Red | – | – |
| | | | L-A6 | Sky blue | – | – |
| | | Wallets | L-B1 | Reddish brown | Thailand | Cowskin |
| | | | L-B2 | Orange | Thailand | Cowskin |
| | | Key holder | L-B3 | Red | Korea | – |
| | | Base neck holder | L-B4 | Orange | – | Cowskin |
| | Hand-crafted leather | Small pieces with nubuck-like treatment | L-C1 | Red | – | Cowskin |
| | | | L-C2 | Deep green | – | Cowskin |
| | | | L-C3 | Navy blue | – | Cowskin |
| | | | L-C4 | Blue-violet | – | Cowskin |
| | | Small pieces of grain leather | L-C5 | Dark brown | – | – |
| | | | L-C6 | Black | – | – |
| | | | L-C7 | Reddish brown | – | – |
| | | | L-C8 | Blue | – | – |
| | | | L-C9 | Brown | – | – |
| | | | L-C10 | Deep green | – | – |
| | | | L-C11 | Dark brown | – | – |
| | | | L-C12 | Navy blue | – | – |
| | | | L-C13 | Black | – | – |

[a] For samples in which the abbreviation of the color name is given in parentheses, each color was analyzed individually.

[b] –: Unknown

for 4-aminoazobenzene, which was analyzed in accordance with EN 17234-2:2011[16]. Using EN 14362-1:2003 and EN 17234-1:2010, o-aminoazotoluene, 5-nitro-o-toluidine, and 4-aminoazobenzene were not detectable because these PAAs were further reduced to other compounds (o-aminoazotoluene: o-toluidine and 2,5-diaminotoluene, 5-nitro-o-toluidine: 2,4-diaminotoluene, 4-aminoazobenzne: aniline and 1,4-phenylenediamine) during reduction treatment.

***Textile samples***

Each textile sample was cut in an appropriate manner and 1 g of the sample was weighed and placed in a reaction tube. Next, 17 mL of citrate buffer solution preheated to 70±2 ℃ was added to the tube. The reaction tube was held for 30 min at 70±2 ℃. After 30 min, 3 mL of aqueous sodium dithionite solution was added in the tube, the reaction tube was shaken vigorously by hand, and immediately held again for 30 min at 70±2 ℃. After 30 min, the reaction tube was cooled to room temperature within 2 min. The sample solution was filtered and transferred to a separating funnel after cooling. Twenty milliliters of MTBE were added to the sample solution, the mixture was shaken by a vertical shaker for 30 min, and the MTBE layer was obtained. This extraction procedure was performed twice and the MTBE layers were combined and filtered through anhydrous sodium sulfate. The filtrate was concentrated to approximately 1 mL with a rotary evaporator maintain the temperature of the water bath below 30℃. The volume of the sample solution was adjusted to 10 mL with MTBE. Finally, 50 μL of MTBE solution containing 2 μg/mL of internal standard was added to 1 mL of the sample solution, and this solution was then analyzed by gas chromatography/mass spectrometry (GC/MS).

***Leather samples***

Each leather sample was cut in an appropriate manner and sieved to 4 mm. One gram of the sample was weighed and placed in a reaction tube. Next, 20 mL of hexane was added to the tube to degrease the leather and the sample was treated in an ultrasonic bath at 40℃ for 20 min. The hexane layer was decanted from the sample after sonication. This degreasing procedure was performed twice and the residual hexane was completely evaporated overnight in an open vessel in a fume hood.

Seventeen milliliters of citrate buffer solution preheated to 70±2 ℃ were added to the reaction tube and the tube was held for 25 min at 70±2 ℃. After 25 min, 1.5 mL of aqueous sodium dithionite solution was added to the tube and the reaction tube was shaken vigorously by hand and immediately held for 10 min at 70±2 ℃. After 10 min, 1.5 mL of aqueous sodium dithionite solution was again added and the reaction tube was held for 10 min at 70±2 ℃. After another 10 min, the reaction tube was cooled to room temperature within 2 min. The sample solution was extracted and treated in the same manner as the textile samples.

For analysis of 4-aminoazobenzene, 9 mL of 2% (w/w) sodium hydroxide aqueous solution was added to the reaction tube. The reaction tube was shaken sufficiently to wet the leather material. Subsequently, 1.0 mL of sodium dithionite aqueous solution was added to the reaction tube and the tube was shaken vigorously by hand and immediately held for 30 min at 40±2 ℃. After 30 min, the reaction tube was cooled to room temperature within 1 min. After cooling, 50 μl of MTBE solution containing an internal standard (10 μg/mL), 5 mL of MTBE, and 7 g of sodium chloride was added to the reaction tube. The reaction tube was shaken by a horizontal shaker for 45 min. The treatment time between cooling and shaking was within 5 min. After shaking, the reaction tube was centrifuged at 3000 rpm for 10 min, and the MTBE layer was obtained. This MTBE layer was filtered through anhydrous sodium sulfate and the filtrate was then analyzed by GC/MS.

***GC/MS analysis***

All samples in this investigation were analyzed with a Focus GC equipped with a DSQ II MS (Thermo Fisher Scientific, Waltham, MA, USA). A DB-35MS fused silica capillary column (length: 30 m, internal diameter: 0.25 mm, film thickness: 0.25 μm; Agilent Technologies, Santa Clara, USA) was used. The carrier gas was He at a flow rate of 1.0 mL/min. The temperatures of the injector, transfer line, and ion source were 250, 250, and 230℃, respectively. The sample solution (1 μL) was injected in the splitless mode. The GC oven temperature was initially maintained at 55℃ for 5 min and increased to 230℃ at a rate of 15℃/min. The temperature was then increased to 290℃ at rate of 5 ℃min and to 310℃ at rate of 20℃/min. The oven temperature was then maintained at 310℃ for 5 min. The MS was operated in the electron ionization (EI) mode at 70 eV, and the analysis was carried out using the selected ion monitoring (SIM) mode. The retention times and the quantifying and qualifying ions are listed in Table 3.

Limits of detection (LODs) and quantification (LOQs) were calculated as 3.3 and 10 times the standard deviation[17] obtained through determination of low-concentration (most compounds, 0.01 μg/mL; 1,4-phenylenediamine, 2,4-diaminotoluene, and 2,4-diaminoanisole, 0.1 μg/mL) standard solutions (n=3). The values thus obtained were converted to per unit weight of sample (Table 3). The LODs obtained in this study were lower than those mandated by the EU (below 5 μg/g). Recovery tests were conducted in accordance with EN14362-1:2003[14] with minor modifications[12]. The recovery tests for 4-aminoazobenzene in leather samples was conducted in accordance with EN17234-2:2011[16]. The recovery results are listed in Table 3. The data obtained in this investigation were not corrected by the obtained recoveries.

## RESULTS AND DISCUSSION

***Textile samples***

Twenty-nine cotton products (39 samples) and two silk products (two samples) were analyzed. The frequencies of detection and concentration ranges for each PAA in the textile samples are listed in Table 4 and the concentrations of PAAs detected in the samples are listed in Table 5. Twelve PAAs were detected at concentrations above the LOD in these samples and 10 of these PAAs are restricted in the EU. The following PAAs were detected with high frequency (expressed as the number of samples detected above the LOD vs. the number of samples analyzed): aniline (29/41), 4-chloroaniline (18/41), o-toluidine (11/41), 4-aminobiphenyl (11/41), and benzidine (11/41) (Table 4).

