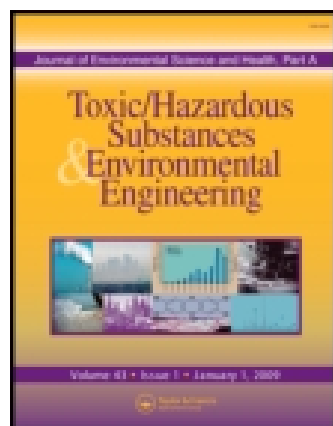


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Analysis of isothiazolinone preservatives in polyvinyl alcohol cooling towels used in Japan

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Recently, cases of contact dermatitis that were related to the use of polyvinyl alcohol (PVA) cooling towels containing isothiazolinone preservatives were reported in Japan. The aim of this investigation was to analyze the concentrations of five different isothiazolinone compounds present in PVA towels and to assess the effectiveness of washing in removing the preservatives from new towels prior to being used for the first time. Twenty-seven PVA towels were used in this study. Two groups (i.e., laboratory-simulation and volunteer) of washing experiments were conducted to evaluate the effect of washing procedures. Qualitative and quantitative analyses were performed by LC/MS/MS, which detected 2-methyl-4-isothiazolin-3-one (MI) and 5-chloro-2-methyl-4-isothiazolin-3-one (CMI) in 23 samples (MI: 0.29–154 $\mu\text{g g-wet}^{-1}$, CMI: 2.2–467 $\mu\text{g g-wet}^{-1}$), 2-*n*-octyl-4-isothiazolin-3-one (OIT) in one sample (478 $\mu\text{g g-wet}^{-1}$). The compounds 4,5-Dichloro-2-*n*-octyl-4-isothiazolin-3-one (2Cl-OIT) and 1,2-benzisothiazolin-3-one (BIT) were not detected in all samples. We confirmed the presence of residual MI, CMI, and OIT in the washed towels, and the residual-to-original content ratio of OIT was higher than that of MI and CMI in PVA towels, due to the higher hydrophobicity of OIT than MI and CMI. A concern has been raised about the occurrence of contact dermatitis being caused by the use of PVA towels. It is suggested that a detailed description of isothiazolinone preservatives in PVA towels and an effective washing procedure for the removal of these preservatives should be provided by the manufacturer. Further, alternative non-sensitizing preservatives might be considered for the manufacture of PVA cooling towels in the future.

Keywords: Isothiazolinone preservatives, polyvinyl alcohol cooling towel, contact dermatitis; household products.

Introduction

Isothiazolinone compounds, comprised of nitrogen- and sulphur-containing heterocyclic aromatic rings, are widely used as preservatives in cosmetics,^[1] paints,^[2] oils,^[3] anti-fouling booster biocides,^[4] wall adhesives,^[5] and moist tissue paper^[6] because of their broad-spectrum antibacterial activities. In particular, a mixture of 2-methyl-4-isothiazolin-3-one (MI; CAS 2682-20-4) and 5-chloro-2-methyl-4-isothiazolin-3-one (CMI; CAS 26172-55-4) is one of the most extensively used isothiazolinone preservatives (e.g., Kathon® CG)^[7] in cosmetics and industrial products, and its first use can be traced back to 1976 in Europe and 1981 in the United States.

However, these preservatives have the potential to cause skin sensitization, and many cases of isothiazolinone

preservative-induced contact dermatitis have been reported.^[1,8] The European Union (EU) recently stipulated that the permissible concentration of MI and CMI mixtures in cosmetic products should be less than 15 $\mu\text{g g}^{-1}$ (MI: 3.75 $\mu\text{g g}^{-1}$, CMI: 11.25 $\mu\text{g g}^{-1}$).^[9] In the United States, the permissible concentration of MI and CMI mixtures for rinse-off products, e.g., shampoo and soap, is similar to that stipulated by the EU; however, it is less than 7.5 $\mu\text{g g}^{-1}$ for leave-on products such as body creams.^[10] The permissible concentration for the MI preservative alone is less than 100 $\mu\text{g g}^{-1}$.^[9,10] In Japan, the cosmetic industry standards require the concentration of MI and CMI mixtures to be less than 15 $\mu\text{g g}^{-1}$ for rinse-off products only, and that of MI alone is to be less than 100 $\mu\text{g g}^{-1}$.^[11]

In recent years, several types of novel household and personal products have been developed that help keep the body cool; these are especially popular during the humid summer season in Japan. These products include: (i) gel-based products that use high-water content superabsorbent polymers that can dissipate heat from skin, and (ii) polyvinyl alcohol (PVA) towels (Fig. 1) that provide a cooling sensation to the skin by absorbing excess heat as a result of hyper-efficient water evaporation. Unfortunately, the use of mattress-type gel products have in mattress

