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Methylisothiazolinones

Diagnosis and prevention of allergic contact dermatitis

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to Per-Olof, Christopher, Sofia and Victoria

CONTENTS

LIST OF PUBLICATIONS

This thesis is based on the following papers, which will be referred to by their Roman numerals:

I Gruvberger B, Persson K, Björkner B, Bruze M, Dahlquist I, Fregert S. Demonstration of Kathon® CG in some commercial products. Contact Dermatitis 1986: 15: 24-27

II Bruze M, Dahlquist I, Fregert S, Gruvberger B, Persson K. Contact allergy to the active ingredients of Kathon® CG. Contact Dermatitis 1987: 16: 183-188

III Bruze M, Dahlquist I, Gruvberger B. Contact allergy to dichlorinated methylisothiazolinone. Contact Dermatitis 1989: 20: 219-220

IV Bruze M, Fregert S, Gruvberger B, Persson K. Contact allergy to the active ingredients of Kathon® CG in the guinea pig. Acta Derm Venereol (Stockh) 1987: 67: 315-320

V Bruze M, Gruvberger B, Persson K. Contact allergy to a contaminant in Kathon CG in the guinea pig. Dermatosen in Beruf und Umwelt 1987: 35: 165-168

VI Gruvberger B, Bruze M, Tammela M. Preservatives in moisturizers on the Swedish market. Acta Derm Venereol (Stockh). Accepted for publication

VII Gruvberger B, Bruze M, Almgren G. Occupational dermatoses in a plant producing binders for paints and glues. Contact Dermatitis. Accepted for publication

VIII Gruvberger B, Bruze M. Can chemical burns and allergic contact dermatitis from higher concentrations of methylchloroisothiazolinone/methylisothiazolinone be prevented? American Journal of Contact Dermatitis. Accepted for publication

IX Gruvberger B, Bruze M. Can glutathione-containing emollients inactivate methylchloroisothiazolinone/methylisothiazolinone? Submitted

ABBREVIATIONS

a.i.	active ingredient/s
BIT	1,2-benzisothiazolin-3-one
CTFA	Cosmetic, Toiletry, and Fragrance Association
GC	Gas chromatography
GPMT	Guinea pig maximization test
GSH	glutathione
HPLC	High performance liquid chromatography
ICDRG	International Contact Dermatitis Research Group
INCI	International Nomenclature of Cosmetic Ingredients
K-CG	Kathon® CG
K-886	Kathon® 886
K-WT	Kathon® WT
MCI	5-chloro-2-methyl-4-isothiazolin-3-one
MI	2-methyl-4-isothiazolin-3-one
45243-K-CG	4,5-dichloro-2-methyl-4-isothiazolin-3-one
2-MP	2-methylol phenol
MS	Mass spectrometry
NMR	Nuclear magnetic resonance
ppm	parts per million

AIMS OF THE STUDY

The purposes of the present investigation were as follow:

- To find a method to demonstrate and quantify methylisothiazolinones in test solutions and products
- To isolate and identify contact allergens in Kathon® CG
- To assess the sensitizing capacities of the allergens by using the guinea pig maximization test
- To study the occurrence of methylisothiazolinones in moisturizers
- To investigate the occurrence and causes of occupational dermatoses in a plant where high concentrations of methylisothiazolinones were handled to produce binders for paints and glues.
- To find methods to prevent chemical burns and allergic contact dermatitis from methylisothiazolinones

INTRODUCTION

Preservatives

Preservatives are biologically active substances required in especially water-based products to inhibit the growth of micro-organisms such as bacteria, fungi and yeasts. The micro-organisms may be pathogenic for humans, but even when harmless for humans they may be undesirable because they may degrade the product. Degradation of constituents may imply loss or change of activity, discoloration, and malodor. To prevent such undesirable events preservatives are added to most cosmetics and toiletries as well as to industrial products such as cutting fluids, paints and glues. Thus, most people are daily exposed to one or more preservatives. Preferably, the preservatives themselves should not constitute any hazard to human health or the environment.

Adverse reactions to preservatives

Preservatives are potential contact allergens. In patients with suspected cosmetic sensitivity they have been identified as among the most important causes of allergic contact dermatitis, after fragrances (1). Irritant reactions, contact urticaria, and stinging are examples of other adverse reactions to preservatives (2).

Kathon preservatives

During the eighties some preservatives, and in particular formaldehyde, were questioned due to suspicions that they possess carcinogenic properties. This may, in part, explain why a new preservative Kathon® CG (K-CG) became widely distributed so rapidly. Also contributing to this development were other factors as effectiveness, degradation in the environment (3, 4), and the marketing claim that K-CG was safe to human health when used as recommended by the manufacturer.