Because many dyes and other chemical compounds contain aniline, the detection frequency and concentrations of aniline may be higher than those of the other PAAs in this study. Although 4-chloroaniline was detected in various textile samples, o-toluidine, 4-amino-

Table 3 Retention times, quantifying and qualifying ions, limits of detection (LODs) and quantifications (LOQs), recoveries, and coeeficients of variation (CVs)

| Compounds | Retention time (min) | Quantifying ion (m/z) | Qualifying ion (m/z) | LOD[a] (μg/g) | LOQ[a] (μg/g) | Liquid–liquid extraction (n = 5)[b] Recovery (%) | CV (%) | Required recovery[b] |
|---|---|---|---|---|---|---|---|---|
| Aniline | 9.27 | 93 | 66 | 0.0090 | 0.027 | 77 | 5.7 | — |
| o-Toluidine | 10.55 | 106 | 107 | 0.0086 | 0.026 | 76 | 6.3 | 50% |
| 2,4-Xylidine | 11.60 | 121 | 120 | 0.029 | 0.089 | 83 | 3.5 | - |
| 2,6-Xylidine | 11.65 | 121 | 120 | 0.014 | 0.042 | 77 | 4.0 | - |
| o-Anisidine | 11.90 | 123 | 108 | 0.021 | 0.064 | 81 | 3.5 | 70% |
| 4-Chloroaniline | 12.26 | 127 | 129 | 0.021 | 0.064 | 75 | 3.9 | 70% |
| p-Cresidine | 12.84 | 137 | 122 | 0.047 | 0.14 | 81 | 3.8 | 70% |
| 2,4,5-Trimethylaniline | 12.90 | 120 | 135 | 0.031 | 0.093 | 83 | 4.7 | 70% |
| 1,4-Phenylenediamine | 13.22 | 108 | 80 | 0.16 | 0.49 | 10 | 3.6 | — |
| 4-Chloro-o-toluidine | 13.25 | 141 | 143 | 0.022 | 0.068 | 78 | 4.8 | 70% |
| 2,4-Diaminotoluene | 14.43 | 121 | 122 | 0.067 | 0.20 | 53 | 4.9 | 50% |
| 2,4-Diaminoanisole | 15.29 | 123 | 138 | 0.11 | 0.32 | 19 | 24 | 20% |
| 2-Naphthylamine | 15.93 | 115 | 143 | 0.021 | 0.063 | 86 | 4.5 | 70% |
| 5-Nitro-o-toluidine | 16.43 | 152 | 106 | 0.057 | 0.17 | - | - | - |
| 4-Aminobiphenyl | 17.56 | 169 | 152 | 0.051 | 0.15 | 90 | 3.6 | 70% |
| 4-Aminoazobenzene | 21.29 | 197 | 120 | 0.070 | 0.21 | - | - | - |
| 4,4'-Oxydianiline | 22.11 | 200 | 171 | 0.051 | 0.15 | 171 | 4.0 | 70% |
| 4,4'-Methylenedianiline | 22.25 | 198 | 197 | 0.047 | 0.14 | 136 | 5.1 | 70% |
| Benzidine | 22.36 | 184 | 167 | 0.042 | 0.13 | 114 | 3.1 | 70% |
| o-Aminoazotoluene | 23.38 | 225 | 106 | 0.059 | 0.18 | - | - | - |
| 4,4'-Methylenedi-o-toluidine | 24.39 | 226 | 211 | 0.049 | 0.15 | 115 | 4.1 | 70% |
| 3,3'-Dimethylbenzidine | 24.79 | 212 | 196 | 0.036 | 0.11 | 99 | 2.5 | 70% |
| 4,4'-Thiodianiline | 26.28 | 216 | 184 | 0.088 | 0.27 | 118 | 3.5 | 70% |
| 4,4'-Methylene-bis-(2-chloro-aniline) | 26.87 | 266 | 268 | 0.026 | 0.079 | 85 | 3.3 | 70% |
| 3,3'-Dichlorobenzidine | 26.87 | 252 | 254 | 0.028 | 0.085 | 88 | 3.1 | 70% |
| 3,3'-Dimethoxybenzidine | 27.06 | 244 | 201 | 0.039 | 0.12 | 99 | 7.0 | 70% |
| Naphthalene-$d_8$[c] | 11.75 | 136 | | | | | | |
| 2,4,5-Trichloroaniline[c] | 15.52 | 195 | | | | | | |
| Anthracene-$d_{10}$[c] | 17.78 | 188 | | | | | | |

[a] Limit of detection (LODs) and quantification (LOQs) were calculated as 3.3 and 10 times the standard deviation[17] obtained through determination of low-concentration (0.01 μg/mL for most compounds; 0.1 μg/mL for 1,4-phenylenediamine, 2,4-diaminotoluene, and 2,4-diaminoanisole) standard solution (n=3). The values thus obtained were converted to the per unit weight of sample.

[b] Conducted in accordance with EN14362-1: 2003[14] with minor modifications (extraction method using diatomaceous earth column was changed to liquid-liquid extraction); the recovery test for 4-aminoazobenzene in leather sample was conducted in accordance with EN ISO 17234-2:2011[16] [n=5, recovery=99%, CV=1.4%])

[c] Internal standard (naphthalene-$d_8$: aniline to 2,4-diaminotoluene, 2,4,5-trichloroaniline: 2,4-diaminoanisole and 2-naphthylamine, anthracene-$d_{10}$: 5-nitro-o-toluidine to 3,3'-dimethoxybenzidine)

biphenyl, and benzidine were mainly detected in scarves, sheets and covers. Almost all of the concentrations of PAAs detected in this study were below 1.0 μg/g; however, high concentrations of benzidine detected in 7 textile products (8 samples) were observed at levels exceeding the EU regulatory limit (below 30 μg/g). These samples (shawl, sheets, and covers) were manufactured in India (5 products) and unknown (2 products). The total ion chromatogram (SIM mode) and mass spectrum (scan mode: m/z=60-300) for benzidine detected in T-B3_O are shown in Fig. 1. According to the International Agency for Research on Cancer (IARC), benzidine is classified as group 1 (carcinogenic to humans). The concentrations of benzidine exceeding the EU regulatory limit were very high (45-593 μg/g) and shawls, sheets, and covers can come into direct contact with human skin. T-B3 and T-E7 (Store A), T-E1 to E5 (Store B), and T-E6, T-E8, and T-E9 (Store C) were obtained from three different retail stores. Because benzidine exceeding the EU regulatory limit were detected in samples purchased in each retail store and high concentrations of benzidine above the EU regulatory limit have also been reported in scarves by RAPEX, these products may contain azo dyes generating carcinogenic PAAs. Thus, an exposure evaluation for benzidine in these products should be performed.

In our previous study, benzidine, 3,3'-dimethoxybenzidine, and 2,4-diaminotoluene were detected above the EU regulatory limit in several placemats manufactured in India[12]. However, 2,4-diaminotoluene was not detected in this study. In the previous study, 4-aminobiphenyl, 3,3'-dimethylbenzidine, and benzidine were detected in the same samples[12] and a similar tendency was observed in this study. In addition, the colors of samples containing high concentrations of PAAs were orange, violet, brown, navy, and blue. In the some of the samples with high concentrations of benzidine, the color of the buffer solution immediately changed from clear to the color of the textile when the sample was placed in the buffer solution. This observation can be attributed to the very low color fastness of the dye used in the samples. These tendencies were also observed in our previous study[12] and it was suggested that the azo dyes used for these products may have been direct dyes.

Although the detection frequency of 4-aminozobenzene was also high in the RAPEX reports, it was not possible to analyze because it degrades to aniline and 1,4-phenylenediamine during the reduction treatment used for sample processing. In this study, 4-aminoazobenzene did not appear to be present in the products studied since 1,4-phenylenediamine was not detected in the textile samples.