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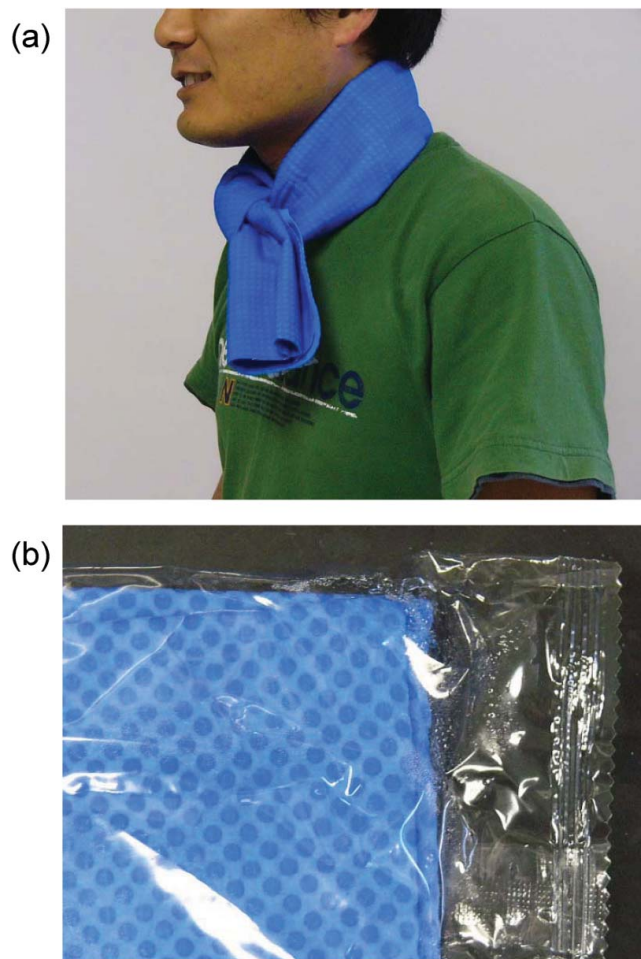


Fig. 1. (a) An example of the usage of PVA cooling towel, and (b) unopened PVA cooling towel (sample A5). Water drops can be seen inside the package and are noticeable.

manufacture have led to several cases of allergic contact dermatitis.^[12]

These gel products used 2-*n*-octyl-4-isothiazolin-3-one (OIT; CAS 26530-20-1) as a preservative, which probably migrated from the gel layer to the textile surface through a polyethylene film and was directly exposed to the patient's skin. One of the cases where the patient required over 30 days to recover was classified as a severe consumer product accident as per the Consumer Safety Product Act legislated by the Ministry of Health, Labour and Welfare, Japan. This motivated us to investigate the amounts of isothiazolinone preservatives in commercial gel products in Japan from April to June, 2010, and OIT, MI, and CMI were detected in these gel products studied.^[13]

On the other hand, cases of contact dermatitis caused by the use of PVA cooling towels were also reported by the National Consumer Affairs Center of Japan (NCAC) in 2012.^[14] Generally, since dried PVA towels are hard and easily broken, these are moistened with water to prevent

them from breaking before they depart the factory (Fig. 1), and the water-containing isothiazolinone preservatives are used in the PVA towels to protect the products against mildew. Although NCAC has confirmed the presence of isothiazolinone preservatives in several PVA towels, the amounts of these preservatives have not been accurately determined.

Although some manufacturers suggest that customers wash new towels before using them to remove the preservatives, the effect of washing on the removal of the preservatives has not been assessed previously. Therefore, it is necessary to study isothiazolinone preservatives in PVA towels for the prevention and control of contact dermatitis, which is caused by the use of these cooling towels.

The aim of this study was to confirm the presence of five isothiazolinone preservatives in the PVA towels, quantify their concentrations, and evaluate the effectiveness of the washing process in removing the preservatives from the PVA towels before their first-time use. We conducted two types of washing experiments: (i) a laboratory-simulated washing procedure, which used a glass tube and mechanical shaker for the washing and elution of preservatives in ultrapure water (Milli-Q water) for analysis, and (ii) volunteer-based washing, where the towels were washed by volunteers as per the products instruction.

Materials and methods

PVA towel samples

Twenty-seven PVA cooling towels were purchased from several online and retail stores in Japan in December, 2011 (sample A1 to A7), and from May to June, 2012 (sample B1 to B20) (Table 1). All the PVA towels were used for skin cooling (Fig. 1), except samples A6 and A7, which were used to wipe the body after swimming. The towels were moist at the time of purchase (Fig. 1), except for sample B19, which was sold in a dry condition.