Kathon® preservatives are manufactured by Rohm and Haas Company, Philadelphia, USA and contain either octylisothiazolinones or methylisothiazolinones. K-CG (cosmetic grade) consists of two a.i., stabilizing salts and water. 5-Chloro-2-methyl-4-isothiazolin-3-one (MCI) (CAS no 26172-55-4) and 2-methyl-4-isothiazolin-3-one (MI) (CAS no 2682-20-4) (Fig. 1) constitute the a.i., and due to conditions present during the synthesis MCI is formed at a 3 times higher concentration than MI. The CTFA-adopted and later on INCI-adopted names for MCI and MI are methylchloroisothiazolinone and methylisothiazolinone, respectively. Rohm and Haas also manufactures other methylisothiazolinone-based preservatives with the same principal composition as K-CG (Table 1). The preservatives are corrosive at high concentrations, but maintain effectiveness against bacteria, fungi, yeasts, and algae at low concentrations. They have a broad spectrum of applications (Table 2). The recommended use concentrations vary between 1 and 15 ppm a.i. in industrial water treatment and up to 35 ppm in electrodeposition systems (5). For cosmetic and toiletries a maximum concentration of 15 ppm a.i. is recommended by the manufacturer (6).

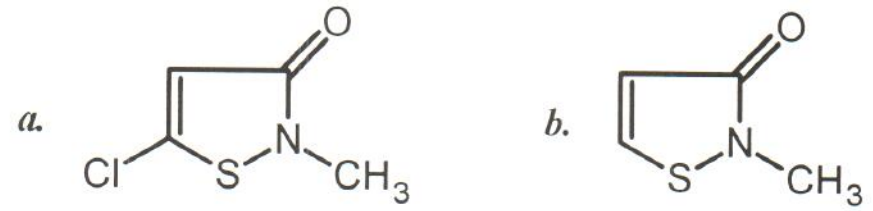

Fig.1. Structures of a) 5-chloro-2-methyl-4-isothiazolin-3-one and b) 2-methyl-4-isothiazolin-3-one

Table 1. Various methylisothiazolinone-based preservatives manufactured by Rohm and Haas Company

Kathon® CG
Kathon® CG/ICP
Kathon® LX
Kathon® 886 MW
Kathon® WT

Table 2. Examples of products and processes where the methylisothiazolinones MCI and MI can be used

cosmetics	glues
cutting fluids	paints
cooling tower water	swimming pool water
detergents	toiletries
fountain solutions	in photoprocessing
moistened toilet papers	in paper mill production

Patch test reactions to Kathon® 886

In the beginning of the eighties, two workers with hand dermatitis were examined at the Department of Occupational Dermatology, General Hospital, Malmö. They were exposed to cutting fluids containing the preservative K-886. At this time K-886 was completely unknown to Swedish dermatologists and nothing was published on its sensitizing capacity in humans or in animals. The patients tested positively to K-886 in water at 0.1 % a.i. (1000 ppm). The morphology of the positive test reactions had features of both an allergic and an irritant reaction. To further elucidate the nature of the reaction, it was decided to perform patch testing with serial dilutions and patch testing of controls (7).

Patch testing of controls and with serial dilutions

K-CG, which according to the manufacturer contains the fewest contaminants of the Kathons, was inserted into the standard patch test series at the Departments of Occupational Dermatology in Malmö and Lund (7). K-CG was diluted in water and the patch test concentration was 300 ppm a.i. A great number of positive test reactions were obtained and the morphology of the reactions was similar for all patients and did not differ from the positive reactions to K-886 that were noted in the 2 metal workers. The result of patch testing with serial dilutions of K-CG indicated an irritant reaction since the capacity to elicit a positive test was lost very fast (7).

Starting with the concentration of an allergen giving a moderate patch test reaction, it is often possible to decrease this concentration 100 times without losing the capacity to elicit a positive patch test reaction (8, 9). On the other hand, the ability to elicit an irritant reaction is usually lost with minute dilution steps from the concentration giving a moderate reaction. Thus, the result with loss of reactivity with a tenfold dilution in the majority of patients was supportive of an irritant nature of the reactions (7).

In summary, extensive investigations to elucidate the nature of the patch test reactions had not been conclusive. Most facts suggested an irritant nature, but an allergic nature could not be ruled out entirely. Still, nothing was published on the sensitizing capacity of K-CG, and the manufacturer did not disclose any information on such a capacity. It was then decided to patch test with K-CG at higher concentrations. If the test reactions in the controls represented irritant reactions it was believed that a concentration increase of more than threefold would at least double the frequency of positive patch test reactions, while it was considered to be unlikely to find a prevalence of hypersensitivity to a virtually unknown preservative at around 10 % (10).