Table 4 Detection frequencies and concentration ranges for PAAs in textile and leather products[a]

| Chemical | Textile | | | Leather | |
|---|---|---|---|---|---|
| | Products (n = 31) | Samples (n = 41) | Concentration range (μg/g) | Products (n = 23) | Concentration range (μg/g) |
| Aniline | 21 | 29 | 0.037 – 631 | 23 | 0.12 – 587 |
| o-Toluidine | 8 | 11 | 0.071 – 0.64 | 2(1) | 0.064 – 430 |
| 2,4-Xylidine | 1 | 5 | tr[b] – 0.95 | 0 | —[c] |
| 2,6-Xylidine | 0 | 0 | — | 0 | — |
| o-Anisidine | 5 | 7 | tr – 0.59 | 0 | — |
| 4-Chloroaniline | 12 | 18 | tr – 5.5 | 10 | 0.10 – 3.9 |
| p-Cresidine | 2 | 2 | 1.4 – 1.9 | 1 | 0.21 |
| 2,4,5-Trimethylaniline | 1 | 1 | 0.18 | 0 | — |
| 1,4-Phenylenediamine | 0 | 0 | — | 3 | 3.6 – 21 |
| 4-Chloro-o-toluidine | 0 | 0 | — | 0 | — |
| 2,4-Diaminotoluene | 0 | 0 | — | 0 | — |
| 2,4-Diaminoanisole | 0 | 0 | — | 0 | — |
| 2-Naphthylamine | 3 | 3 | 0.13 – 2.7 | 1 | 0.44 |
| 5-Nitro-o-toluidine | 0 | 0 | — | 0 | — |
| 4-Aminobiphenyl | 9 | 11 | 0.21 – 11 | 4 | 0.81 – 2.0 |
| 4-Aminoazobenzene | 0 | 0 | — | 0 | — |
| 4,4'-Oxydianiline | 0 | 0 | — | 0 | — |
| 4,4'-Methylenedianiline | 0 | 0 | — | 0 | — |
| Benzidine | 9(7) | 11(8) | 0.23 – 593 | 4(1) | 0.38 – 31 |
| o-Aminoazotoluene | 0 | 0 | — | 0 | — |
| 4,4'-Methylenedi-o-toluidine | 0 | 0 | — | 0 | — |
| 3,3'-Dimethylbenzidine | 5 | 5 | 0.17 – 5.5 | 7(1) | 0.15 – 40 |
| 4,4'-Thiodianiline | 0 | 0 | — | 1 | 0.66 |
| 4,4'-Methylene-bis-(2-chloro-aniline) | 0 | 0 | — | 0 | — |
| 3,3'-Dichlorobenzidine | 0 | 0 | — | 0 | — |
| 3,3'-Dimethoxybenzidine | 2 | 2 | 0.40 – 2.3 | 1 | 15 |

[a] The numbers in parentheses denote the number of samples in which the PAA concentration exceeded the EU regulatory limit of 30 μg/g.

[b] Between LOD and LOQ. [c] Not detected.

Table 5 Concentrations of PAAs detected in textile samples (μg/g)[a]

| | Aniline | o-Toluidine | 2,4-Xylidine | o-Anisidine | 4-Chloroaniline | p-Cresidine | 2,4,5-Trimethylaniline | 2-Naphthylamine | 4-Aminobiphenyl | Benzidine | 3,3'-Dimethylbenzidine | 3,3'-Dimethoxybenzidine | Munsell Color[b] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| T-A1 | 0.037 | —[c] | — | — | — | — | — | — | — | — | — | — | |
| T-A2 | 1.1 | — | — | — | tr[d] | — | — | — | — | — | — | — | |
| T-A3 | 0.48 | — | — | — | 0.37 | — | — | — | — | — | — | — | |
| T-A4 | — | — | — | — | — | — | — | — | — | — | — | — | |
| T-A5 | — | — | — | — | — | — | — | — | — | — | — | — | |
| T-B1 | 94 | — | — | 0.12 | — | — | — | — | — | — | — | — | |
| T-B2 | 0.25 | — | — | — | — | — | 0.18 | — | — | — | — | — | |
| T-B3_SB | 0.040 | 0.13 | — | — | — | — | — | — | — | — | — | — | |
| T-B3_YG | 0.35 | 0.28 | — | 0.59 | 0.37 | — | — | — | — | — | — | — | |
| T-B3_O | 24 | 0.64 | — | 0.23 | 0.15 | — | — | — | 5.9 | 263 | 2.7 | — | 10YR 4/8 |
| T-C1 | 0.079 | — | — | — | — | — | — | — | — | — | — | — | |
| T-C2 | 0.21 | — | — | — | 0.91 | — | — | 0.20 | — | — | — | — | |
| T-C3 | 0.15 | — | — | — | — | — | — | — | — | — | — | — | |
| T-C4 | — | — | — | — | — | — | — | — | — | — | — | — | |
| T-C5 | — | — | — | — | — | — | — | — | — | — | — | — | |
| T-C6 | — | — | — | — | tr | — | — | — | — | — | — | — | |
| T-C7 | — | — | — | — | tr | — | — | — | — | — | — | — | |
| T-C8 | — | — | — | — | — | — | — | — | — | — | — | — | |
| T-C9 | — | — | — | — | 2.1 | — | — | 2.7 | — | — | — | — | |
| T-C10 | 631 | 0.18 | — | — | — | — | — | — | 0.43 | — | — | — | |
| T-D1 | 1.1 | — | — | — | 5.5 | — | — | — | — | — | — | — | |
| T-D2_BL | — | — | — | — | 3.0 | — | — | — | — | — | — | — | |
| T-D2_R | 0.32 | — | — | — | 0.48 | 1.4 | — | — | — | — | — | — | |
| T-D3 | — | — | — | — | — | — | — | — | — | — | — | — | |
| T-D4 | — | — | — | — | 0.21 | — | — | — | — | — | — | — | |
| T-E1 | 17 | 0.29 | — | — | — | — | — | — | 1.2 | 141 | 3.6 | — | 2.5YR 5/8 |
| T-E2 | 33 | 0.071 | — | — | — | — | — | — | 9.8 | 351 | — | 2.3 | 2.5PB 3/4 |
| T-E3 | 13 | 0.35 | — | 0.076 | — | — | — | — | 0.21 | 2.5 | 0.17 | — | |
| T-E4 | 6.3 | 0.19 | tr | tr | — | 1.9 | — | — | 2.8 | 413 | 5.5 | — | 5R 4/8 |
| T-E5_O | 0.10 | — | — | — | 0.14 | — | — | — | — | — | — | — | |
| T-E5_V | 0.28 | — | — | — | 0.30 | — | — | — | — | 0.23 | — | — | |
| T-E6_B | 0.36 | — | 0.12 | 0.072 | 0.18 | — | — | — | — | — | — | — | |
| T-E6_G | 0.23 | — | 0.95 | 0.12 | 0.072 | — | — | — | — | — | — | — | |
| T-E6_V | 0.50 | — | tr | — | 0.098 | — | — | — | — | — | — | — | |
| T-E6_Y | 0.78 | — | tr | — | 0.066 | — | — | — | — | — | — | — | |
| T-E7_B | 0.61 | 0.078 | — | — | — | — | — | — | 2.1 | 100 | — | — | 2.5PB 4/6 |
| T-E7_DB | 0.70 | 0.26 | — | — | — | — | — | — | 1.1 | 45 | — | — | 5PB 2/1 |
| T-E7_SB | — | — | — | — | — | — | — | — | — | — | — | — | |
| T-E7_ST | 0.33 | — | — | — | — | — | — | — | 0.76 | 30 | — | — | |
| T-E8 | 3.3 | 0.082 | — | — | — | — | — | — | 1.1 | 134 | 2.8 | — | 10R 6/10 |
| T-E9 | 130 | — | — | — | — | — | — | 0.13 | 11 | 593 | — | 0.40 | 2.5PB 2/4 |

[a] Boldface denotes values that exceed the EU regulatory limit of 30 μg/g. [b] Classified according to the Munsell color system: hue value/chroma. [c] —: Not detected. [d] tr: Between the LOD and LOQ.

***Leather samples***

Twenty-three leather products (23 samples) were analyzed. The frequencies of detection and concentration ranges of each PAA in the leather samples are listed in Table 4 and the concentrations of PAAs detected in the samples are listed in Table 6. Eleven PAAs were detected at concentrations above the LOD in these samples and 9 of these PAAs are restricted in EU. The following PAAs were detected with high frequency: aniline (23/23), 4-chloroaniline (10/23), 3,3'-dimethylbanzidine (7/23), 4-aminobiphenyl (4/23), benzidine (4/23), and 1,4-phenylenediamine (3/23) (Table 4). 4-Aminoazobenzene was analyzed in accordance with EN17234-2 and was not detected in any of the leather samples.

