

Persistence of Isothiazolinones in Clothes After Machine Washing

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Background: Sensitization to methylchloroisothiazolinone (MCI) and methylisothiazolinone (MI) is a worldwide problem. Washing machine detergents are suspected to cause cutaneous symptoms in highly sensitized patients. Little is known about the persistence of isothiazolinones in clothes that have been washed.

Objective: The aim of the study was to analyze the possible persistence of MI, MCI, benzisothiazolinone, and octylisothiazolinone in common fabrics after machine washing.

Methods: Different clothes (cotton, polyester, linen, and wool) were collected, and 4 types of wash were done (control, standard, standard + conditioner, and standard + double rinse). The samples were analyzed using ultrahigh-performance liquid chromatography.

Results: The results showed that the concentrations of isothiazolinones were very low, independent of the type of material or wash. The highest levels were found in the control wash (hand wash), reaching a maximum of 0.4 ppm in the linen.

Conclusions: Our findings suggest that it is not necessary to recommend that patients sensitized to MI avoid isothiazolinones in machine detergents or fabric conditioners or to double rinse. However, after using the detergent for hand washing (the control in our study), there may remain sufficient concentrations of isothiazolinones in clothes to produce symptoms in highly sensitized patients.

Sensitization to methylchloroisothiazolinone (MCI) and methylisothiazolinone (MI) is a worldwide problem. Fabric conditioners and washing machine detergents are suspected to cause cutaneous symptoms in highly sensitized patients.^{1,2} Little is known about the persistence of isothiazolinones in clothes that have been machine washed.

Generalized dermatitis is not uncommon in isothiazolinone-sensitized patients, and spreading symptoms can occur via contact with cosmetics, paints, or airborne sprays. As isothiazolinones are present in many laundry detergents, avoidance methods or double rinsing after machine may be recommended. In the present study, we attempted to determine the degree of persistence of isothiazolinones in clothes after machine washing. Two patients

(1 man and 1 woman) were patch tested with the standard Spanish series³ because of a generalized cutaneous eruption. Both were very intensely positive for MI (0.2% aq.) and MCI/MI (0.2% aq.; Chemotechnique Diagnostics, Vellinge, Sweden) and negative for other possible allergens. The woman was also patch tested with benzisothiazolinone (BIT) and octylisothiazolinone (OIT), with negative results. The cutaneous symptoms improved in both patients after they started avoiding substances containing isothiazolinones (cosmetics and cleaning products). Both patients provided the same detergent (Colon detergent; Reckitt Benckiser S.L., Barcelona, Spain) and fabric conditioner (Flor Azul; Reckitt Benckiser S.L.) they used to wash their clothes. In a previous study, our group determined high concentrations of MI and BIT in the detergent (29,942.7 and 22,451.4 ppm, respectively),⁴ so it was decided to study the possible persistence of these preservatives in common fabrics using liquid chromatography–tandem mass spectrometry.

Reagents and Instrumentation

Liquid chromatography–mass spectrometry–grade water and analytical-grade methanol were purchased from Fisher Scientific (Pittsburgh, PA). MI, MCI, BIT, and OIT were purchased from Dr. Ehrenstorfer GmbH (Germany). All chemicals were purchased with 96% purity. All analyses were carried out using a high-performance liquid chromatography system (Agilent series 1290; Agilent Technologies, Santa Clara, CA) equipped with a

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TABLE 1. Characteristics of the Washes

	Control (in a Bucket of Water)	Standard	Standard + Fabric Conditioner	Standard + Double Rinse
Detergent (52 mL)	Yes	Yes	Yes	Yes
Fabric conditioner (30 mL)	No	No	Yes	No
Total time, min	30	44	44	63
No. rinses	1	1	1	2
Amount of water, L	5	70	70	70

reversed-phase column and coupled to triple quadrupole (tandem mass spectrometry).