Irritant reactions and patch test sensitization

Forty patients were simultaneously patch tested with K-CG at 1000 ppm and 300 ppm a.i. When the tests were read after 3 days, no reaction of the previously mentioned type (A) was noted (7). However, a positive reaction of a different type (B) was noted in 10 patients. Macroscopically, these reactions were sharply demarcated and brownish red, with a slightly shiny and dry surface. Thus, this morphology was consistent with what usually is considered to be a typical irritant reaction. However, the other type of reaction (A) appeared on the back of some patients 10 to 30 days after the application of the first patch test. These reactions were located on that part of the back where K-CG had been applied. All test preparations which had been patch tested on that part of the back were retested. Positive reactions to only K-CG were noted in all retested patients at the regular reading time (after 3 days). By definition, a negative patch test reaction followed by a flare-up reaction after 10 to 30 days and then a positive reaction after 3 days at retesting means that sensitization was induced by the patch test procedure (11). For the first time, K-CG was shown to be a sensitizer.

Even though K-CG was shown to be a contact sensitizer it did not mean that the other positive patch test reactions to K-CG at 300 ppm a.i. necessarily represented allergic reactions. However, the morphology of the flare-up reactions to K-CG and of the positive patch test reactions to K-CG after 3 days when retested as well as of the positive reactions after 3 days when tested the first time, was the same. Furthermore, the strength of reactivity was the same for the patch test-sensitized individuals and the patients reacting positively when routinely patch tested the first time; i.e. it was impossible to elicit a positive reaction also in the actively sensitized individuals when patch testing with the test solution diluted tenfold (7).

The frequency of contact allergy to K-CG in patients with suspected contact dermatitis routinely tested with our standard test series was obviously as high as 4 - 5 % in our region.

How had these patients become sensitized? Which substance/substances in K-CG were allergens? Actually, these questions initiated the first studies in this thesis.

MATERIAL AND METHODS

Substances/products

The main substances and products used in the studies (I-VI, VIII, IX) are listed in Table 3.

Substances and products representing the work environment in a plant producing binders for paint and glues and used at patch testing are listed in paper VII.

Table 3. Substances and products with manufacturers

Substance/product	Manufacturer	Paper
Kathon® CG	Rohm and Haas Company, Philadelphia, USA	I - IX
Kathon® WT	Rohm and Haas Company, Philadelphia, USA	VIII
Kathon® 893	Rohm and Haas Company, Philadelphia, USA	IV, V
1,2-Benzisothiazolin-3-one	ICI, Manchester, Great Britain	IV, V
2-Methylol phenol	Merck, Darmstadt, Germany	IV, V
Methyl, ethyl, propyl, and butyl paraben	Fluka Chemica, Buchs, Switzerland	VI
Imidazolidinyl urea	ICN Biomedicals Inc., Costa Mesa, USA	VI
Diazolidinyl urea	ICN Biomedicals Inc., Costa Mesa, USA	VI
Methyldibromoglutaronitrile	Schülke & Mayr, Hamburg, Germany	VI
Quaternium 15	Sigma Chemicals Co, St. Louis, USA	VI
Formaldehyde	Acros Organics, Geel, Belgium	VI
DMDM hydantoin	McIntyre Group LTD, University Park, USA	VI
2-Bromo-2-nitropropane-1,3-diol	Boots Company, Nottingham, England	VI
Sodium bisulfite	Janssen, Chimica, Beerse, Belgium	VIII
Glutathione	ICN Biomedicals Inc., Costa Mesa, USA	IX
Fenuril	Pharmacia Upjohn, Stockholm, Sweden	IX
Essex	Schering-Plough, Stockholm, Sweden	IX
Locobase	Yamanouchi, Malmö, Sweden	IX

Chemical investigations

High performance liquid chromatography (HPLC)

HPLC was performed to demonstrate the a.i. of K-CG in solutions and products (I-VI, VIII, IX). Columns packed with Nucleosil C_{18} from Machery-Nagel & Co, Düren, Germany were used. The length of the columns were 150 to 250 mm and the i.d. was 4 or 5 mm with particle size 3 or 5 µm. The eluents consisted mainly of mixtures of ethanol or methanol and acetic acid (0.07 mole $\times$ l^{-1}).

Mobile phases concerning identification and quantification of other preservatives are described in paper VI.

Two solvent delivery systems consisting of a ConstaMetric III pump and a SpectroMonitor III (both from Laboratory Data Control, Riviera Beach, USA) as well as an SP Spectra Series P200 pump and an SP Spectra System UV1000 detector (Spectra Physics, Riviera Beach, USA) were used. Both systems were equipped with Rheodyne injectors, model 7125 (Rheodyne, Catoti, USA). The eluate was monitored at 280 nm.

