

Occurrence and concentrations of isothiazolinones in detergents and cosmetics in Switzerland

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Summary

Background. In recent years, the frequency of contact allergy to isothiazolinones has increased alarmingly in Europe, but only limited data are available on concentrations of isothiazolinones in consumer products.

Objectives. To examine the current frequency of isothiazolinones [methylisothiazolinone (MI), methylchloroisothiazolinone (MCI), benzisothiazolinone (BIT), and octylisothiazolinone (OIT)] in a wide array of detergents and cosmetics relevant for the Swiss population.

Methods. By means of a market survey, the occurrence of isothiazolinones was investigated in 1948 consumer products. Of these, 88 products were analysed by liquid chromatography–high-resolution mass spectrometry after ultrasonic extraction.

Results. Only 7.6% of all cosmetics contained isothiazolinones, but the prevalence in detergents was much higher (42.9%). The measured concentration ranges in detergents were 4.3–10, 3.5–279, 3.8–186 and 7.9 ppm (one product only) for MI, MCI, BIT, and OIT, respectively. For cosmetics, these were 1.3–133 and 4.8 ppm (one product only) for MI and MCI, respectively.

Conclusions. Our study has shown that high concentrations of isothiazolinones (including MI) can be found in a large variety of products, in particular in detergents. Therefore, the safe use of these preservatives should be re-evaluated by including detergents in the exposure assessment.

Key words: allergic contact dermatitis; benzisothiazolinone; biocides; cosmetics; household cleaning products; market survey; methylchloroisothiazolinone; methylisothiazolinone; octylisothiazolinone; personal care products.

Detergents and cosmetics with high water contents are easily contaminated by microorganisms, which can spoil the product or even pose a health risk in cases of

pathogenic microorganisms (1). To prevent contamination, manufacturers add preservatives to their products. Isothiazolinones are among the most frequently used preservatives, owing to their activity against a broad spectrum of bacteria, fungi and yeasts over a wide range of pH levels (1).

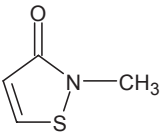
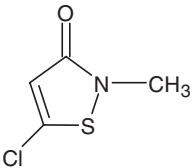
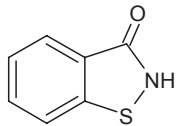
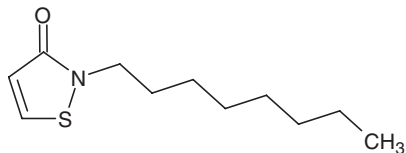
Isothiazolinones are heterocyclic organic chemicals containing vicinal sulfur and nitrogen atoms. The substances methylchloroisothiazolinone (MCI) and methylisothiazolinone (MI) are used as a 3:1 mixture (MCI/MI; CAS no.: 55965-84-9) or MI is used as single substance in a wide range of consumer products, such

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Table 1. Chemical structures, names, CAS numbers, molecular weights and regulations for the investigated isothiazolinones

Chemical structure	IUPAC compound name (common name)	Abbreviation	CAS no.	Molecular weight (g/mol)	Regulation
	2-Methylisothiazol-3(2H)-one (2-Methyl-3-isothiazolinone)	MI, MIT	2682-20-4	115.1	A
	5-Chloro-2-methyl-4-isothiazolin-3-one (5-Chloro-2-methyl-3-isothiazolinone)	MCI, CMI, MCIT	26172-55-4	149.6	A
	1,2-Benzisothiazol-3(2H)-one (1,2-Benzisothiazolinone)	BIT, Bzl	2634-33-5	151.2	B
	2-Octyl-3(2H)-isothiazolinone (2-Octyl-3-isothiazolinone)	OIT, OI	26530-20-1	213.3	B

A, According to Regulation (EC) No. 1223/2009, the presence of the chemical must be indicated in the list of ingredients. The maximum concentration allowed is 0.0015% for MCI/MI (3:1) or 0.01% for MI alone only in rinse-off products. The presence of MCI/MI in leave-on cosmetics has been prohibited since 16 April 2016 [Regulation (EU) No. 1003/2014]. The presence of MI alone in leave-on cosmetics has been prohibited since 12 February 2017 [Regulation (EU) No. 2016/1198].

B, Substance cannot be used as a preservative in cosmetic products, because it is not listed in Annex V of Regulation (EC) No. 1223/2009.

as detergents, cosmetics, several do-it-yourself products, and indoor water-based paints (2). In consumer products other than cosmetics, further isothiazolinones are used, including benzisothiazolinone (BIT) and octylisothiazolinone (OIT) (see Table 1 for chemical structures). MCI, MI, BIT and OIT are all classified as skin sensitizers in the harmonized classification according to (EC) 1272/2008 (Regulation on classification, labelling and packaging of substances and mixtures – CLP regulation) (3). In the 1980s, a first ‘epidemic’ of allergy caused by MCI/MI occurred (4). Subsequently, maximum allowed concentrations were set for MCI/MI (in the EU, 15 ppm for all cosmetics; in the United States, 15 ppm for rinse-off products and 7.5 ppm for leave-on products), and this resulted in a decrease in the prevalence of sensitized patients (5). However, in 2005, MI was introduced as a stand-alone preservative in cosmetics, and this was followed by a new ‘epidemic’ of contact dermatitis cases caused by MI and MCI/MI (5, 6). In their first assessment of MI, the