There exists a one-to-one correspondence between samples A1–A5 and samples B1–B5, e.g., A1 corresponds to B1, because they are the same product purchased at different times. An additional 19 towels (same product as sample B8) were purchased in December 2012, of which 5 were used to quantify the amounts of preservatives in PVA towels, and the other 14 were distributed to volunteers for washing to assess the effectiveness of the washing procedure in removing preservatives.

Materials and reagents

The mixture of MI and CMI (MI: 3.63%, CMI: 10.85%) was obtained from Waterstone Technology (Carmel, IN, USA). 1,2-Benzisothiazolin-3-one (BIT; CAS 2634-33-5) and OIT were purchased from Tokyo Chemical Industry

Table 1. List of PVA towel samples used in this study.

<i>Year of purchase</i>	<i>Sample name</i>	<i>Country of manufacture</i>	<i>Instruction notes as displayed^a</i>
2011	A1	China	To remove the preservative, wash the towel in water before using it for the first time (soak and wring it several times). The preservative is harmless.
	A2	Thailand	Wash out the towel in warm water before using it for the first time.
	A3	China	Wash and lightly wring the towel before using it for the first time. The product is soaked in water containing a harmless preservative.
	A4	China	Wash the towel in tap water before using it for the first time.
	A5	China	To remove the specific smell and prevent deterioration, wash the preservative (using cosmetic products) in warm water before using it for the first time.
	A6 ^b	Japan	Hand-wash the towel before using it for the first time to remove storage solution.
	A7 ^b	China	Sufficiently wring the towel before using it for the first time.
2012	B1	China	Sufficiently wash before using the towel for the first time to remove the soaking water containing the preservative. Isothiazolinones are used for cosmetic products and quasi-drugs as preservative and have the potential to cause allergic contact dermatitis.
	B2	Thailand	Benzoic acid is used to prevent mold. Wash the towel in water or warm water before using it for the first time.
	B3	China	Wash the towel before using it for the first time. The harmless preservative (sodium diacetate) is used.
	B4	China	Wash the towel before using it for the first time. The harmless preservative (sodium diacetate) is used.
	B5	China	Wash the towel in warm water before using it for the first time to remove the preservative used for preventing deterioration. Isothiazolinone preservatives are used to prevent mold. "These preservatives with the potential of skin sensitization" are described.
	B6	China	Soak the product in warm water (about 40°C) before using the towel for the first time.
	B7	China	Sufficiently wash the towel before using it for the first time.
	B8	China	Wash the towel in tap water and lightly wring it before using it for the first time.
	B9	China	Wash and lightly wring the towel before using it for the first time. The product is soaked in the water containing harmless preservative.
	B10	China	Wash the towel in tap water and lightly wring it before using it for the first time. The attached sheet enclosed with the product instructs, "Isothiazolinones are used as preservatives. To remove the preservatives, wash by warm water before first use." The preservatives with the potential of skin sensitization are described.
	B11	China	Since the PVA towel contains preservatives (MI and CMI), wash in running warm water and wring the PVA towel two or three times (about 60 sec) before using the towel for the first use to completely remove the preservatives. These preservatives have the potential of causing allergic contact dermatitis.
	B12	China	To remove the isothiazolinone preservatives (MI and CMI), sufficiently wash the towel in the water or warm water before using it for the first time. The note about allergic contact dermatitis is described.
	B13	China	Preservatives (MI and CMI) are used for preventing deterioration. Sufficiently wash the towel in warm water before using it for the first time to remove the preservatives. The note about allergic contact dermatitis is described.
	B14	China	Wash the towel in water before using it for the first time. The towel is moistened in the water containing the harmless preservative to prevent dryness.
	B15	Japan	Wash the towel before using it for the first time because the towel is soaked in the water containing the preservative.
	B16	China	Wash the towel in water before using it for the first time. The product contained preservatives (isothiazolinones).
	B17	China	The towel is soaked in the water containing preservatives (MI and CMI). To remove the preservatives, sufficiently wash it in warm water before using it for the first time. Allergic contact dermatitis can be caused by isothiazolinone is described in the notes.
	B18	China	The towel is soaked in the water contained harmless sodium diacetate. Wash it in water and lightly wring it before using it for the first time.
	B19 ^c	China	Wash the towel before using it for the first time.
	B20	China	The preservatives for cosmetic grade were used and the treatment of preservation performed in Japan. Wash the towel in water or warm water before using it for the first time in order to prevent the allergic contact dermatitis caused by the preservatives. "Isothiazolinone preservatives were not used in this product." is presented.

^aSummary of the note described on the product.^bFor wiping body after swimming.^cSold in a dry condition.