For preparative applications a gradient system with automatic sample injection and automatic sample collection was used. The system could also be used manually. The automatic process was controlled by an F-AV electronic process control unit (Film AV, Malmö, Sweden). The solvent delivery system consisted of an LDC Gradient Master (Laboratory Data Control) and 2 Constametric III pumps. The injector was pneumatic, Rheodyne model 7010 A (Rheodyne) and samples were collected by a pneumatic six position valve, Rheodyne model 5011 A. The UV detector, Spectromonitor D (Laboratory Data Control) was operated at 280 nm. The columns (100 mm $\times$ 11 mm i.d. semipreparative or 200 mm $\times$ 5 mm i.d. analytical) were packed with Nucleosil C_{18} (5 µm, Macherey-Nagel & Co).

Mass Spectrometry (MS)

The isolated allergens described in papers II and V were investigated by MS at Lund University, Sweden.

Nuclear Magnetic Resonance Spectrometry (NMR)

The isolated sensitizers (II, V) were also investigated by NMR at Lund University.

Preparation of samples from products

About 1 g of the product was accurately weighed and 2-10 ml of the mobile phase was added. The mixture was stirred and then filtered through a Millipore filter of the Millex® HV type or an ordinary paper filter. The solutions were analyzed by HPLC (I, VI).

Isolation of sensitizers from Kathon®CG

K-CG was separated into 5 fractions using gradient HPLC system and the semipreparative column (II) (Fig. 2). Fractions FII, FIV and FV were of high purity so studies were performed to determine their structures by MS and NMR (II, V).

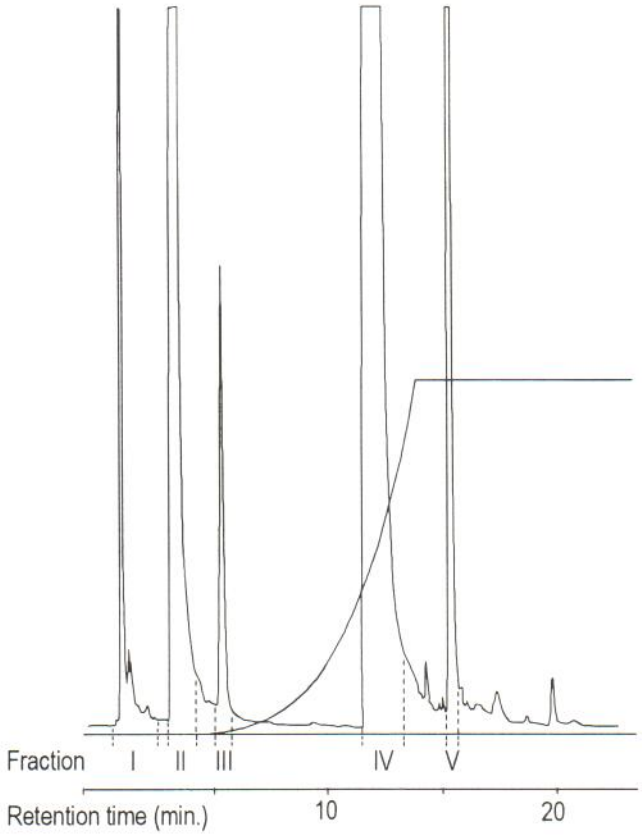

Fig. 2. HPLC separation of K-CG into 5 fractions

Inactivation of MCI and MI by sodium bisulfite

1.9 ml of aqueous sodium bisulfite 11.0 % w/v, freshly prepared, was mixed with 0.1 ml K-WT (VIII). The reaction was studied with regard to the changes of the concentrations of MCI and MI. Samples from the reaction mixtures were injected into the HPLC at various reaction times.

Inactivation of MCI and MI by glutathione in emollients

Preparation of glutathione-containing emollients

Various amounts of GSH were added to Fenuril, Essex, and Locobase, giving 3 preparations of each emollient containing 0.10, 0.50, and 2.0 % w/w GSH, respectively (IX). A half of each preparation was kept in room temperature (20 - 22° C) and the other half was refrigerated (6 - 7° C).

Inactivation of 15 ppm MCI and MI

20 µl of a dilution of K-CG containing 1500 ppm MCI and MI was added to 2.00 g of a GSH-containing emollient and mixed for 1 minute (IX). An extraction solution was added and the extract was injected into the HPLC. This procedure was repeated every 2 to 4 weeks during a period of 6 months.