Sample Preparation

Sample fabrics were purchased in a specialized textile store at Las Palmas de Gran Canaria (Spain). The specimens collected were as follows: 100% cotton, 100% polyester, 80% linen/20% cotton, and 100% wool, either white or light colored. Two samples of each material measuring 10 × 20 cm each were used (the woolen samples shrunk after washing to half the original size). A market detergent (Colón) and a fabric conditioner (Flor Azul) were used. The washes were done in a standard washing machine (model WA10J5710SW/GG; Samsung, Thailand), without mixing with any other clothes. The washes were done with the standard program, in cold water, with rinses of 10 minutes and centrifuge for 1 minute. Details of the various types of wash are given in Table 1. The samples were then hung up outside to dry for 16 to 20 hours, and then each sample was placed in an individual plastic bag and kept in darkness until

its analysis 48 hours later. As a control, we used the same fabrics immersed in water with the detergent for 30 minutes and hung up for the same time.

A liquid-liquid extraction procedure with methanol was carried out. To do this, a piece of each cloth was put into a Falcon tube, and then 45 mL of methanol was added. To extract all the compounds, the Falcon tubes were stirred overnight. After that, the same volume of methanol was taken from each sample and then evaporated until dryness. Finally, 15 mL of methanol was added to wash the tubes, and then 1 mL was transferred into a chromatogram vial. All samples were analyzed using ultrahigh-performance liquid chromatography. A previous study by our group found the limit of detection to be lower than 1 ng/g for all compounds in a detergent matrix, this being more difficult than in the present study.⁴

RESULTS

The analysis of the detergent and the fabric conditioner showed concentrations of MI of 29,942.7 and 43,187.9 ng/g and of BIT of

TABLE 2. Concentration of Isothiazolinones According to the Washing Method Used

Concentration in ng/g Extracted From the 10 × 20-cm sample (Except Wool, 5 × 10 cm)	BIT		MCI		MI		OIT	
	Mean, ng/g	SD	Mean, ng/g	SD	Mean, ng/g	SD	Mean, ng/g	SD
Control (n = 2)								
Polyester	7.8	5.5	0	0	77.4	33.3	2.6	1.7
Wool	0	0	0	0	14.6	1.8	0	0
Cotton	6.1	1.4	0	0	548	89.6	0	0
Linen	3.59	0.01	0	0	457.8	14.8	0	0
Standard wash (n = 2)								
Polyester	0	0	0	0	5.5	1.4	3.4	2.2
Wool	0	0	0	0	17.7	4.6	0	0
Cotton	1	0.2	0	0	9.5	3.1	0	0
Linen	3.8	0.4	0	0	4.7	0.8	3.6	2.9
Standard wash + conditioner (n = 2)								
Polyester	10.1	2	0	0	8.9	1.1	2.4	0.5
Wool	0.9	1.3	0	0	16.6	0.4	0	0
Cotton	5.48	0.05	0	0	19.9	2.3	0	0
Linen	15.8	3.3	0	0	23.6	5.2	0	0
Standard wash double rinse (n = 2)								
Polyester	1.6	2.3	0	0	36.4	15.6	2.6	1.1
Wool	0	0	0	0	14.3	2.1	3.3	2.2
Cotton	0	0	0	0	10.1	3.7	1.1	0.1
Linen	5.8	1.5	0	0	13.2	7.1	2.5	0.9

BIT, benzisothiazolinone; MCI, methylchloroisothiazolinone; MI, methylisothiazolinone; OIT, octylisothiazolinone.

22,451.4 and 34,708.5 ng/g, respectively. No MCI or OIT was found. These data agree with those indicated on the product labels.

After machine washing, the results show that the concentrations of all the isothiazolinones were very low, independently of the type of material or wash. The highest levels were found in the control wash (water immersion), reaching a maximum of 0.4 ppm in the linen (Table 2).