Cosmetic Ingredient Review in the US and the EU’s Scientific Committee on Consumer Safety (SCCS) concluded that a concentration of up to 100 ppm in cosmetics was safe for the consumer (7, 8). Nonetheless, thereafter the first cosmetic-related cases of contact allergy were published in 2010 (9), so that the EU’s SCCS updated its opinion on MI and indicated that, for leave-on cosmetic products, no safe concentrations of MI had been adequately demonstrated, whereas rinse-off products could be considered safe at a limit of 15 ppm from the viewpoint of the induction of contact allergy (sensitization) (10). As a result, it was decided that MCI/MI should not be present in leave-on cosmetics from 16 April 2016 (11). However, MI alone was still allowed in cosmetics up to 100 ppm until 12 February 2017 (8), and for detergents there is no restriction. Regarding MCI/MI, in the most recent amendments it was decided that MI alone should not be present in leave-on cosmetics (8). The increasing prevalence of contact allergy caused by isothiazolinones

raised concern among dermatologists (12), and created the need for more information on the occurrence and concentrations of these compounds in industrial and consumer products.

To date, only limited data on the current concentrations of isothiazolinones in detergents and cosmetics have been published for Switzerland (13) and other European countries (4, 14–17). Moreover, the available information refers mainly to cosmetics, which are frequently reformulated, and the majority of which stay on the market for < 5 years (14). The methodology for the analytical determination of isothiazolinones has been developed mainly for environmental water matrices (18–22), food packaging materials (23, 24), detergents (14, 16, 17), and cosmetic matrices (14, 17, 20, 25–27), using either liquid chromatography (LC), high-performance liquid chromatography (HPLC), or, to a lesser extent, gas chromatography.

The objective of this study was to determine the frequencies of occurrence and the concentrations of the four major isothiazolinones (MI, MCI, OIT, and BIT) in a wide array of consumer product categories (rinse-off and leave-on cosmetics for adults and babies, and detergents) sold in Switzerland. Note that, in this study, baby products are discussed as a separate group, even if regulation does not specifically address them, because children use different products and might be more sensitive than adults to chemical exposure. These assessments are part of a larger project aimed at a comprehensive exposure assessment for the Swiss population. The investigated detergents and cosmetics were selected because they are commonly used in Switzerland, as revealed by a product-use questionnaire (28).

Materials and Methods

Occurrence of four isothiazolinones in different consumer products in Switzerland according to a market survey

The ingredient labels of 1948 products were screened for the presence of isothiazolinones, including MI, MCI, BIT, and OIT, in four different shops between March and May 2015. These shops included the largest retailers in Switzerland with national coverage: Migros, Coop, Aldi, and Lidl. In addition, product information was collected from the online websites of Migros, Coop, and Unilever. Various manufacturers were also contacted by email to inquire whether their products may contain isothiazolinones. In response to a change in regulations (11), the labels were checked again in February 2016.

Analytical determination of isothiazolinones in detergents and cosmetics

Sample selection. Between May and June 2015, a survey was conducted in Switzerland to assess the use-patterns of various consumer products, including 12 detergents (all-purpose cleaner, dishwashing detergent, glass cleaner, etc.) and 22 cosmetics (shampoo, body lotion, make-up products, etc.). Use-patterns were collected among the Swiss population ($n = 759$; aged 0–91 years) by postal questionnaire. The core survey questions focused on specific brand names of the most commonly used products and on the frequencies of detergent and cosmetic use. To correctly identify the products, respondents could provide either the brand and product name or the product barcode (28).

On the basis of the product brands identified in the questionnaire as being the most frequently used, and the detergent and cosmetic categories that were preserved with isothiazolinones (as identified in the occurrence study; Fig. 1), a sample of 69 detergents and 19 cosmetics was selected for analysis (of these 19 cosmetics, one was a baby product, namely baby wipes; Fig. 1). Three products labelled as containing isothiazolinones in the first screening, but not in the second, were selected to test the change of formulation; that is, a total of three cosmetics were bought both before and after the formulation change. For analysis, these 88 products were purchased from several Swiss retailers (Migros, Denner, Manor, Coop, Aldi Suisse, and Lidl), and drug-stores in the area of Zurich. The manufacturers were located both in Switzerland and abroad, such as in Germany, France, and the UK. The selected samples are summarized in Table 2. Many of these detergents and cosmetics were labelled as containing at least one of the four isothiazolinones (56%). Until analysis, the samples were kept at room temperature in their original sealed packages.

Chemicals and reagents. BIT (97% purity) was obtained neat from Sigma-Aldrich (St Louis, MO, USA). OIT (> 98.0% purity) was obtained neat from Tokyo Chemical Industry (Tokyo, Japan). MCI/MI was obtained as a 2.7:1 mixture (14.2% active ingredient; 10.4% MCI and 3.8% MI; no single standard available for MCI) from ACBR (Karlsruhe, Germany). The chemical structures of the four isothiazolinones are shown in Table 1. Methanol and water (pro analysi) were obtained from Merck (Darmstadt, Germany). Formic acid (HCOOH) ($\geq 98\%$ purity) and acetonitrile (ACN) (HPLC grade) were obtained from Sigma Aldrich.