The high concentrations of o-toluidine (430 μg/g), benzidine (31 μg/g), and 3,3'-dimethylbenzidine (40 μg/g) detected in LC-4, LC-3, and LC-1, respectively, were observed at values exceeding the EU regulatory limit. The total ion chromatogram (SIM mode) and mass spectrum (scan mode: m/z=60-300) for o-toluidine detected in L-C4 are shown in Fig. 2. Products containing PAAs above the EU regulatory limit were handcrafted pieces with nubuck-like treatment (raising the surface and complete dyeing to the inside). Although relatively high concentrations of 1,4-phenylenediamine were detected in the leather samples, 4-aminobenzene was not detected in any of the samples by EN 17234-2:2011. Thus, it is believed that 1,4-phenylenediamine detected in the leather samples did not originate from 4-aminoazobenzene.

## CONCLUSIONS

Twenty-six PAAs originating from azo dyes in commercial textile products that can potentially come into direct contact with human skin (31 products; 41 samples) and leather products (31 products; 31 samples) in Japan were investigated. Twelve and 11 PAAs were detected in the textile and leather products, respectively, with nearly all of the PAAs detected at low concentrations (below 1.0 μg/g). However, the concentrations of benzidine (45-593 μg/g) in one shawl products and 6 sheet and cover products (7 samples) exceeded EU regulatory limits (below 30 μg/g). In addition, the concentrations of o-toluidine (430 μg/g), benzidine (31 μg/g), and 3,3'-dimethylbenzi-

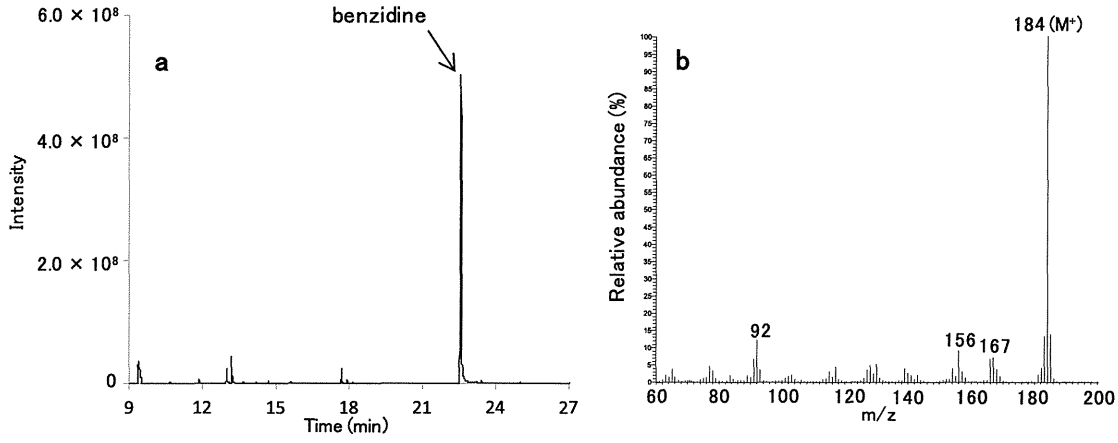


Fig. 1 Benzidine detected in sample T-B3_O in the (a) total ion chromatogram (SIM mode) and (b) mass spectrum (scan mode: m/z=60-300)

Table 6 Concentrations of PAAs detected in leather samples (μg/g)[a]

| | Aniline | o-Toluidine | 4-Chloroaniline | p-Cresidine | 1,4-Phenylenediamine | 2-Naphthylamine | 4-Aminobiphenyl | Benzidine | 3,3'-Dimethylbenzidine | 4,4'-Thiodianiline | 3,3'-Dimethoxybenzidine | Munsell Color[b] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| L-A1 | 587 | —[c] | 3.9 | — | 21 | — | — | 1.0 | 0.37 | 0.66 | — | |
| L-A2 | 22 | — | — | — | — | — | — | — | — | — | — | |
| L-A3 | 1.7 | — | — | — | — | — | — | — | — | — | — | |
| L-A4 | 0.83 | — | — | — | — | — | — | — | — | — | — | |
| L-A5 | 0.14 | — | — | — | — | — | — | — | — | — | — | |
| L-A6 | 0.13 | — | — | — | — | — | — | — | — | — | — | |
| L-B1 | 15 | — | — | — | — | 0.44 | — | — | 0.15 | — | — | |
| L-B2 | 24 | — | 0.12 | — | — | — | — | — | — | — | — | |
| L-B3 | 0.45 | — | — | — | — | — | — | — | — | — | — | |
| L-B4 | 16 | 0.064 | — | 0.21 | — | — | — | — | — | — | — | |
| L-C1 | 93 | — | — | — | — | — | 1.2 | 0.38 | 40 | — | — | 7.5R 3/10 |
| L-C2 | 92 | — | 0.17 | — | — | — | 1.1 | 16 | 0.74 | — | — | |
| L-C3 | 78 | — | 0.27 | — | — | — | 2.0 | 31 | 11 | — | 15 | 7.5PB 2/2 |
| L-C4 | 27 | 430 | 0.11 | — | — | — | — | — | 1.7 | — | — | 10PB 2/8 |
| L-C5 | 2.2 | — | — | — | — | — | — | — | 4.8 | — | — | |
| L-C6 | 81 | — | 0.34 | — | 8.6 | — | 0.81 | — | — | — | — | |
| L-C7 | 0.78 | — | — | — | — | — | — | — | — | — | — | |
| L-C8 | 0.12 | — | — | — | — | — | — | — | — | — | — | |
| L-C9 | 61 | — | — | — | — | — | — | — | — | — | — | |
| L-C10 | 39 | — | 0.10 | — | — | — | — | — | — | — | — | |
| L-C11 | 53 | — | 0.16 | — | — | — | — | — | — | — | — | |
| L-C12 | 33 | — | 0.27 | — | — | — | — | — | — | — | — | |
| L-C13 | 0.64 | — | 0.24 | — | 3.6 | — | — | — | — | — | — | |

[a] Boldface denotes values that exceed the EU regulatory limit of 30 μg/g. [b] Classified according to the Munsell color system: hue value/chroma. [c] —: Not detected.

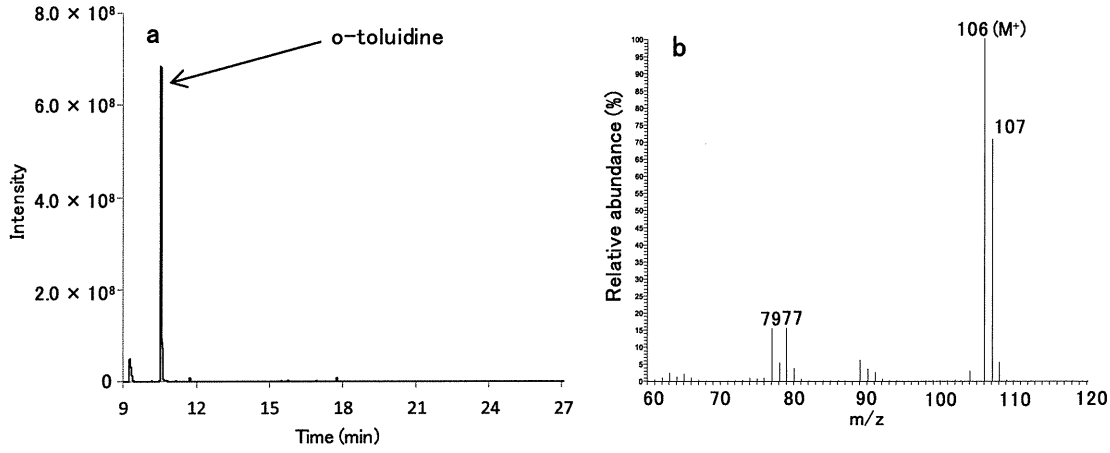


Fig. 2 o-Toluidine detected in sample L-C4_O in the (a) total ion chromatogram (SIM mode) and (b) mass spectrum (scan mode: m/z=60-300).

dine (40 μg/g) in leather products were found to exceed EU regulatory limits. Shawl, sheets, and covers can come into direct contact with human skin. Thus, exposure evaluations for benzidine should be performed for these products.