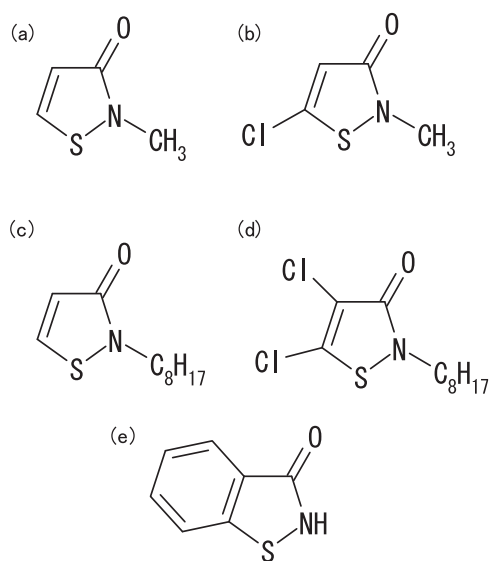


Fig. 2. Chemical structures of isothiazolinone preservatives investigated in this study. (a) 2-methyl-4-isothiazolin-3-one (MI), (b) 5-chloro-2-methyl-4-isothiazolin-3-one (CMI), (c) 2-*n*-octyl-4-isothiazolin-3-one (OIT), (d) 4,5-dichloro-2-*n*-octyl-4-isothiazolin-3-one (2Cl-OIT), and (e) 1,2-benzisothiazolin-3-one (BIT).

Co., Ltd. (Tokyo, Japan), and 4,5-dichloro-2-*n*-octyl-4-isothiazolin-3-one (2Cl-OIT; CAS 64359-81-5) was obtained from AK Scientific, Inc. (Union City, IN, USA). The chemical structures of these isothiazolinone preservatives are shown in Figure 2. Reserpine, used as an internal standard, and HPLC-grade acetonitrile and trifluoroacetic acid (TFA) were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Pesticide residue-grade methanol was obtained from Sigma-Aldrich Japan Inc. (Tokyo, Japan). Ultrapure water was produced by a Milli-Q Advantage A10 water purification system (Merck Millipore, Tokyo, Japan).

Sample processing

A 0.5 g sample was collected from each towel by scission and placed in a glass tube filled with 5 mL methanol, followed by a 30-min shaking on a horizontal shaker. The sample solution was then filtered on a suction funnel with a glass filter, and the residue was washed with methanol. The filtrate was combined with the wash, and the volume of the combined solution was adjusted to 10 mL using methanol. To dilute the sample solution to an appropriate concentration with methanol, 1 mL of the sample solution was mixed with 1 mL of ultrapure water, and the solution was combined with 50 μ L of methanol containing 2 μ g mL⁻¹ of reserpine. Subsequently, the sample solution was filtered by using a PTFE filter (pore size: 0.20 μ m, Merck Millipore) and analyzed by liquid chromatography/tandem mass spectrometry (LC/MS/MS).

Assessing the effectiveness of the washing step in the removal of isothiazolinone preservatives

Laboratory-simulated washing procedure

Samples B8 and B9 were used for examining the effect of a laboratory-simulated washing procedure on the removal of isothiazolinone preservatives from new PVA towels. Each sample was divided into 2 cm $\times$ 2 cm squares, of which 4 pieces were placed into a glass tube. Afterwards, 20 mL of ultrapure water (25 $\pm$ 2°C) was added to the glass tube, which was then shaken on a horizontal shaker at 300 rpm for 30 sec. After the shaking, the sample solution was filtered, and the volume of the sample solution was adjusted to 25 mL by adding ultrapure water. This washing process was repeated three times, and the sample solution obtained after each wash was analyzed individually.

The sample solution was diluted to the appropriate concentration with ultrapure water. Afterwards, 1 mL of the sample solution was combined with methanol, mixed with 50 μ L of the methanol solution containing 2 μ g mL⁻¹ of reserpine, and filtered through a PTFE filter prior to LC/MS/MS measurements. In addition, the towel sample was extracted by methanol using the aforementioned method after the laboratory-simulated washing process. Furthermore, we performed additional analysis on sample B8, which was washed by using warm ultrapure water (40 $\pm$ 2°C). In these laboratory-simulated experiments, the experiments were carried out in quadruplicate for every sample.

Washing by volunteers

The volunteers washed the samples as per the product notes found on the product case, which said 'Wash and wring the towel using tap water before its first usage,' without providing any additional instructions. After the wash, the PVA towels were collected and analyzed using the same procedure described here.

LC/MS/MS analysis

All LC/MS/MS analysis was carried out on an LC800 system (GL Sciences, Inc., Tokyo, Japan) equipped with a QTRAP 4000 mass spectrometer (AB SCIEX, Framingham, MA, USA) using two mobile phase solvents: ultrapure water containing 0.02% TFA (solvent A) and acetonitrile containing 0.02% TFA (solvent B). The gradient elution started from 25% of eluent B, which was held constant for 2 min, and increased linearly to 90% over 2 min, which was held constant for another 12 min.