Table 2. General overview of the selected detergents and cosmetics for chemical analysis and their categorization

Category	Use	Product	Number of samples (n)
Detergents	Laundry	Fabric conditioner	3
		Detergent liquid	3
	Dishwashing	Hand dishwashing detergent	8
		All-purpose cleaners	12
	Bathroom	Bathroom cleaning spray/liquid	13
		Toilet cleaner liquid	6
	Floor-carpet care	Floor cleaning liquid	5
		Carpet cleaning liquid	2
	Kitchen	Kitchen cleaning spray/liquid	7
		Glass cleaner	6
Cosmetics	Cleaning wet tissue	Cleaning wet tissue	4
	Hair care	Shampoo*	3
		Conditioner*	3
		Styling gel†	1
	General hygiene	Shower gel*	3
		Liquid soap (hands)*	1
	Skin care	Face cream†	3
		Facial cleanser†	2
		Hand cream†	2
	Baby care	Baby wipes†	1

*Rinse-off cosmetics.

†Leave-on cosmetics.

Instrumentation. For LC, an Agilent 1200 system (Agilent, Waldbrunn, Germany) equipped with a Sonoma Phenyl-Hexyl column (100 × 4.6 mm internal diameter; particle size, 3 µm) was used. The mobile phases included a water phase containing 0.1% HCOOH (phase A) and an ACN phase containing 0.1% HCOOH (phase B). A 10-min gradient program was used for elution, with a flow rate of 1.0 mL. The gradient parameters were: 0–1.5 min, linear gradient from 10% to 30% B; 1.5–4 min, linear gradient to 90% B; 4–6 min, hold at 90% B; 6–7 min, linear gradient back to 10% B; and 7–10 min of equilibration at 10% B. The column temperature was set to room temperature. Samples were injected with a volume of 10 µL. Detection was performed with an ESI-Q-TOF system (maXis; Bruker Daltonics, Bremen, Germany). The instrument was operated in wide-pass quadrupole mode. The TOF data were collected between m/z 20 and m/z 600 with a low-collision energy of 10 eV and an ion energy offset of 4 eV. The optimized source conditions were: drying gas, 8.0 L/h (nitrogen 99.99% purity) at a temperature of 200°C; nebulizer pressure, 1.6 bar; capillary and endplate voltages, 500 and 4500 V, respectively; TOF flight tube voltage, 9870 V;

reflection voltage, 2000 V; pusher voltage, 1642 V; and microchannel plate detector voltage, 1671 V. The resolving power of the instrument was between 20 000 and 45 000 measured with full width at half-maximum with a spectral rate of 2.5 Hz, depending on the sample concentration and peak width. For LC–high-resolution mass spectrometry (LC-HRMS) experiments, the ESI-TOF mass spectrometer was calibrated in the positive mode with a solution of sodium formate in methanol Na(NaCOOH) n clusters in the enhanced quadratic algorithmic mode. Mass spectra were recorded in centroid mode with the use of independent reference lock-mass ions (methyl stearate, m/z 299.2945) via the lock-mass interface to ensure mass accuracy and reproducibility.

Data acquisition and quantification were performed with DATA ANALYSIS 4.0 (Bruker Daltonics) together with QUANT ANALYSIS 2.1 (Bruker Daltonics). The signals of the four target isothiazolinones were integrated in the extracted ion chromatogram. Quantification of the four isothiazolinones was performed with external calibration curves (quadratic fit, no weighting factor).

Sample preparation. Samples were prepared according to a standard operating procedure of the cantonal laboratory of Basel city based on (25), with minor adjustments. The extraction procedure was performed as follows. A 0.5 g piece of the selected sample was accurately weighed in a 15-ml centrifuge tube, and 10 ml of extraction solution was added. For the wet wipes, a piece of ~0.5 g was weighed and analysed. Another piece of tissue was used to determine the liquid content (weighing wet and dry), on which the concentrations were based. For the extraction of MI and MCI, 10 ml of water containing 1% HCOOH was added, whereas for BIT and OIT 10 ml of methanol was used (note that BIT and OIT were analysed and therefore extracted only for detergents, as the regulation does not allow the use of these compounds in cosmetics). Each sample was prepared in duplicate. One of the duplicates was spiked as a quality check (approximately 50 ppm for MCI, BIT, and OIT, and 20 ppm for MI). The solutions were homogenized with a vortex, and placed in an ultrasonic bath for 15 min. After 5 min of centrifugation at 1914 g, a 1 ml aliquot was pressed through a syringe filter (hydrophilic PP membrane; Acrodisc Pall Corporation, Port Washington, USA, New York, 0.45 µm, 13 mm, GHP membrane). The samples were stored at –18°C until analysis.

Quantification and quality assurance. Stock solutions of 1000 ng/µL were prepared by weighing ~10 mg of each compound into a 10 ml volumetric flask and filling it up with methanol. Calibration solutions were