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# Analysis of Isothiazolinones and Other Preservatives in Gel-Products Used for Cooling in Japan

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**Summary**

Recently, two cases of contact dermatitis caused by 2-n-octyl-4-isothiazolin-3-one (OIT) used as a preservative in cooling gel-products has been reported in Japan, and one of the cases was declared a serious product accident based on the "Consumer Safety Product Act." In this study, the concentrations of three isothiazolinone preservatives (OIT, 2-methyl-4-isothiazolin-3-one [MIT], and 5-chloro-2-methyl-4-isothiazolin-3-one [Cl-MIT]), seven different parabens, carbendazim (MBC), and tebuconazole (Teb) in 24 cooling gel-products were investigated. OIT was detected in two samples (0.14 μg/g and 2.2 μg/g). MIT was detected in 11 samples at concentrations in the range of 0.12-115 μg/g and Cl-MIT was detected in six samples in concentrations ranging from a trace amount to 16 μg/g. The EU cosmetic limits were used to consider the risk of skin sensitization and the concentrations of MIT and Cl-MIT detected in several samples were over these limit. It is possible for the gel to cause contact dermatitis when the consumer's weight presses on the gel-product because OIT might penetrate from the gel to the textile surface in the case of a serious product accident. Furthermore, it is possible that using the gel product for the forehead or neck has a similar risk of skin sensitization if the gel-product's surface tears and the gel containing isothiazolinone preservative leaks out. It is advisable to replace the preservatives in cooling gel-products with non-sensitizing preservatives. All parabens were detected in the gel-products, except benzylparaben, and their concentrations were 12-696 μg/g. MBC and Teb were detected in three samples with concentrations in the ranges of 0.82-54 μg/g and 1.5-25 μg/g, respectively.

**Key words:** gel-product for cooling, isothiazolinone compound, contact dermatitis, preservative

## INTRODUCTION

Recently, some kinds of gel-products have been used to cool the body during the humid summer season in Japan. These gel-products use superabsorbent polymers that contain large volumes of water to take heat away from the surface of skin. Cases of allergic contact dermatitis related to the gel-product (a mattress gel-sheet) used for cooling have been reported[1)]. In these reports, two patients (adult women) slept on the mattress gel-sheet used for cooling, and both patients developed itchy edematous erythema on their arms, abdomen, legs etc., when the gel sheet came in contact with their skin for 1-3 months of use. A patch test was performed and the gel used in the mattress was identified as the causative product. Furthermore, the preservative used for this gel-sheet also revealed a positive reaction (0.1% pet.). The preservative contained 2-*n*-octyl-4-isothiazolin-3-one (OIT), 2-methyl-4-isothiazolin-3-one (MIT), and carbendazim (MBC), and patch testing showed a positive reaction for OIT (0.1% pet.) and negative reactions for MIT (0.1% aq.) and MBC (1% pet.) in both patients' cases. Although this gel-sheet was composed of three layers (inside: gel, center: polyethylene film, outside: textile made of polyester), OIT was detected in the gel and outside the textile surface. Thus, it was thought that OIT might have penetrated from the gel layer to the textile surface. Since one of the patients needed over 30 days for healing, this case was declared a serious product accident based on the "Consumer Safety Product Act" at 24 March 2010 by the Ministry of Health, Labour and Welfare[2)].

Although many cases of contact dermatitis caused by OIT used as a preservative have been reported through occupational exposure to paints, adhesives, etc.[3, 4)], cases of contact dermatitis caused by OIT exposure through household products such as leather shoes[5)] and gel-products used for cooling[1)] have rarely been reported. Furthermore, the amounts of OIT in gel-products used for cooling have not been investigated. Therefore, we investigated the concentrations of OIT in gel-products used for cooling in Japan.

In addition, other isothiazolinone compounds are also well-known skin sensitizers, especially MIT and 5-chloro-2-methyl-4-isothiazolin-3-one (Cl-MIT) which are ingredients of preservative (for example, Kathon™ CG[6)]) and these isothiazolinones are widely used in cosmetic products, wall adhesives, and water-based paints. Furthermore, many cases of contact dermatitis related to MIT and Cl-MIT have been reported[7, 8)]. Thus, the European Union (EU) has regulated the allowable concentrations of MIT and Cl-MIT in cosmetic products to less

than 15 μg/g as a mixture (MIT: 3. 75 μg/g, Cl-MIT: 11. 25 μg/g), and 100 μg/g for MIT only[9]. These two isothiazolinones compounds were also investigated in this study. Furthermore, parabens (PBs), MBC, and tebuconazol (Teb), which are used as biocides were also investigated because these compounds were detected in the gel-product when a preliminary experiment was conducted.

## MATERIALS AND METHODS

### *Samples*

Gel-products used for cooling were purchased from several retail and online stores in Japan from April to June 2010. The details of the gel-product samples are shown in Table 1 and a total of 24 gel-product samples were analyzed. Sample A1 was the same product that caused contact dermatitis and that was reported in a serious product accident, though the lot number of the product used in this study was different. Since the gels composed of superabsorbent polymers were wrapped in plastic sheet, these gels were separated from the gel-product. "Allergy checked" was printed on the product container of sample A6 only. Part of the gel-product sample used for elucidation of contact dermatitis reported in the serious product accident was provided by the National Institute of Technology and Evaluation (NITE). This sample was used as a control for developing the analytical method used in this study.

### *Materials*

OIT and PBs [methylparaben (Me-PB), ethylparaben (Et-PB), propylparaben (Pr-PB), isopropylparaben (Iso-Pro-PB), butylparaben (Bu-PB), isobutylparaben (Iso-Bu-PB), and benzylparaben (Be-PB)] were obtained from Tokyo Kasei Kogyo Co., Ltd. MIT and Cl-MIT were purchased from Waterstone Technology, LLC as a mixture (MIT: 3. 63%, Cl-MIT: 10. 85%). MBC and Teb were obtained from Sigma-Aldrich and Dr.Ehrenstorfer GmbH, respectively. Reserpine and bisphenol A-$d_{16}$ (BPA-$d_{16}$) were obtained from Wako Pure Chemical Industries, Ltd., and Cambridge Isotope Laboratories, Inc., respectively. These compounds were used as internal standards. HPLC grade trifluoroacetic acid (TFA) and acetonitrile were purchased from Wako Pure Chemical Industries, Ltd. Pesticide residue analysis grade methanol was obtained from Sigma-Aldrich. Milli-Q water was produced using a Milli-Q Synthesis A10 system (Merck Millipore, Tokyo, Japan).

### *Sample Processing*

A 0. 5 g sample of the gel was placed into a glass tube with 10 mL of methanol and shaken in the absence of light for 16 hours at 30℃. After shaking, the sample was filtered using a suction funnel with a glass filter. The residue was washed with methanol and the wash was combined with the filtrate. Next, the volume of the sample solution was adjusted to 25 mL. The sample solution (1 mL) and Milli-Q water (1 mL) were combined and 50 μL of a methanol solution containing 2 μg/ml of the internal standard (positive mode: reserpine, negative mode: BPA-$d_{16}$) was added to the sample solution. The sample solution was analyzed by liquid chromatography/tandem mass spectrometry (LC/MS/MS) after filtration using a PTFE filter (pore size 0. 20 μm, Merck Millipore, Tokyo, Japan).