Inactivating capacity of glutathione in emollients

In order to determine the total inactivating capacity of GSH in emollients various amounts of

MCI and MI were added to GSH-containing preparations until there was no more inactivating capacity according to HPLC analyses (IX). This procedure was repeated every 2 to 4 weeks during a period of 6 months.

Testing in humans

Patients

In the study II and III, 28 and 12 individuals hypersensitive to K-CG, respectively, were tested with fractions and isolated substances from K-CG. In study VIII seven other individuals allergic to K-CG were patch tested with reaction mixtures from K-WT and sodium bisulfite.

Controls

Twenty dermatitis patients without allergy to K-CG were tested with the isolated substances (II, III) and 5 dermatitis patients were tested with inactivated K-WT solutions (VIII).

Employees

At a plant producing binders for paints and glues 76 present and 11 former employees participated in an investigation consisting of an interview, clinical investigation and patch testing with both a standard test series and a series with products and chemicals representing the work environment (VII). Since the binders are water-based, preservatives have to be added to prevent growth of micro-organisms. Most of the added preservatives are based on MCI and MI. Of the present employees, 51 were production workers with skin exposure to the chemicals, 17 laboratory workers with possible skin exposure, and 8 office workers with virtually no skin exposure to the chemicals representing the work environment.

Patch test technique

The patients in studies II, III, and VIII were patch tested with Al-test (Astra Agency, Södertälje, Sweden) and Scanpor® tape (Norgesplaster AS, Kristiansand, Norway). Thirty µl of the test solutions was applied on each test unit. The test patches were removed after 48 h and evaluated after a further 24 h according to ICDRG criteria.

Finn Chambers® (Epitest, Helsinki, Finland) attached to Scanpor® tape and IQ Chambers (Chemotechnique Diagnostics, Malmö, Sweden) attached to 3M tape (3M, Medical Specialities, 3M Health Care, St Paul, USA) were used in study VII. The tests were removed after 48 h and evaluated after a further 24 or 48 h according to ICDRG criteria. A second reading was done 6 or 7 days after application of the patches (12).

Testing in animals

Test method

The guinea pig maximization test (GPMT) was performed in accordance with the original descriptions (13-15) but with some modifications in order to increase the standardization of the test and also to create conditions for objective evaluation, including statistical calculations of the patch test reactions and the inclusion of a positive control group (9). Rechallenge was performed according to a method previously described (16).

Induction, challenge and rechallenge

Induction and challenge were performed with MCI, MI, 45243-K-CG, and 2-MP (IV,V). Equimolar concentrations of MCI, MI, and 45243-K-CG were used for intradermal induction (0.0067 mole $\times$ l^{-1}) and for epidermal induction (0.0033 mole $\times$ l^{-1}) as well as for challenge (0.0013 mole $\times$ l^{-1}). Rechallenge was performed with MCI, MI, 45243-K-CG, BIT and Kathon® 893 containing 45 % of the a.i. 2-n-octyl-4-isothiazolin-3-one. The structures of 45243-K-CG, BIT and 2-n-octyl-4-isothiazolin-3-one are shown in Fig. 3.

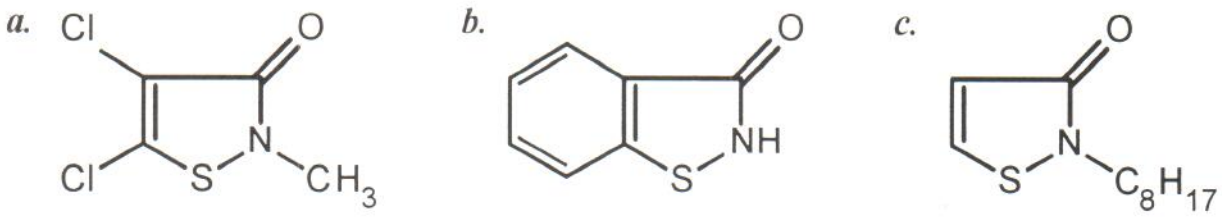

Fig.3. Structures of a) 45243-K-CG, b) BIT and c) 2-n-octyl-4-isothiazolin-3-one

Animals

Albino female guinea pigs of the Dunkin-Hartley strain (J A Sahlin, Malmö, Sweden) weighing 300 - 400 g were used. For each series 42 animals were used. Thirty-six animals were included in each sensitization study (12 in the control group and 24 in the test group), while the remaining 6 animals constituted an additional control group. These 6 guinea pigs were sensitized to and challenged with the known sensitizer 2-MP as a positive control group to eliminate the possible influence of expectations on the evaluation of the test reactions resulting in underestimation (9). The test and control animals, and the animals in the positive control group, were randomly distributed to the cages.