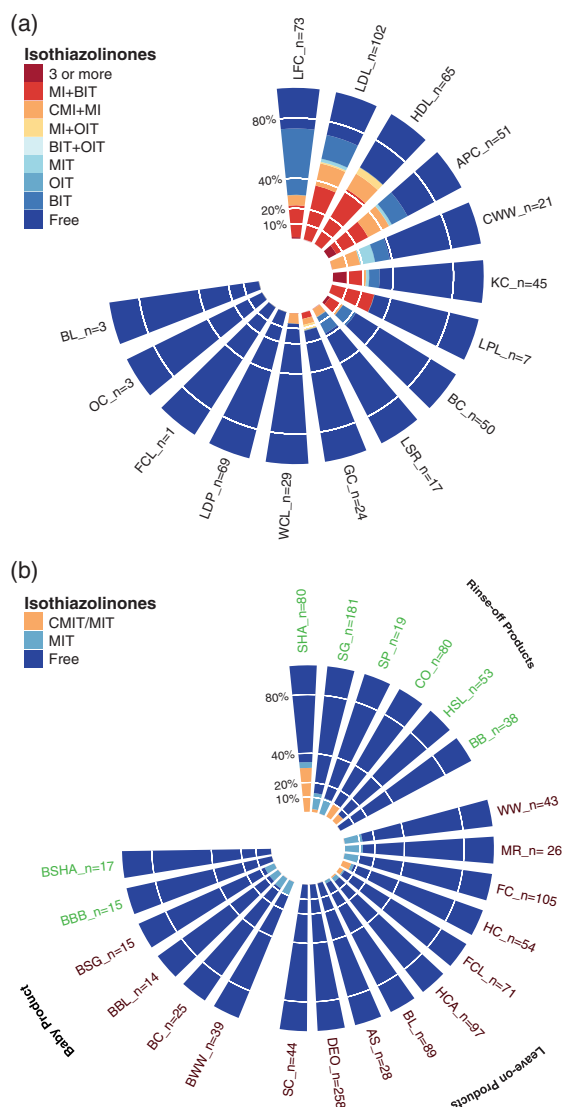


Fig. 1. (a) Occurrence of isothiazolinones in detergents and (b) cosmetics for leave-on, rinse-off, and baby products (results of the first screening in 2015). APC, all-purpose cleaner; AS, aftershave; BB, bubble bath; BBB, baby bubble bath; BBL, baby body lotion; BC, bathroom cleaner; BL, bleach (a); BL, body lotion (b); BSG, baby shower gel; BSHA, baby shampoo; BWV, baby wet wipes; CO, conditioner; CWW, cleaning wet wipes; DEO, deodorant; FC, face cream; FCL, floor cleaning liquid (a); FCL, facial cleanser (b); GC, glass cleaner; HC, hand cream; HCA, hair care; HDL, hand dishwashing detergent; HSL, hand soap liquid; KC, kitchen cleaner; LDL, laundry detergent powder; LDP, laundry detergent powder; LFC, laundry fabric detergent; LPL, laundry pretreatment liquid; LSR, laundry spot remover; MR, make-up remover; OC, oven cleaner; SG, shower gel; SHA, shampoo; SP, shaving products; WCL, toilet cleaner; WW, wet wipes; MI, methylisothiazolinone; MCI, methylchloroisothiazolinone; BIT, benzisothiazolinone; OIT, octylisothiazolinone; green: rinse-off products, brown: leave-on products; SC, sunscreen; BC, baby cream.

prepared by subsequent dilution of the stock solutions with water containing 1% HCOOH for MCI and MI, and with methanol for BIT and OIT. The concentrations were 0.5–16.0 ng/μl and 0.2–5.9 ng/μl for MCI and MI, respectively, and 1.0–8.0 and 0.5–4.1 ng/μl for BIT and OIT, respectively. The quadratic calibration curves were based on six points of increasing concentrations for MCI and MI, and on five points for BIT and OIT. Correlation coefficients (*R*) of the calibration curves were greater than 0.99 for MCI, MI, and BIT, and 0.98 for OIT, when the average over the 17 investigation days was taken.

The limits of detection (LODs) and limits of quantification (LOQs) were manually established on the basis of the signal-to-noise ratio (3:1 for the LOD, 10:1 for the LOQ). The limits were calculated by the use of chromatograms from the lowest calibration solutions and three samples (labelled as not containing isothiazolinones), and, finally, by taking the average of three different days (*n* = 3). In detergents, the LODs were 0.94 ppm for MCI, 1.04 ppm for MI, 1.15 ppm for BIT, and 2.03 ppm for OIT, and the LOQs were 3.1 ppm for MCI, 3.5 ppm for MI, 3.8 ppm for BIT, and 3.4 ppm for OIT. In cosmetics, the LODs and LOQs were 0.42 and 1.4 ppm for MI, and 0.38 and 1.3 ppm for MCI, respectively.

The accuracy of the method was assessed by spiking five isothiazolinone-free matrices (a floor cleaner, a kitchen cleaner, a laundry fabric conditioner, a body lotion, and a shampoo) at three concentration levels. The recovery values were obtained as ratios of the measured concentration to the spiked concentration, and expressed as percentages. Recovery rates were: 115–135%, 92–113%, 93–110% and 87–101% for MCI, MI, BIT and OIT, respectively, in the floor cleaner matrix; 75–90%, 113–135%, 134–144% and 52–82% for MCI, MI, BIT and OIT, respectively, in the kitchen cleaner matrix; 133–147%, 93–130%, 81–101% and 58–77% for MCI, MI, BIT and OIT, respectively, in the laundry fabric conditioner matrix; 98–137% and 115–119% for MCI and MI, respectively, in the body lotion matrix; and 111–115% and 111–131% for MCI and MI, respectively, in the shampoo matrix. In this article, the concentrations are given in ppm, which corresponds to mg/kg.