An Inertsil ODS-4 column (50 mm $\times$ 2.1 mm id, 3 μ m; GL Sciences, Inc.) was used for the separation of the target compounds. The flow rate, column oven temperature, and

Table 2. Molecular weight (M.W.), retention time, and MS/MS conditions for the studied chemicals.^a

Chemical	M.W.	Retention time (min)	Q1 ^b (m/z)	Q2 ^c (m/z)	CE ^d (Volt)
MI	115.0	1.03	116	101	33
CMI	149.6	1.45	150	135	33
BIT	151.2	1.78	152	134	37
OIT	213.3	5.72	214	102	21
2Cl-OIT	282.2	6.52	282	170	23
Reserpine ^e	608.7	5.23	609	448	41

^aPositive mode.^bQ1: Precursor ion.^cQ2: Product ion.^dCollision energy.^eInternal standard.

injection volume were 0.2 mL min⁻¹, 40°C, and 10 µL, respectively. The retention times of the target compounds are listed in Table 2. The target compounds were ionized by electrospray ionization (ESI) in the positive mode. Multiple reaction monitoring (MRM) mode was used for the qualitative and quantitative analysis of all the compounds. The precursor (Q1) and product (Q2) ions of the target compounds are shown in Table 2.

Recovery, limit of detection (LOD), and limit of quantification (LOQ)

Recovery tests were performed to confirm the validity of the analytical method used in this study. Each low-concentration (0.5 µg g⁻¹, MI: 0.17 µg g⁻¹) and high-concentration (10 µg g⁻¹, MI: 3.3 µg g⁻¹) compound was added to 0.5 g of the sample that did not contain the target compounds (n = 4).

Results and discussion

Recovery, limit of detection (LOD), and limit of quantification (LOQ)

The recoveries are shown in Table 3. When the isothiazolinone compounds were present at low concentrations,

although the recovery of 2Cl-OIT was slightly low (78%), good recoveries (97–109%) were obtained for all the other isothiazolinone compounds. The values of coefficient of variation (CV) of all the compounds was below 10%, except that of MI (11%). When the isothiazolinone compounds were present in high concentrations, good recoveries (96–110%) were obtained for all isothiazolinone preservatives, and their CV values were below 3.3%. LOD was calculated using the standard deviation (ρ) and t-value (t = 4.71 for n = 4) obtained from the recovery tests carried out at low concentrations of the compounds,^[15] and LOQ was calculated based on 10 times the value of ρ .^[16] The LODs and LOQs of isothiazolinone preservatives were in the range of 0.071–0.20 µg g-wet⁻¹ and 0.15–0.42 µg g-wet⁻¹, respectively (Table 3).

Concentration of isothiazolinone preservatives in PVA cooling towels

MI and CMI were detected in 23 samples, corresponding to a detection frequency of 85%. OIT was detected only in sample B8. In contrast, BIT and 2Cl-OIT were not detected in any sample. The concentrations of MI, CMI, and OIT, measured based on the wet or dry weight of each sample, are summarized in Table 4. The concentration of MI and CMI in the identified samples was in the range of

Table 3. Limit of detection (LOD), limit of quantification (LOQ), and recoveries of isothiazolinone preservatives.

Chemical	LOD ^a	LOQ ^b	Recoveries (n = 4) ^c			
	(µg/g-wet)	(µg/g-wet)	Low (%)	CV (%)	High (%)	CV (%)
MI	0.095	0.20	109	11	103	3.1
CMI	0.20	0.42	102	8.2	110	2.1
BIT	0.095	0.20	100	4.1	103	2.6
OIT	0.071	0.15	97	3.1	96	3.3
2Cl-OIT	0.085	0.18	78	4.6	100	3.1

^aLOD was calculated according to JIS K0136 using the standard deviation (ρ) and t-value (t = 4.71 for n = 4) obtained from the recovery test using low-concentration chemicals.

^bLOQ was calculated based on 10 times the value of ρ .

^cLow: 0.5 µg g⁻¹ (MIT: 0.17 µg g⁻¹), high: 10 µg g⁻¹ (MIT: 3.3 µg g⁻¹), CV: coefficient of variation.

Table 4. Concentrations of isothiazolinone preservatives in PVA towels.