Evaluation

The reactions were evaluated blindly 24 h after removal of the patches. The minimum criterion of an allergic (positive) reaction was a confluent erythema. Within each test group, the number of positive animals was statistically compared to the number of positive animals in the corresponding control group and also to the number of positive animals tested with the vehicle alone. For the rechallenge, a comparision was made only between the number of positive animals in the test and control groups.

Statistical calculation

Fisher's exact test (one-sided) for two proportions was used.

RESULTS

The results of the various studies are described in details in the 9 separate papers but they will also be commented on briefly in this section.

Demonstration of MCI and MI in products

With HPLC technique the 2 a.i. of K-CG (MCI and MI) were demonstrated in commercial products (Fig. 4) (I). Thirty-eight out of 123 (30.9 %) investigated products of both leave-on type (16/56, 28.6 %) and rinse-off type (22/67, 32.8 %) were shown to contain MCI and MI and the concentrations varied between 1 - 15 ppm (I). About 10 years later, 6.0 % of 100 investigated leave-on products were demonstrated to contain MCI and MI at concentrations not exceeding 15 ppm (VI).

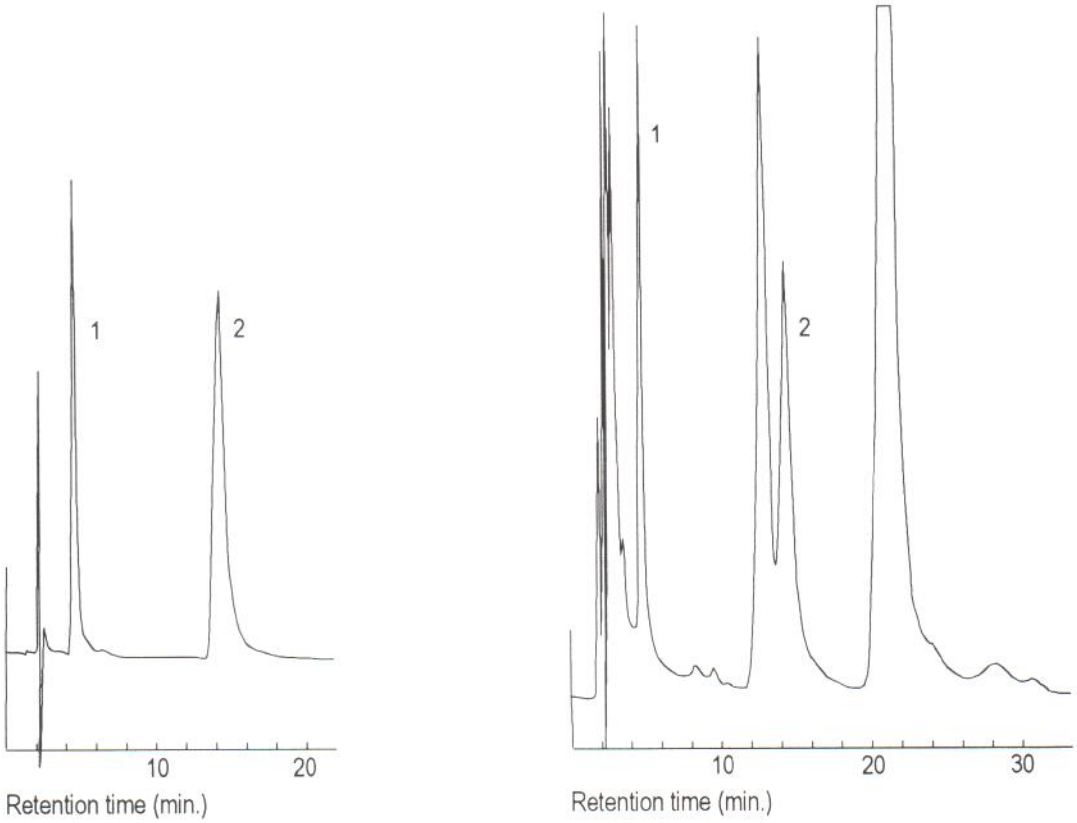

Fig 4. HPLC chromatograms of a) K-CG and b) a commercial product containing K-CG. Peak 1 corresponds to MI and peak 2 corresponds to MCI.

Demonstration of contact allergens in Kathon® CG

The results of patch testing with serial dilutions of K-CG and its 5 fractions in 28 individuals hypersensitive to K-CG are given in Table 4 (II). All tested individuals reacted to fraction FIV and the strength of the reactivity was almost the same for K-CG and fraction FIV. Two individuals reacted to fraction FII. The patient with a positive reaction to the most diluted fraction FIV (10 ppm) had also the highest degree of reactivity to the most diluted fraction FII (100 ppm). One patient tested positively to all 5 fractions.