Results

Occurrence of isothiazolinones in detergents and cosmetics in Switzerland

In total, the labels of 1948 products (1266 cosmetics, 557 detergents, and 125 baby products) were manually checked. To avoid duplicates, the barcodes were

photographed and noted. The investigation showed that 239 detergents (42.9%), 97 cosmetics for adults (7.7%) and eight cosmetic baby products (6.4%) were preserved with at least one isothiazolinone. Figure 1 shows the percentages of products containing isothiazolinones in each product category for detergents (Fig. 1a) and cosmetics (Fig. 1b). A higher percentage of detergents (42.7%) than of cosmetics contained isothiazolinones; and, in contrast to cosmetics, BIT and OIT are permitted and were labelled on detergents. BIT (31.2%) and MI (31.1%) were the most frequently found isothiazolinones, followed by MCI (9.9%). Apart from BIT, which was found as the sole isothiazolinone in 11.5% of the preserved products, the isothiazolinones were mainly found in combination. The most frequent combination was MI with BIT (18.1%), followed by MI with MCI (8.1%), and finally MI with OIT (0.9%). Isothiazolinones occurred most often in the following detergent categories: laundry fabric conditioner (72.6%), liquid laundry detergent (72.5%), all-purpose cleaner (58.8%), and hand dishwashing detergent (58.5%).

Ninety-six cosmetics out of 1266 were labelled with MI or MCI/MI. As expected, neither BIT nor OIT was labelled on cosmetics. MI alone was more often labelled on cosmetics (4.0%) than MCI/MI (3.6%). Shampoo was the product category with the highest percentage of isothiazolinones (33.8%), followed by shower gel (13.3%), wet wipes (11.6%), and make-up remover (11.5%). Only MI alone could be found on the labels of 6.4% of the investigated baby products ($n=125$), with the top baby cosmetics being baby wipes (10.3%, $n=39$), cream (8.0%, $n=25$), lotion (7.1%, $n=14$), and shower gel (6.7%, $n=15$). Some cosmetic product categories were free of isothiazolinones, such as deodorant, aftershave, and sunscreen. The 96 cosmetics labelled as containing isothiazolinones during the first screening were checked again 2 months before the implementation of the new directive of the European Commission, which prohibits the use of MCI/MI in leave-on cosmetics starting from 16 April 2016 (11). A change of formulation was observed for 60 of 97 cosmetics. The preservatives MI and MCI/MI were replaced mainly with 1,3-dimethylol-5,5-dimethylhydantoin (DMDM hydantoin), ethylparaben, methylparaben, sodium benzoate, or phenoxyethanol. Among leave-on cosmetics, only one face cream preserved with MI could be found in February 2016. However, in rinse-off cosmetics isothiazolinones (MI alone and MCI/MI) were still used, mainly in shampoo, conditioner, and liquid hand soap, but in fewer products. Wet wipes for adults were no longer preserved with isothiazolinones, but one baby wipes product was still labelled as containing MI. All other baby products were free of isothiazolinones.

Concentrations of isothiazolinones in consumer products

Detergents. Isothiazolinones were detected in 36 of 69 detergents (52%) (Tables 3 and 4; raw data see Tables S1–S2). MI and BIT were the most frequently detected compounds in 27 and 29 products, respectively, followed by MCI in 7 products (but at a concentration above the LOQ in only two all-purpose cleaners), and OIT in six products (but at a concentration above the LOQ in only one floor cleaning detergent (7.9 ppm)). The concentrations for each isothiazolinone were 4.3–10, 3.5–279, 3.8–186 and 7.9 ppm for MCI, MI, BIT, and OIT, respectively. This corresponds to average concentrations of 6.9 ppm for MCI, 44 ppm for MI, and 75 ppm for BIT. MCI was only used in combination with MI. Carpet cleaner and toilet cleaner did not contain isothiazolinones.

Of the 69 detergents analysed, eight (12%) were mislabelled: the labels of two floor cleaning detergents only mentioned BIT, but MI was also detected in both products, and, in one product, all four isothiazolinones were found. Although a bathroom cleaning detergent spray and one laundry detergent were labelled as not containing isothiazolinones, BIT could be detected in both (below the LOQ). MCI and MI were mentioned on the label of one toilet cleaner, but chemical analysis did not detect either. Similarly, one all-purpose cleaner was labelled as containing MI and BIT, but neither of these could be identified. In another all-purpose cleaner, only BIT was detected, although the label stated that it contained BIT and MI. Although a hand dishwashing detergent liquid was labelled as containing MI and BIT, MCI was detected as well (below the LOQ).

Cosmetics. Among the 19 investigated cosmetics, isothiazolinones were detected in 13 products (Table 4). MI and MCI were measured in 12 and 6 products, respectively. The concentration ranged between 1.3 and 133 ppm for MI and 4.8 ppm for MCI (only quantified in one shampoo). The average concentration of MI was 58 ppm. Similarly to detergents, MCI was only used in combination with MI. Although 16 cosmetics were labelled as containing isothiazolinones, they could not be detected in four products, owing either to the LOD or faulty declaration.