Sample	Concentrations ($\mu\text{g/g-wet}$)			Concentrations ($\mu\text{g/g-dry}$)		
	MI	CMI	OIT	MI	CMI	OIT
A1	18	77	— ^a	26	113	—
A2	—	—	—	—	—	—
A3	123	362	—	173	509	—
A4	17	82	—	38	178	—
A5	29	113	—	64	246	—
A6	9.3	77	—	18	152	—
A7	57	42	—	108	79	—
B1	0.94	3.0	—	1.8	5.6	—
B2	—	—	—	—	—	—
B3	—	—	—	—	—	—
B4	33	158	—	70	331	—
B5	18	77	—	34	145	—
B6	57	223	—	106	417	—
B7	29	153	—	44	233	—
B8	1.9	11	478	2.9	16	721
B9	129	359	—	223	619	—
B10	20	79	—	35	135	—
B11	154	341	—	285	631	—
B12	105	236	—	197	441	—
B13	0.29	2.2	—	0.50	3.9	—
B14	80	467	—	150	877	—
B15	9.4	45	—	17	84	—
B16	38	149	—	106	409	—
B17	22	80	—	56	203	—
B18	—	—	—	—	—	—
B19 ^b	—	—	—	132	401	—
B20 ^a	6.9	16	—	26	58	—

^aNot detected.^bDry weight basis only.

0.29–154 and 2.2–467 $\mu\text{g g-wet}^{-1}$, respectively, and the concentration of OIT in sample B8 was 478 $\mu\text{g g-wet}^{-1}$.

We also confirmed that the products used for wiping the body after swimming (A6 and A7) and sold in dry condition (B19) contained MI and CMI. Therefore, it was suggested that the isothiazolinone preservatives might be used for PVA towel in general, regardless of the purpose of usage or the condition of preservation. It is worth noting that the MI and CMI concentrations differed between the same product purchased at different times (e.g., A1 and B1). This might be because some manufactures changed the composition and/or amounts of preservatives used in the PVA towels following an announcement about the health risks of isothiazolinone preservatives used in PVA towels made by NCAC in January 2012. The isothiazolinone preservatives were present at various concentrations in the towels that carried an indication regarding the use of isothiazolinone preservatives. However, in the case of sample B20, although the product description claimed “isothiazolinone preservatives were not used in this product”, we also detected MI and CMI in this sample.

Recently, MI was solely used in cosmetic products and paints without being combined with CMI, and there were

reported cases of contact dermatitis after using these products.^[17] In our study, MI was found with CMI in the samples, and its concentrations were lower than those of CMI. Therefore, we can assume that the mixture of MI and CMI, such as Kathon CG, might be used as a preservative for the PVA towels. The concentrations of isothiazolinone preservatives detected in this study were higher than those in gel products (trace–115 $\mu\text{g g}^{-1}$) used for cooling reported in our previous study,^[13] and also higher than the concentrations of MI and CMI in other household products reported as follows: 10–15 $\mu\text{g g}^{-1}$ in moist toilet paper (Kathon CG),^[6] 5–28.4 $\mu\text{g g}^{-1}$ in household paint,^[5] and 4.4–10.4 $\mu\text{g g}^{-1}$, 13.7–26.5 $\mu\text{g g}^{-1}$, and 5.9–133 $\mu\text{g g}^{-1}$ of MI, CMI, and OIT in wall paper adhesives, respectively.^[18]

Examination of washing effect on the removal of isothiazolinone preservatives

Laboratory-simulated washing procedure

A washing procedure to be performed by consumers before the first-time use of new PVA towels is

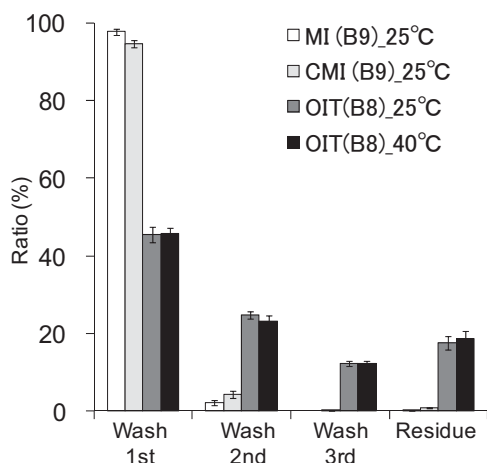


Fig. 3. The removal ratio of isothiazolinone preservatives after each washing cycle, and the ratio of residue to its initial amount in PVA towel before washing. (Error bars indicate $\pm$ standard deviation.)

recommended by most manufactures. Therefore, sample B9 containing MI and CMI, and sample B8 containing OIT were used for examining the effect of the washing procedure. The content ratios of MI and CMI in the washing solution after each washing cycle to the total amount of the preservatives in towel sample B9 before washing are shown in Figure 3. The residual ratios of the preservatives in the washed samples are also shown in Figure 3. After the first wash, 98% of MI and 95% of CMI were removed from the towel; after the entire 3-cycle wash, MI was not detected in the washing solution, and only 0.12% of MI and 0.95% of CMI remained in the PVA towels after the washing procedure.