Fractions FII and FIV were identified to be the 2 a.i. of K-CG, MI and MCI, respectively (II) and fraction FV to be 45243-K-CG (dichlorinated methylisothiazolinone) (V). The structural formula of 45243-K-CG is shown in Fig. 3.

The results of patch testing with serial dilutions of equimolar concentrations of MCI, MI, and 45243-K-CG in 12 individuals hypersensitive to K-CG are given in Table 5 (III).

Table 4. Results of patch testing with serial dilutions of K-CG and fractions FI, FII, FIII, FIV, and FV in 28 individuals hypersensitive to K-CG

	Number of patients with positive test reactions					
Concentration* ppm	K-CG	FI	FII	FIII	FIV	FV
300	28	1	2	1	28	1
100	13	0	1	0	16	0
30	5	0	0	0	7	0
10	1	0	0	0	1	0

* The concentrations indicate the concentration of a.i. in the respective test solution of K-CG. The concentrations of the fractions correspond to the concentrations of the respective fraction in the test solution of the a.i. at 300, 100, 30, and 10 ppm, respectively.

Table 5. Results of patch testing with serial dilutions of equimolar concentrations of MCI, MI, and 45243-K-CG in 12 individuals hypersensitive to K-CG. + = positive reaction of allergic nature, (+) = erythema or papules in a peripheral ring, - = negative reaction

Substance	Concentration		Patient no.											
	mmole × l^{-1}	ppm	1	2	3	4	5	6	7	8	9	10	11	12
MCI	1.0	150.0	+	+	+	+	+	+	+	+	+	+	+	+
	0.5	75.0	+	+	+	+	+	-	+	-	+	(+)	+	+
	0.25	37.5	+	+	+	(+)	+		-		+	-	+	(+)
	0.12	18.7	-	+	+	-	(+)				+		-	+
	0.06	9.3		(+)	(+)		-				-			-
	0.03	4.6		-	-									
MI	1.0	115.0	-	(+)	(+)	-	-	-	-	-	-	-	-	(+)
	0.5	57.5		-	-									(+)
	0.25	28.7												-
45243-K-CG	1.0	184.0	+	+	+	+	+	+	+	(+)	+	+	+	+
	0.5	92.4	+	+	+	+	+	(+)	+	(+)	+	+	+	+
	0.25	46.2	+	+	+	(+)	(+)	-	-	-	+	(+)	+	(+)
	0.12	23.1	(+)	+	+	-	-				+	-	(+)	-
	0.06	11.5	-	-	(+)						-		-	
	0.03	5.7			(+)									

Sensitizing capacities of the allergens

The results of the sensitization to and challenge with MI, MCI and 45243-K-CG (IV, V) in the guinea pig are shown in Table 6. The difference in the number of positive animals between test and control groups for the first series of MI was not significant. When, however, the sensitization was repeated with a new series, it was ($p < 0.01$). The differences in the number of positive animals for MCI and for both series of 45243-K-CG, were statistically significant ($p < 0.001$). Based on a great number of sensitization studies in guinea pigs, the number of animals testing positively to 2-MP should equal or exceed 2 to be considered a study without demur (9). Table 6 shows that the positive controls equalled or exceeded 2 animals in all sensitization studies except for series 1 in the sensitization study with 45243-K-CG; that is why a second sensitization study with 45243-K-CG was performed.

The rechallenge with chemically related compounds indicated cross reactions between some substances (Table 7).

Table 6. Test reactions after sensitization to and challenge with MI, MCI, and 45243-K-CG. (C = test reactions to the suspected sensitizer in control animals, T = test reactions to the suspected sensitizer in test animals , V = test reactions to the vehicle in test animals, P = positive control animals sensitized to and challenge with 2-MP, n = number of tested animals in the 4 groups C, T, V, P.

		Number of positive animals			
		C	T	V	P
Sensitization substance	n	12	24	12	6
MI					
series 1		0	4	0	3
series 2		0	11	0	4
MCI		1	19	1	2
45243 K-CG					
series 1		0	19	1	1
series 2		0	23	0	5

Table 7. Cross-reacting (statistically significant) and possible cross-reacting (indicated, but non-significant) substances to MI, MCI, and 45243-K-CG in the guinea pig. (+ = cross-reactivity, (+) = possible cross-reactivity, and - = no indication of cross-reactivity).