Discussion

Occurrence of isothiazolinones in consumer products in Switzerland

The results of the occurrence study showed that Switzerland has a high proportion of detergents

Table 3. Isothiazolinones [methylisothiazolinone (MI), methylchloroisothiazolinone (MCI), benzisothiazolinone (BIT), and octylisothiazolinone (OIT)*] concentrations in the investigated detergents (ppm)

		All-purpose cleaners n = 12	Dishwashing detergent n = 8	Kitchen cleaner n = 7	Bathroom cleaner n = 13	Toilet cleaner n = 6	Floor cleaner* n = 5	Carpet cleaner n = 2	Glass cleaner n = 6	Wet cleaning wipes n = 4	Fabric conditioner n = 3	Laundry detergent liquid n = 3
MCI	Mean (excluding < LOQ [†])	6.9	< 3.1	< 3.1	< 3.1	< 3.1	< 3.1	< 3.1	< 3.1	< 3.1	< 3.1	< 3.1
	Median (excluding < LOQ [†])	6.5	< 3.1	< 3.1	< 3.1	< 3.1	< 3.1	< 3.1	< 3.1	< 3.1	< 3.1	< 3.1
	Range (min.–max.)	4.3–10.0	< 3.1	< 3.1	< 3.1	< 3.1	< 3.1	< 3.1	< 3.1	< 3.1	< 3.1	< 3.1
	No. of < LOQ products	9	8	7	13	6	5	2	6	4	3	3
MI	Mean (excluding < LOQ [†])	14.6	48.9	26.6	41.6	< 3.5	15.3	< 3.5	53.1	154.5	47.4	41.1
	Median (excluding < LOQ [†])	7.8	51.0	26.6	43.1	< 3.5	15.3	< 3.5	53.1	154.5	47.4	41.1
	Range (min.–max.)	< 3.5–39.9	6.0–74.0	19.5–33.7	26.8–53.2	< 3.5	< 3.5–15.3	< 3.5	< 3.5–53.1	< 3.5–279.3	< 3.5–47.4	16.4–65.7
	No. of < LOQ products	6	2	5	9	6	4	2	5	2	2	1
BIT	Mean (excluding < LOQ [†])	59.3	59.3	65.4	67.6	< 3.8	161.7	< 3.8	43.2	186.2	52.7	82.2
	Median (excluding < LOQ [†])	34.3	50.1	65.4	46.6	< 3.8	161.7	< 3.8	43.2	186.2	50.9	82.2
	Range (min.–max.)	25.1–163.0	27.4–109.6	< 3.8–65.4	23.4–118.7	< 3.8	154.5–168.8	< 3.8	< 3.8–43.2	< 3.8–186.2	29.0–78.3	37.0–127.4
	No. of < LOQ products	5	4	6	8	6	3	2	5	3	0	0

LOQ, limit of quantification.

*OIT was detected in six products, but only in one floor cleaning detergent (7.9 ppm) at a concentration above the LOQ.

[†]For detergents, the LOQs were 3.1 ppm for MCI, 3.5 ppm for MI, 3.8 ppm for BIT, and 3.4 ppm for OIT.**Table 4.** Methylisothiazolinone (MI) and methylchloroisothiazolinone (MCI) concentrations in the investigated cosmetics (ppm)

		Shampoo n = 3	Conditioner n = 3	Styling gel n = 1	Shower gel n = 3	Liquid soap n = 1	Face cream n = 3	Facial cleanser n = 2	Hand cream n = 2	Baby wipes n = 1
MCI	Mean (excluding < LOQ [*])	< 1.3	< 1.3	< 1.3	4.8	< 1.3	< 1.3	< 1.3	< 1.3	< 1.3
	Median (excluding < LOQ [*])	< 1.3	< 1.3	< 1.3	4.8	< 1.3	< 1.3	< 1.3	< 1.3	< 1.3
	Range (min.–max.)	< 1.3	< 1.3	< 1.3	< 1.3–4.8	< 1.3	< 1.3	< 1.3	< 1.3	< 1.3
	No. of < LOQ products	3	3	1	2	1	3	2	2	1
MI	Mean (excluding < LOQ [*])	16.6	< 1.4	< 1.4	33.8	< 1.4	129.6	< 1.4	113.3	19.5
	Median (excluding < LOQ [*])	16.6	< 1.4	< 1.4	2.16	< 1.4	129.6	< 1.4	113.3	19.5
	Range (min.–max.)	< 1.4–31.1	< 1.4	< 1.4	< 1.4–97.5	< 1.4	< 1.4–132.6	< 1.4	< 1.4–113.3	< 1.4–19.5
	No. of < LOQ products	3	3	1	0	1	1	2	1	1

LOQ, limit of quantification.

*For cosmetics, the LOQs were 1.3 ppm for MCI and 1.4 ppm for MI.

containing isothiazolinones (42.9%). Generally, powder or other dry products are not preserved with isothiazolinones, as due to their low water content they are not prone to be contaminated by microorganisms. MI and BIT were found in 31.1% and 31.2% of the liquid detergents, respectively; these exceed the values reported in literature (29–31). In contrast, MCI/MI was used less frequently, that is, in only 9.9% of the products. Older studies conducted before 2010 (29–31) showed higher percentages of products preserved with the mixture: MCI/MI was

found in 35.7% (n = 291) of the detergents in Italy (30), in 11.4% (n = 697) of the detergents in Switzerland (31), and in 9% (n = 97) of the detergents in Sweden (29). A study conducted in Germany between 2006 and 2009 also showed that MCI/MI was used more frequently (4%) than MI alone (0.9%) for the preservation of cosmetics (32). Thus, possibly because of new regulations, MCI/MI now seems to be used less frequently in Switzerland; this may, however, result in the increased use of MI as stand-alone preservative. In Table S3, a review

of the occurrence studies conducted in Europe in the last 15 years is shown. A decrease in the use of isothiazolinones (both MCI/MI and MI) for the preservation of cosmetics was observed in the second occurrence screening, which was conducted in February 2016; further changes in the cosmetic formulation are expected during the year 2016. However, consumers may still be exposed to leave-on cosmetics containing MI or MCI/MI in the next few years, because they have already bought them.