In contrast, the removal ratios of OIT were 45%, 25%, and 12% for the first, second, and third wash, respectively

(Fig. 3), and 18% of residual OIT remained in the PVA towel after the washing procedure. Next, similar experiments were conducted on sample B8 by washing with warm water ($40 \pm 2^\circ\text{C}$) as recommended by several manufactures. However, no obvious difference was observed when compared to the results obtained by using room-temperature water, or the expected effect was not detected in our experiments (Fig. 3). The octanol-water partition coefficients ($\log P_{ow}$) of MI, CMI, and OIT calculated by Pallas ver. 3.6.2.1 (CompuDrug Chemistry, Inc., Bal Harbor, FL, USA) were 0.28, 1.50, and 3.79, respectively. This result suggests that the adsorption affinity of OIT to PVA material is higher than that of MI and CMI because of its hydrophobic property.

Washing by volunteers

Five PVA towel samples (product B8) purchased in December 2012 were analysed to quantify the amounts of preservatives. Although these towels were obtained by blanket purchase, they were divided into two groups: “group WO” that mainly contained a high concentration of OIT ($n = 2$) and “group WM” that mainly contained high concentrations of MI and CMI ($n = 3$). In the group WO, OIT was detected in both the samples at a concentration ranging from 208 to 531 $\mu\text{g g-wet}^{-1}$, which was similar to the result obtained earlier (sample B8: 478 $\mu\text{g g-wet}^{-1}$). A small amount of OIT was also detected in one of the group WM samples (0.44 $\mu\text{g g-wet}^{-1}$), probably arising from contamination during the manufacturing process. The concentration of MI and CMI in group WM samples was in the range of 99–141 and 157–239 $\mu\text{g g-wet}^{-1}$, respectively ($n = 3$).

The 14 other towels were distributed and washed by volunteers as per product instructions. The concentrations of isothiazolinone residuals in PVA towels after the wash are

Table 5. Concentrations of studied chemicals in PVA towels after washing and some washing conditions in volunteer tests.

Code of sample washed by volunteer	Concentrations ($\mu\text{g/g-wet}$)			Concentrations ($\mu\text{g/g-dry}$)			The number of times to soak and wring the towel	Remarks
	MI	CMI	OIT	MI	CMI	OIT		
WO-1	— ^a	—	9.9	—	—	28	3	Use of neutral detergent
WO-2	—	—	82	—	—	266	10	
WO-3	—	—	83	—	—	208	3	
WO-4	—	—	146	—	—	423	1	Use of warm water
WO-5	—	—	14	—	—	55	3	
WO-6	—	—	49	—	—	183	3	
WO-7	—	—	93	—	—	218	4	
WO-8	—	—	281	—	—	778	2	
WO-9	—	—	195	—	—	591	3	
WM-1	2.6	14	0.24	6.2	34	0.58	3	Use of warm water
WM-2	16	37	0.12	50	117	0.37	1	Rinsing with running water
WM-3	7.4	18	—	21	52	—	5	
WM-4	11	26	—	49	119	—	2	Weakly wring
WM-5	2.2	9.5	—	8.0	35	—	2	

^aNot detected.

presented in Table 5. The isothiazolinone preservatives were detected in all the samples at concentrations above the LOQs. The concentration of OIT in group WO was in the range of 9.9–281 $\mu\text{g g-wet}^{-1}$, and the concentration of MI and CMI in group WM was in the range of 2.2–16 and 9.5–37 $\mu\text{g g-wet}^{-1}$, respectively. In addition, OIT was also detected in two group WM samples (0.12–0.14 $\mu\text{g g-wet}^{-1}$).

Furthermore, we observed that the removal of OIT was less efficient than the removal of MI and CMI; a similar result was also noted in the laboratory-simulated washing experiments. In the case of sample WO-1, which was washed with a neutral detergent, the concentration of OIT was lower than that in other group WO samples, indicating that the use of a neutral detergent might be more effective for the removal of OIT from PVA towels than washing with water alone. However, we could not confirm the effect of the number of washings and the use of warm water on the removal of isothiazolinone preservatives during the washing procedure for PVA towels.