DISCUSSION

The increasing use of MI in cosmetic and cleaning products earned the preservative the title of contact allergen of the year by the American Contact Dermatitis Society in 2013.⁵ Cleaning products often contain high concentrations of MI, which may facilitate contact sensitization.⁶ Studies have shown that the lowest dose level of chloromethylisothiazolinone that was able to induce significant lymphocyte proliferation was 100 ppm using murine local lymph node assay⁷ and 25 ppm in the guinea pig using the modified Buehler protocol.⁸

An Italian study detected the presence of isothiazolinones in 90.7% of the laundry detergents analyzed, with the most common preservative being MCI/MI, followed by MI and BIT.⁹ Pastor-Nieto et al² also determined the presence of isothiazolinones in fabric conditioners in the Spanish market. Although isothiazolinones contained in clothes are able to cause dermatitis,¹⁰ little is known about the persistence of these substances after washing. As far as we are aware, the only previous study to examine this issue is that of Hofmann et al,¹¹ which, with a detection limit of 0.5 ppm, detected no isothiazolinones. Our study, however, which had a detection limit of 1 ng/g (0.001 ppm), found traces of isothiazolinones at concentrations having no clinical relevance.

Because of the particular setting of each case, we cannot be sure that all washing machines or cycles can totally clear isothiazolinones. However, the findings of Hofmann et al¹¹ together with our results suggest that it is not necessary to recommend that patients sensitized to MI avoid it in machine detergents or fabric conditioners or to double rinse, as a first approach in standard cycles regardless of the composition of the clothes. Isothiazolinones are soluble substances in water, so it seems that standard programs in washing machines are able to decrease their concentrations to levels without

clinical relevance. However, after using the detergent for hand washing (similar conditions to our control), there may remain sufficient concentrations of isothiazolinones in clothes to produce symptoms in highly sensitized patients.

REFERENCES

1. Aerts O, Goossens A, Lambert J, et al. Contact allergy caused by isothiazolinone derivatives: an overview of non-cosmetic and unusual cosmetic sources. *Eur J Dermatol* 2017;27:115–122.
2. Pastor-Nieto MA, Alcántara-Nicolás F, Melgar-Molero V, et al. Conservantes en productos de higiene y cosméticos, medicamentos tópicos y productos de limpieza doméstica en España. *Actas Dermosifiliogr* 2017;108:758–770.
3. Hervella-Garcés M, García-Gavin J, Silvestre-Salvador JF. The Spanish standard patch test series: 2016 update by the Spanish Contact Dermatitis and Skin Allergy Research Group (GEIDAC). *Actas Dermosifiliogr* 2016;107:559–566.
4. Marrero-Alemán G, Borrego L, Antuña AG, et al. Isothiazolinones in cleaning products: Analysis with liquid chromatography tandem mass spectrometry of samples from sensitized patients and market. *Contact Dermatitis* 2020;82:94–100.
5. Castanedo-Tardana M, Zug K. Methylisothiazolinone. *Dermatitis* 2013;24:2–6.
6. Aerts O, Meert H, Goossens A, et al. Methylisothiazolinone in selected consumer products in Belgium: adding fuel to the fire? *Contact Dermatitis* 2015;73:142–149.
7. Botham P, Hilton J, Evans C, et al. Assessment of the relative skin sensitizing potency of 3 biocides using the murine local lymph node assay. *Contact Dermatitis* 1991;25:172–177.
8. Chan PK, Baldwin RC, Parsons RD, et al. Kathon biocide: manifestation of delayed contact dermatitis in guinea pigs is dependent on the concentration for induction and challenge. *J Invest Dermatol* 1983;81:409–411.
9. Magnano M, Silvani S, Vincenzi C, et al. Contact allergens and irritants in household washing and cleaning products. *Contact Dermatitis* 2009;61:337–341.
10. Umekoji A, Fukai K, Sowa-Osako J, et al. Allergic contact dermatitis caused by the preservative 4,5-dichloro-2-n-octyl-4-isothiazolin-3-one in black trousers. *Contact Dermatitis* 2016;75:326–328.
11. Hofmann MA, Giménez-Arnau A, Aberer W, et al. MI (2-methyl-4-isothiazolin-3-one) contained in detergents is not detectable in machine washed textiles. *Clin Transl Allergy* 2018;8:1.