The occurrence study reported above focused only on detergents and cosmetics. However, isothiazolinones can be used as active ingredients in biocides (2), which then contain much higher concentrations of isothiazolinones than used for preservation, so that biocides may contribute more to exposure than other consumer products. To examine this aspect further, in February 2016 an additional occurrence study was conducted on the three main suppliers of do-it-yourself products in Switzerland (Jumbo, Coop Bau und Hobby, and Migros do it), regarding the labels of 60 biocidal products (algaecides, pesticide/insecticides, and fungicides). Only one biocidal product of 60 was labelled as containing OIT (0.05 g/100 g). The low concentration indicates that OIT is used for the preservation of this product, rather than as an active ingredient. Therefore, even if isothiazolinones are present as preservatives in a number of different product categories (2), it can be concluded that isothiazolinones are very rarely used as active ingredients in biocides available on the Swiss market.

Concentrations of isothiazolinones in detergents and cosmetics (analytical study)

For analysis, we used LC-HRMS after ultrasonic extraction. Table 5 shows that ultrasonic extraction is a well-established extraction method, and that LC is widely used for analysis. Only two studies have investigated the four isothiazolinones in detergents, but with very limited sample sizes. For both detergents and cosmetics, the concentrations are difficult to compare, owing to the limited number of studies, different analytical methods, intercountry product variability, and the small sample size.

MCI was only found in combination with MI, because MCI can only be purchased as a mixture with MI. One of the commercial mixtures of MCI/MI is Kathon® CG (~3:1). The MCI/MI ratio in the investigated products was not always the expected ratio of 3:1. Previous studies showed a similar pattern (26, 33), and the authors suggested that MCI might react with other formulation ingredients. Another explanation for the unexpected ratios is

that some products preserved with MCI/MI could contain ingredients preserved with MI alone, thus changing the ratio.

For detergents, BIT and MI were the most commonly detected compounds (usually in combination). BIT was used in relatively high concentrations (average of 75 ppm; nine products exceeding a content of 100 ppm). The MI average concentration was only half as high as that of BIT (44 ppm), with one product exceeding 100 ppm. In comparison, MCI and OIT concentrations were relatively low (6.9 and 7.9 ppm respectively). These values are comparable to the findings of Alvarez-Rivera et al. (17) (Table 5), although some of our LODs/LOQs are higher. In their work, BIT was detected at the highest average concentration (150 ppm) and OIT at the lowest (0.7 ppm). They found both MCI and MI in low concentrations (average of 5.9 ppm for MCI and 7.5 ppm for MI), whereas our study shows much higher concentrations of MI (44 ppm on average) than of MCI (6.9 ppm), because many more products contained MI. Nevertheless, high concentrations of MI were also found by Aerts et al. (14). This Belgian survey indicated that MI had the highest concentrations (average value of 97 ppm), and also was the most frequently found isothiazolinone. MCI and OIT were not detected in any of the products, which was probably attributable to the very small sample size ($n=6$). Although the study of Alvarez-Rivera et al. (17) showed a similar limitation as that of Aerts et al. (14) in terms of a small sample size ($n=8$), MCI was found in three of the eight detergents, in contrast to only 10% in our study. The high MI concentrations may present a concern, as a high concentration of MI (used in combination with BIT) in one detergent was reported by van Steenkiste et al. (34) to have caused a case of severe airborne-induced contact dermatitis (34). Also, a Swiss investigation showed that BIT is used in high concentrations (50–500 ppm) in tattooing inks, which may present an additional risk (35).

In cosmetics, MI alone was detected more often than in combination with MCI. When used alone, MI was detected in relatively high concentrations (>100 ppm; average of 58 ppm); three leave-on products (two face creams and one hand cream) apparently contained MI in concentrations above the legal limit of 100 ppm; however, these are still within the uncertainty of the method. After banning MCI/MI in leave-on products, in two of these products MI was no longer labelled. Therefore, we checked the labels in a second round of analysis, and confirmed the change of formulation. Nevertheless, leave-on products with MI exceeding the legal limits were also reported by Aerts

Table 5. Studies that have investigated isothiazolinones [methylisothiazolinone (MI), methylchloroisothiazolinone (MCI), benzisothiazolinone (BIT), and octylisothiazolinone (OIT)] in detergents or cosmetics

Study	Country	Compound	Matrix	Extraction	Analysis	Concentration in ppm (range, n)
Speksnijder et al. (20)	Netherlands	MI, MCI, and DCMI	Five shampoos	–	HPLC-MS/MS	MCI (<0.003–1.7), MI (0.08–1.3)
Alvarez-Rivera et al. (17)	Spain	MI, MCI, BIT, and OIT	Cosmetics (15 rinse-off; six leave-on) and detergents (four laundry products; one dishwasher; three surface cleaners)	MSPD1	HPLC-MS/MS	Rinse-off cosmetics: MCI (0.71–41.8), MI (0.35–111) Leave-on cosmetics: MCI (0.18–0.22), MI (0.72–26.1) Detergents: MCI (4.19–8.44), MI (1.79–29.0), BIT (116–255), OIT (0.56–1.21)
Aerts et al. (14)	Belgium	MI, MCI, BIT, and OIT	Cosmetics (18 leave-on; 12 rinse-off), eight detergents, and paints	Ultrasonic extraction	HPLC-UV	Leave-on cosmetics: MCI (<1.7), MI (39–188) Rinse-off cosmetics: MCI (<1.7), MI (<1.3–128) Detergents: MCI (<1.7), MI (<1.3–181), BIT (<0.9–26), OIT (<1.5)
Wittenberg et al. (26)	United States	MI and MCI	Cosmetics (11 shampoos/conditioners; 13 skin care products)	Ultrasonic extraction	UHPLC-MS/MS	Cosmetics: MCI (0.3–10.3), MI (0.4–89.6)
This study	Switzerland	MI, MCI, BIT, and OIT	19 cosmetics* and 69 detergents	Ultrasonic extraction	HPLC-MS	Cosmetics: MCI (1.4–4.8), MI (1.3–133) Detergents: MCI (4.3–10.0), MI (3.5–279), BIT (3.8–186), OIT (7.9)