Possibility of increasing of allergic contact dermatitis by the PVA cooling towels

Emmett et al. recommended that the concentration of OIT for patch tests should be 0.025% because skin sensitization can be induced at OIT concentration of 0.05–0.1%.^[19] However, Mose et al.^[20] performed patch tests at OIT concentration of 0.1% because the use of 0.025% OIT might underestimate the positive results.^[20] Because the use of 150 ppm of MI and CMI mixture can induce contact dermatitis, Watanabe et al.^[21] recommended that the concentration of the mixture to perform the patch test is 0.01% (100 ppm).^[21] Furthermore, a 0.01% mixture of MI and CMI has been used for patch tests of standard Japanese allergens^[22] and the European baseline series.^[23] To avoid the underestimation of positive results, the use of 0.02% mixture of MI and CMI is advised,^[24] and the use of 0.2% MI in water was recommended for patch tests of the European baseline series.^[25]

In this study, we found that the concentration of OIT in sample WO 8, which was washed by a volunteer, was above 0.025%, and the concentrations of MI and CMI detected in some towels washed by volunteers were higher than the EU permissible limits for cosmetic products (mixture: 15 $\mu\text{g g}^{-1}$, MI: 3.75 $\mu\text{g g}^{-1}$, CMI: 11.25 $\mu\text{g g}^{-1}$). The occurrence of allergic contact dermatitis on the patient using cosmetic cream containing 12 $\mu\text{g g}^{-1}$ of Kathon CG was reported.^[25] Further, a positive patch test reaction has been obtained from patient using cosmetic products containing about 1 $\mu\text{g g}^{-1}$ Kathon CG.^[26]

Therefore, it is necessary to carefully use the PVA cooling towel, especially for patients who are already sensitive to the isothiazolinone preservatives, because of the possible long-time direct contact of the PVA towel with the skin. However, the instruction notes provided on the

product's packing case or as a piece of enclosed paper also varied, where some described the use of isothiazolinone preservatives and the necessity of removal before the towel's first usage, whereas others only stated "wash before using the towel for its first usage" without stating the purpose of the washing (Table 1).

To prevent the occurrence of allergic contact dermatitis, a detailed description of the composition and amount of isothiazolinone preservatives in the PVA towels, and an effective washing method for removing these chemicals should be provided by the manufactures. Alternatively, it is important to consider the use of non-sensitizing preservatives in the manufacture of PVA towels.

Some cases of contact dermatitis caused by MI and CMI-containing wall paint used in house and office renovation have been reported in the European countries.^[5,27] In these cases, the patients had already been sensitized to MI and CMI by cosmetics and other products containing the same isothiazolinones. In these cases, the patient had not only developed into airborne contact dermatitis, but also showed symptoms like coughing, nasal and conjunctival irritations.

In recent years, cases of contact dermatitis caused by ironing water^[28] and waist-reducing belts^[29] that contain MI and CMI have been also reported. In Japan, we assume the isothiazolinone-sensitized population to be smaller than that in EU countries^[30] due to the exposure to low amounts of isothiazolinone preservatives. However, household wall paper adhesive containing isothiazolinone preservatives are available in the commercial market,^[18] and the case of occupational contact dermatitis for house painter has been reported in Japan.^[31] Furthermore, in this study, we confirmed the use of high concentrations of isothiazolinone preservatives in popular PVA cooling towels in Japan.

A concern has been raised about the increasing incidence rate and risk of airborne contact dermatitis induced by isothiazolinone preservatives by using PVA towels, which might be in direct contact with human skin for a long time. From the perspective of public health, it is necessary to investigate the isothiazolinone preservatives in the various household products that can be potentially exposed to humans.

Conclusion

We investigated the concentrations of five isothiazolinone preservatives (MI, CMI, OIT, 2CI-OIT, and BIT) in 27 PVA cooling towels purchased in Japan. Furthermore, the washing effect of the removal of MI, CMI, and OIT preservatives was examined on the PVA towels before their first usage. MI and CMI were detected in 23 samples in the concentration range of 0.29–154 $\mu\text{g g-wet}^{-1}$ and 2.2–467 $\mu\text{g g-wet}^{-1}$, respectively, corresponding to a detection frequency of 85%. OIT was detected in one sample at a concentration of 478 $\mu\text{g g-wet}^{-1}$, and 2CI-OIT and BIT

were not detected in any sample. We confirmed the presence of residual MI, CMI, and OIT in the washed towels.

The number of washings and the use of warm water have not shown obvious effects on the removal of isothiazolinone preservatives. The residual ratio of OIT was higher than that of MI and CMI in the washed PVA towel, and this was probably because the hydrophobic property of OIT affects the adsorption affinity of OIT with the PVA material. It is however concerning that certain manufacturers do not indicate the purpose and details of the washing procedure sufficiently, as this may induce contact dermatitis if the customers have already been sensitized to isothiazolinone. Therefore, it is suggested to provide a full account of the preservative contents and concentrations, as well as detailed washing instructions for the PVA cooling towels, or to substitute the isothiazolinone preservatives with non-sensitizing preservatives in the towel manufacturing process.

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