### *LC/MS/MS analysis*

All samples were analyzed using a QTRAP4000 mass spectrometer (AB SCIEX) equipped with an Agilent 1100 Series HPLC (Agilent Technologies, Inc., Santa Clara, CA, USA) consisting of a G1376A CapPump, G1377A μ-WPS, G1318A Oven, and G1379A Degasser. Ionization of the target compounds was achieved using electrospray ionization (ESI) in the positive mode (MIT, Cl-MIT, OIT, MBC) or negative mode (PBs and Teb). All compounds were qualified and quantified using multiple reaction mode (MRM). The conditions for the MS/MS analysis, and the precursor (Q1) and product (Q2) ions of the target compounds are shown in Table 2. For LC separation of analytes in the positive mode, eluent A was Milli-Q water containing 0. 02% TFA and eluent B was acetonitrile containing 0. 02% TFA. The gradient elution started from 25% eluent B, which was held for 3 min, then increased linearly to 90% over the course of 2 min, and held for an additional 7 min. An Inertsil® ODS-4 column (length 50 mm, internal diameter 2. 1 mm, particle size 3 μm; GL Sciences, Inc., Tokyo, Japan) was used for separation of the target chemicals. For LC separation of analytes in the negative ion mode, eluent A was Milli-Q water and eluent B was acetonitrile. The gradient elution started at 35% eluent B, which was held for 0. 5 min, and then increased linearly to 60% over the course of 14. 5 min. Next, concentration increased linearly to 90% over the course of 1 min, and then, was held for another 6 min. An Inertsil® ODS-4 column (length 150 mm, internal diameter 2. 1 mm, particle size 3 μm; GL Sciences, Inc., Tokyo, Japan) was used for separation of the target chemicals. Flow rate, column oven temperature, and injection volume were 0. 2 mL/min, 40℃, and 10 μL, respectively. Retention times of the target compounds are shown in Table 2.

## RESULTS AND DISCUSSION

### *Examination of sample processing*

Sharp peaks and good separations were observed the mass chromatograms obtained under the chromatographic conditions of this study. At first, the preliminary experiment for extraction was conducted using the sample provided by NITE. A certain amount of the gel sample was placed into a glass tube with 10 mL of methanol and shaken in the dark for 16 hours at 30℃. After shaking, the samples were centrifuged and the supernatant was analyzed. Then, the residues were re-extracted with 10 mL of methanol in the same manner for 2 hours, the samples were centrifuged, and the supernatant was analyzed. From the results of the first extraction, MIT, OIT, MBC, Me-PB, Bu-PB, and Teb were detected in the sample provided by NITE. From the results of the second extraction, the concentrations of the compounds measured from the second extraction were 0. 6% less than the concentrations measured from the first extraction. Thus, the extraction time was set at 16 hours.

The recovery test was conducted by adding every compound to the samples. Low concentrations (0. 1 μg/g, MIT: 0. 033 μg/g) and high concentrations (20 μg/g, MIT: 6. 7 μg/g) of each compound were added to 0. 5 g of the sample that did not contain the target compounds (n=5). The results of the recoveries are listed in Table 3. At the low concentration, although the recoveries of MIT, Cl-MIT, OIT, and MBC were slightly low (54-77%), the other compounds were obtained in good recoveries (79-111%) and the coefficient of varia-

Table 1 List of the gel products studied

| Usage | No[a] | Country | Materials[b] | Remarks |
|---|---|---|---|---|
| Mattress and pillow | A1* | Japan | superabsorbent polymer/glycerin/water/paraben/moldproof agent/flavor | same product of serious product accident, but lot number is different |
| | A2 | Japan | water/acryl resin/preservative/food colors | keep in refrigerator before using |
| | A3** | China | superabsorbent polymer, water, moldproof agent, etc. | |
| | A4 | Japan | water/sodium sulfate/polymer | keep in refrigerator before using |
| | A5 | Japan | polyhydric alcohol/superabsorbent high polymer/water | keep in freezing chamber before using |
| | A6* | Japan | superabsorbent polymer/glycerin/water/paraben/moldproof agent/flavor | "Allergy checked" printed |
| | A7* | Japan | superabsorbent polymer/glycerin/water/paraben/moldproof agent/flavor | |
| | A8 | China | water/sodium sulfate/polymer | |
| | A9 | Japan | water/antifreezing solution/gelling agent/preservative | keep in freezing chamber before using |
| | A10 | China | carboxyvinyl polymer/glycerin/triethanolamine/moldproof agent/coloring material | keep in freezing chamber before using |
| | A11*** | Japan | water/antifreezing solution/gelling agent/preservative | keep in freezing chamber before using |
| | A12 | China | Unknown | keep in freezing chamber before using |
| | A13 | Unknown | saccharide/superabsorbent polymer/water | keep in freezing chamber before using |
| | A14 | China | superabsorbent polymer/water/moldproof agent/propylene glycol | keep in freezing chamber before using |
| | A15 | Japan | high polymer/water/preservative | keep in refrigerator before using |
| | A16 | China | water/polymer thickener/preservative | keep in refrigerator before using |
| | A17 | Japan | Unknown | keep in refrigerator before using |
| Forehead and neck | B1*** | Japan | water/antifreezing solution/gelling agent | keep in freezing chamber before using |
| | B2*** | Japan | water/antifreezing solution/gelling agent | keep in freezing chamber before using |
| | B3** | China | water, sodium chloride, glycerin, moldproof agent, etc. | keep in refrigerator before using |
| | B4 | Japan | polyhydric alcohol/high polymer superabsorbent resin/water/polyethylene | keep in freezing chamber before using |
| | B5 | Japan | Unknown | keep in freezing chamber before using |
| | B6 | China | water/polymer thickener/preservative | keep in freezing chamber before using |
| Pet animal | C1 | Japan | superabsorbent polymer/water/preservative | |

[a] samples with the same number of asterisks are from the same manufacturer

[b] displayed on the label of the product

Table 2 Molecular weight (M.W.), MS/MS conditions and retention times of chemicals studied

| Chemicals | M.W. | Q1 (m/z) | Q2 (m/z) | DP (volt) | EP (volt) | CE (volt) | CXP (volt) | Retention time (min) |
|---|---|---|---|---|---|---|---|---|
| MIT | 115.0 | 116 | 101 | 96 | 10 | 33 | 18 | 1.10 |
| MBC | 191.2 | 192 | 160 | 56 | 5 | 25 | 10 | 1.11 |
| Cl-MIT | 149.6 | 150 | 135 | 71 | 7.5 | 33 | 6 | 1.39 |
| OIT | 213.3 | 214 | 102 | 46 | 7.5 | 21 | 4 | 8.75 |
| Reserpine | 608.7 | 609 | 448 | 126 | 15 | 41 | 12 | 8.20 |
| Me-PB | 152.2 | 151 | 136 | -25 | -10 | -20 | -7 | 5.43 |
| Et-PB | 166.2 | 165 | 136 | -45 | -10 | -20 | -9 | 8.28 |
| Iso-Pro-PB | 180.2 | 165 | 136 | -45 | -10 | -20 | -9 | 11.51 |
| Pro-PB | 180.2 | 179 | 136 | -30 | -10 | -22 | -9 | 12.01 |
| Iso-Bu-PB | 194.2 | 179 | 136 | -30 | -10 | -22 | -9 | 15.34 |
| Bu-PB | 194.2 | 193 | 136 | -30 | -7.5 | -24 | -9 | 15.64 |
| Be-PB | 228.3 | 227 | 136 | -35 | -7.5 | -22 | -7 | 16.04 |
| Teb | 307.8 | 306 | 223 | -35 | -5 | -12 | -13 | 19.34 |
| BPA-$d_{16}$ | 244.4 | 241 | 223 | -75 | -7.5 | -26 | -13 | 11.80 |

Positive mode (MIT, MBC, Cl-MIT, OIT, Reserpine): TEM (600), CUR (20), GS1 (70), GS2 (80), CAD (6), IS (4500)
Negative mode (Me-PB, Et-PB, Iso-Pro-PB, Pro-PB, Iso-Bu-PB, Bu-PB, Be-PB, Teb, BPA-$d_{16}$): TEM (400), CUR (40), GS1 (60), GS2 (70), CAD (3), IS (-4500)