DCMI, Dichloromethylisothiazolinone; HPLC, high-performance liquid chromatography; MS, mass spectrometry; MSPD, matrix solid-phase dispersion; UHPLC, ultra-high-performance liquid chromatography; UV, ultraviolet detection.

*Cosmetic categories investigated only for the preservatives MCI and MI.

et al. (36) and Neza and Cetini (37), and represent a serious concern. The MCI concentrations in cosmetics were relatively low, with an average value of 4.8 ppm, which is comparable to the most recent studies in Belgium, Spain, and the United States (14, 17, 26). As expected, the MI concentrations (average value of 58 ppm) were higher than those of MCI in cosmetics, and these findings are comparable with those of (14, 26), but higher than those of (20), in which a sample of five shampoos had been investigated. The results of Alvarez-Rivera et al. (17) are comparable for rinse-off products but not for leave-on products, which showed higher MI concentrations in Switzerland (average of 98 ppm) than in Spain (average of 7.2 ppm). As a new regulation prohibiting the use of MCI/MI in leave-on products was introduced during this study, three products (one face cream, one facial cleanser, and one hand cream) were purchased twice, once before and once after the implementation of the new regulation, in order to verify the impact on product formulation. The analysis showed that, after the introduction of the new regulation, isothiazolinones had been removed from all three products. A more representative follow-up investigation may give insight into the compliance with the evolving regulation. Note that, in this study, the illegal presence of BIT and OIT in cosmetic products was not checked, so we cannot exclude their presence.

Schwensen et al. (5) have reported a decreasing trend in the use of MCI/MI in recent years, probably because of the restrictions on the use of this mixture in cosmetics and their classification as skin sensitizers in chemical products; they also observed the presence of MI and BIT (single or combined together) in high concentrations for some product categories, such as water-based paints. Similarly to the findings of Schwensen et al. (5), MI and BIT could be found in a considerable proportion of the investigated detergents of our study (52%), with some concentrations exceeding 100 ppm. Currently, no limit concentrations for detergents are set, which is of concern, given that they constitute the second most important allergen source of MI after cosmetics (14). However, for exposure assessment, not only the concentrations in the products are relevant; various other factors need to be considered, such as the type of contact between consumer and product, and the frequency of use (38, 39). For example, although many laundry detergents were labelled as containing isothiazolinones, they are unlikely to come directly into contact with the user's skin. In contrast, all-purpose cleaners or hand dishwashing detergents more likely reach the skin. Furthermore, all-purpose cleaner was the only category of products containing all four isothiazolinones (up to three together in a single product), with average concentrations of 6.9 ppm for MCI, 17 ppm for MI, 62 ppm for

BIT, and <3.4 ppm for OIT. However, considering that many cleaners are normally diluted in water prior to use, the associated exposure might be less important. As a result, kitchen cleaners and wet cleaning wipes might constitute a more important source of exposure regarding allergic contact dermatitis. However, other factors, such as accidental skin contact, inappropriate product use (e.g. insufficient dilution with water), and airborne elicitation of dermatitis are additional pathways of exposure. Furthermore, although detergents might not be used as often as cosmetics, this may be compensated for by the higher proportion of products containing isothiazolinones. For this reason, detergents might constitute as important a source of exposure as cosmetics when aggregate exposure is considered (36, 40). Moreover, according to patch test results, cross-reactivity between different isothiazolinones cannot be excluded (36, 41). In recent years, only a few studies have investigated the potential cross-reactions between isothiazolinones (42, 43), and have suggested that the concomitant reactions observed for some isothiazolinones are probably attributable to co-exposure rather than cross-reactivity.

The frequencies of occurrence and the concentrations of isothiazolinones in this study cannot be generalized, owing to geographical variation in the availability of products and brands on the market. However, Switzerland is subject to the same regulations as many other European countries, and, for detergents and cosmetics, has a similar product portfolio, with only 20% of the investigated detergents and cosmetics being from regional brands. Therefore, the trends observed may well be applicable to other European countries.

Conclusion

Detergents constitute an important source of allergens, in particular of MI and BIT, which were found in relatively high concentrations. For this reason, future risk assessments for isothiazolinones should include detergents in the exposure assessment. Moreover, given the frequent occurrence and high concentrations of BIT in detergents, clinicians should be aware of this exposure and patch test with BIT, when appropriate. Labelling compliance is essential for consumers who are already sensitized and have to avoid the allergen for secondary prevention. The fact that the chemical analysis showed that four products contained isothiazolinones that were not labelled emphasizes the need for regular screening by authorities to verify product labels and ensure manufacturer compliance with regulations.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1–S2. Single values for concentrations in the products. Section 2.

Table S3. review of published occurrence studies on isothiazolinones.

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