Table 3 Limit of detection (LOD), limit of quantification (LOQ) and recoveries of chemicals studied

| Chemicals | LOD[a] | LOQ[b] | Recoveries (n = 5)[c] | | | |
|---|---|---|---|---|---|---|
| | ($\mu$g/g) | ($\mu$g/g) | low (%) | CV (%) | high (%) | CV (%) |
| MIT | 0.0074 | 0.017 | 69 | 6.1 | 94 | 3.7 |
| Cl-MIT | 0.044 | 0.10 | 68 | 11 | 90 | 3.3 |
| OIT | 0.012 | 0.029 | 77 | 4.0 | 95 | 3.7 |
| Me-PB | 0.035 | 0.081 | 79 | 7.6 | 99 | 2.5 |
| Et-PB | 0.024 | 0.057 | 88 | 5.1 | 99 | 3.1 |
| Iso-Pro-PB | 0.023 | 0.055 | 95 | 4.9 | 86 | 4.6 |
| Pro-PB | 0.010 | 0.024 | 100 | 2.0 | 97 | 3.3 |
| Iso-Bu-PB | 0.020 | 0.047 | 108 | 3.4 | 100 | 4.2 |
| Bu-PB | 0.024 | 0.057 | 111 | 4.2 | 96 | 4.4 |
| Be-PB | 0.021 | 0.049 | 107 | 3.3 | 109 | 3.6 |
| MBC | 0.0079 | 0.018 | 54 | 5.8 | 89 | 2.3 |
| Teb | 0.047 | 0.11 | 109 | 7.5 | 101 | 5.6 |

[a] LOD was caluculated according to JIS K0136 using the standard deviation ($\rho$) and t-value (t = 4.26 for n = 5) obtained from the recovery test of low concentration.
[b] LOQ was calculated as ten times of $\rho$.
[c] low: 0.1 $\mu$g/g (MIT: 0.033 $\mu$g/g), high: 20 $\mu$g/g (MIT: 6.7 $\mu$g/g), CV: coefficient of variation

tion (C.V.) of all compounds was less than 10%, except Cl-MIT (11%). At high concentrations, good recoveries (89-109%) of all compounds were obtained and the C.V. of all compounds was below 5.6%. The limit of detection (LOD) was calculated according to JIS K 0136[10] using the standard deviation ($\rho$) and t-value (t=4.26 for n=5) obtained from the recovery tests at low concentration. LOQ was calculated as ten times $\rho$[11]. The LODs and LOQs are listed in Table 3 and the LODs and LOQs of the isothiazolinones were in the ranges of 0.0074-0.012 μg/g and 0.017-0.10 μg/g, respectively. The other compounds were also obtained with good LODs (0.0079-0.047 μg/g) and LOQs (0.018-0.11 μg/g).

***Concentrations and detection frequencies of the target compounds in the gel product samples***

The concentrations of the target compounds in the gel-product samples are shown in Table 4. The mass chromatograms of MIT, Cl-MIT, and OIT obtained from A11 are shown in Fig. 1. OIT was detected in samples A7 and A11, and these concentrations were 0.14

Table 4 Concentraions (μg/g) and detection frequenies (%) of chemicals studied in the gel products used for cooling

| Samples | Isothiazolinones | | | Parabens | | | | | | | Others | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | MIT | Cl-MIT | OIT | Me-PB | Et-PB | Pro-PB | Iso-Pro-PB | Bu-PB | Iso-Bu-PB | Be-PB | MBC | Teb |
| A1 | 0.12 | – | – | 298 | – | – | – | 12 | – | – | 54 | 1.5 |
| A2 | –[a] | – | – | 431 | 285 | – | – | – | – | – | – | – |
| A3 | – | – | – | – | – | – | – | – | – | – | – | – |
| A4 | – | – | – | – | – | – | – | – | – | – | – | – |
| A5 | – | – | – | – | – | – | – | – | – | – | – | – |
| A6 | – | – | – | 696 | – | – | – | 223 | – | – | 0.82 | 25 |
| A7 | – | – | 0.14 | 648 | – | – | – | 212 | – | – | 1.4 | 15 |
| A8 | 0.87 | – | – | – | – | – | – | – | – | – | – | – |
| A9 | – | – | – | – | – | – | – | 26 | – | – | – | – |
| A10 | – | – | – | 509 | 413 | 33 | – | – | – | – | – | – |
| A11 | 4.2 | 12 | 2.2 | – | – | – | – | – | – | – | – | – |
| A12 | – | – | – | 471 | 500 | 74 | – | – | – | – | – | – |
| A13 | – | – | – | – | – | – | – | – | – | – | – | – |
| A14 | 8.1 | 16 | – | – | – | – | – | – | – | – | – | – |
| A15 | 115 | – | – | – | – | – | – | – | – | – | – | – |
| A16 | 6.4 | 8.9 | – | – | – | – | – | – | – | – | – | – |
| A17 | 5.4 | tr[b] | – | – | – | – | – | – | – | – | – | – |
| B1 | – | – | – | 19 | – | – | – | – | – | – | – | – |
| B2 | – | – | – | – | – | – | 233 | 167 | 172 | – | – | – |
| B3 | 8.6 | 15 | – | – | – | – | – | – | – | – | – | – |
| B4 | – | – | – | – | – | – | – | – | – | – | – | – |
| B5 | 5.3 | – | – | – | – | – | – | – | – | – | – | – |
| B6 | 7.8 | 0.11 | – | – | – | – | – | – | – | – | – | – |
| C1 | 11 | – | – | – | – | – | – | – | – | – | – | – |
| Detection frequency (%)[c] | 46 | 25 | 8.3 | 29 | 13 | 8.3 | 4.2 | 21 | 4.2 | 0 | 13 | 13 |

[a] –: not detected [b] Between LOD and LOQ [c] Detection frequency (%) = 100×(number of sample above LOD)/(number of all sample)

μg/g and 2. 2 μg/g, respectively. The detection frequency of OIT was 8. 3%. MIT was detected in 11 samples, and the concentration range and detection frequency were 0. 12–115 μg/g and 46%, respectively. Cl-MIT was detected in six samples, with concentration ranging from trace amount to 16 μg/g and a detection frequency of 25%. Detection frequency of MIT was highest among all the target compounds. All parabens, except Be-PB, were detected in gel-products and their concentrations were 12–696 μg/g. It was observed that three kinds of PBs were detected in the same sample in the cases of A10 and A12. Furthermore, isomers Bu-PB and Iso-Bu-PB were detected in the same sample (B2). The detection frequency of Me-PB (29%) was highest among the PBs. However, the detection frequency of PBs (38%) was lower than that of MIT (46%). MBC and Teb were detected in three samples and these concentrations were 0. 82–54 μg/g and 1.5–25 μg/g, respectively.

Although the minimum inhibitory concentration (MIC) of OIT is 2 mg/L for some kinds of mold and yeast, the MIC of OIT was reported to be over a few hundred mg/L in the case of some types of fungus [12]. On the other hand, it was reported that the MIC of MIT for some kinds of freshwater mold was 31 mg/L[13]. Thus, the concentrations of OIT and MIT detected in this study might not be sufficient for use as anti-mold agents if these preservatives are used separately. In some cases, isothiazolinone preservatives were used as a mixture, for example, OIT and MBC (for example, ROCIMA™ 367N[14]). Furthermore, Cl-MIT was only detected in samples that contained MIT and the concentrations of Cl-MIT detected in the samples were two or three times higher than those of MIT, except for in A17 and B6. A similar tendency was also observed in wallpaper adhesives[15]. It was thought that the reason for this tendency was that the preservatives always contained Cl-MIT as a mixture with MIT (for example, Kathon™ CG[6]) and the concentration of Cl-MIT was three or four times higher than MIT in these preservatives. Since Cl-MIT was not chemically stable, Cl-MIT in A17 and B6 might decompose in gel-products

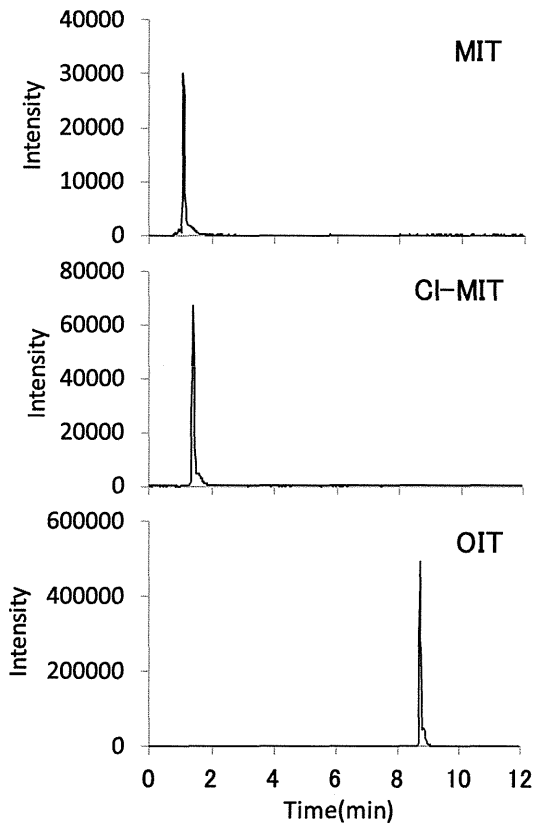


Fig. 1 Mass chromatograms of MIT, Cl-MIT, and OIT obtained from sample A11

during storage.

The concentration of OIT in A1 was below LOD even though A1 was the same product used for the elucidation of contact dermatitis reported in the serious product accident (with a different lot number) and OIT was detected in the control sample provided by NITE. Therefore, the difference in lot numbers might affect this result. A1, A6, and A7 were manufactured by same company, and Me-PB, Bu-PB, MBC, and Teb were detected in these samples. Notably, MBC and Teb were only detected in the samples manufactured by this company. On the other hand, none of the target compounds were detected in A3, and MIT and Cl-MIT were detected in B3 even though these two samples were manufactured by the same company. Although A11, B1, and B2 were manufactured by the same company, A11 contained MIT, Cl-MIT, and OIT, while B1 and B2 contained PB. These results suggest that different kinds of preservatives were used in gel-products manufactured by the same company, depending on the intended use of the gel-product.

***Safety of gel products used for cooling***

It was reported that the ranking of sensitization potential was MIT/Cl-MIT > OIT[16]. However, since OIT is a moderate sensitizer[3], the recommended concentration of OIT used to perform the patch test was reported (0. 011–0. 1% in pet.)[4]. It was reported that a sewing machine operator presented with contact dermatitis caused by contact with mattress textile, and 40–50 μg/g of OIT was detected in the mattress textile. The concentrations of OIT detected in the present study were lower than those in the case of the mattress textile[4]. The EU has regulated the concentrations of MIT and Cl-MIT in cosmetic products to less than 15 μg/g as a mixture (MIT: 3. 75 μg/g, Cl-MIT: 11. 25 μg/g), and 100 μg/g (MIT only)[9]. Although the gel-product used for cooling was not involved in this cosmetic regulation, the EU limit was used to evaluate the risk of skin sensitization. The concentrations of MIT and Cl-MIT detected in A11, A14, and B3 were over the EU regulation limit. On the other hand, MIT was detected in A1, A8, A15, B5, and C1, although Cl-MIT was not detected in these samples. MIT is used for the preservative that does not contain Cl-MIT (for example, NEOLONE™ DsP[17]). The contact dermatitis caused by MIT has been reported[18]. The concentration of MIT in A15 was over the EU regulation limit. There is no existence of cross-reactivity between MIT/Cl-MIT and OIT, although cross-reactivity between MIT and Cl-MIT has been reported[4, 16]. The concentration of PBs used in cosmetic products has also been regulated in the EU to less than 0. 4% (individually) and 0. 8% (total amount of PBs)[9]. All the concentrations of PBs detected in this study were below this regulation value. MBC was not a skin sensitizer because it was reported that the result of the patch test for MBC (1. 0% pet.) was negative[19]. Teb also was not a skin irritant based on the material safety data sheet provided from supplier.

In general, gel-products used for cooling are put into a plastic bag and it is recommended by the manufacturer that sheet or pillow be used as a cover. This notification is described on the surface of the product. Thus, gel-products containing preservatives usually does not come in direct contact with skin. However, in the case of the serious product accident, the patient was exposed to OIT for a long time because the OIT from the gel might have penetrated to the textile surface[1]. It is possible that similar contact dermatitis can occur when the consumer's weight presses on the cooling gel-product used for a pillow as in A14 and A15, for which the concentrations of MIT and Cl-MIT were over the EU regulation value. Furthermore, it is thought that the cooling gel-products used on the forehead or neck (such as B3) have a similar risk of skin sensitization if the gel-product surface tears and the gel containing isothiazolinone preservative leaks out. Thus, it is advisable to replace preservatives used in gel-products for cooling with non-sensitizing preservatives. The components in almost all the samples investigated in this study were described only as "preservative" and did not show any information about the specific chemical components (Table 1). It is likely that the identification of the causative substance may be delayed when contact dermatitis related to these products occurs. Thus, it is desirable that manufacturers provided information about the components of the gel-products used cooling directly on the surfaces of the products.

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報　文

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# 有害物質含有家庭用品規制法のトリフェニル錫(TPT)およびトリブチル錫(TBT)分析法改定過程において観察されたTPTの分解について

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# Study on the Degradation of Triphenyltin (TPT) observed in the Process for the Revision of the Official Analytical Method for TPT and Tributyltin (TBT) in Household Products

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**Abstract**

The round-robin test to evaluate the method for the simultaneous determination of triphenyltin (TPT) and tributyltin (TBT) in household products developed by National Institute of Health Sciences in Japan was performed by six laboratories. Samples of three types (textile, water-based adhesive, and oil-based paint) were prepared by addition of known amounts of TPT and TBT (0.1, 1.0, 10 μg/g) and sent to the participants. They were analyzed in our laboratory five month later by GC-MS after ethyl-derivation with sodium tetraethylborate. TBT in the samples showed acceptable recoveries of 66-120%, while TPT concentrations were considerably low in all samples. In the textile and water-based adhesive, diphenyltin (DPT), the degradation product of TPT, was detected, and the total amounts of DPT and TPT were comparable to the added TPT. Thus, it was confirmed that TPT converted to DPT by dephenylation in these samples. In the case of oil-based paint, however, DPT was not detected. It seemed that DPT was adsorbed strongly onto the silica cartridge column used for the clean-up of oil before the ethyl-derivation step.

**Keywords:** tributyltin, triphenyltin, household product, GC-MS, diphenyltin

## I　緒言

トリフェニル錫(TPT)およびトリブチル錫(TBT)化合物は、藻類や貝類に殺生物作用を有し、船底や漁網の防汚剤として使用されたことから、1980年代には海洋汚染物質となり、牡蠣やムール貝の養殖事業に大打撃を与えた[1]。1985年頃から巻貝のインポセックスなど環境ホルモン作用が問題[2,3]となり、安全性評価がすすめられ、免疫や生殖に関わる毒性が明らかにされた[4]。現在、TPTおよびTBTは、化学物質管理の考えが導入され、日本では生産、輸出入[5]および排出[6]に関して規制され、2012年にEUにおいてもREACHによって規制が始まった[7]。

また、TPTおよびTBTは、皮膚刺激性を有し、経皮吸収されやすく、生殖機能障害を引き起こすことから、「有害物質を含有する家庭用品の規制に関する法律(家庭用品規制法)」によって、TPTは1979年に、TBTは1980年に、人体に直接接触する繊維製品などの家庭用品にこれら化合物を使用させないことを目的に規制された[8, 9]。家庭用品規制法におけるTPTおよびTBTの分析法は、家庭用品からこれらの化合物を溶媒抽出した後、精製、硝酸分解を行い、フレームレス原子

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