Cross-reacting substance	Sensitization substance		
	MI	MCI	45243-K-CG
MI		-	-
MCI	(+)		+
45243-K-CG	-	(+)	
Kathon® 893	-	-	-
BIT	-	-	-

Occupational dermatosis in a plant producing binders and glues

An occupational dermatosis was diagnosed in 22 present workers (28.9 %) (VII). Irritant and allergic contact dermatitis was demonstrated in 9 (11.8 %) and 13 (17.1 %) present employees,

respectively. Occupational contact allergy to acrylates and formaldehyde was detected in 3 and 1 worker, respectively. Considering both present and former employees 12 individuals (9 present and 3 former production workers) were demonstrated to have an occupational contact allergy to MCI and MI. K-CG, the screening substance for contact allergy to MCI and MI, was the only sensitizer in the standard test series for which there was a statistically significant difference in contact allergy rates between production workers and other workers. Contact allergy rates for the 6 most common sensitizers in the standard series are given in Table 8.

Four of the present production workers had spilled Kathon® LX on their skin resulting in chemical burns and allergic contact dermatitis (Table 9). In total, the figure for occupational skin diseases among all present production workers was 40.4 %.

Table 8. Contact allergies to the 6 most common sensitizers in the standard test series in 30 present and 4 former employees out of 76 present and 11 former employees.

			Present employees				Former employees	
			Workers				Workers	
	Total		Production		Laboratory and Office		Production	Laboratory
	87 patch tested		51 patch tested		25 patch tested		7 patch tested	4 patch tested
	positive	%	positive	%	positive	%	positive	positive
K-CG	12	13.8	9	17.6	0	0	3	0
Gold sodium thiosulfate	9	10.3	5	9.8	2	8.0	2	0
Nickel sulfate	5	5.7	3	5.9	2	8.0	0	0
Balsam of Peru	4	4.6	2	3.9	2	8.0	0	0
Fragrance mix	4	4.6	3	5.9	1	4.0	0	0
Thimerosal	4	4.6	3	5.9	1	4.0	0	0

Table 9. Chemical burn and contact allergy to K-CG in present production workers

		Chemical burn		
		+	-	Total
Contact allergy	+	4	5	9
to K-CG	-	0	42	42
	Total	4	47	51

$p = 0.0005$ (Fisher's exact test)

Inactivation of MCI and MI by sodium bisulfite

The concentrations of MCI and MI rapidly decreased after addition of sodium bisulfite to K-WT (VIII) (Table 10). The degradation products did not elicit any irritant test reactions, neither in test patients nor in controls. Five out of 7 individuals hypersensitive to K-CG reacted to a reaction mixture being about 30 s old and containing MCI and MI at 14 ppm, while 4 and 2 individuals tested positively to the reaction mixtures being 30 min and 48 h old, respectively (Table 11). The controls tested negatively to all reaction mixtures. All tested individuals reacted negatively to sodium bisulfite.

Table 10. The concentration of MCI and MI in the reaction mixture of 1.9 ml sodium bisulfite and 0.1 ml of K-WT at reaction times 0 s, 30 s, 30 min and 48 h.

	Concentration of MCI and MI (ppm)		
Reaction time	MCI	MI	MCI + MI
0 s	5148	2102	7250
30 s	10	4	14
30 min	7	3	10
48 h			< 3

Table 11. Results of patch testing with serial dilutions of K-CG, sodium bisulfite and reaction mixtures of K-WT and sodium bisulfite being about 30 s, 30 min and 48 h old in 7 individuals hypersensitive to K-CG.

(+ = positive patch test reaction of allergic nature; - = negative reaction)

	Case no:	1	2	3	4	5	6	7
MCI and MI	200 ppm	+	+	+	+	+	+	+
	100	-	-	+	+	+	+	+
	50	-	-	-	+	+	+	+
	25	-	-	-	-	-	+	+
	12.5	-	-	-	-	-	-	+
	6.25	-	-	-	-	-	-	+
Sodium bisulfite	11.0 %	-	-	-	-	-	-	-
Reaction mixture								
Time	30 s	-	+	-	+	+	+	+
	30 min	-	-	-	+	+	+	+
	48 h	-	-	-	-	+	-	+

Inactivation of MCI and MI by glutathione

The inactivation of MCI and MI by GSH in emollients, containing different amounts of lipids was studied with HPLC (IX). The results of inactivation of 15 ppm MCI and MI by GSH in emollients are shown in Fig. 5. The total inactivating capacities of 0.10 %, 0.50 % and 2.0 % w/w GSH in Fenuril, Essex and Locobase, respectively, were very similar within each concentration group and independent of type of storage and age. One emollient from each concentration group is shown in Fig. 6. GSH at 2.0% in Locobase was shown to inactivate up to 2400 ppm MCI and MI independent of age (Fig. 6c) and GSH at 0.50 % in Essex was capable to inactivate a few hundred ppm MCI and MI (Fig. 6b). GSH at 0.10% in Fenuril had a limited inactivation capacity (Fig. 6a).

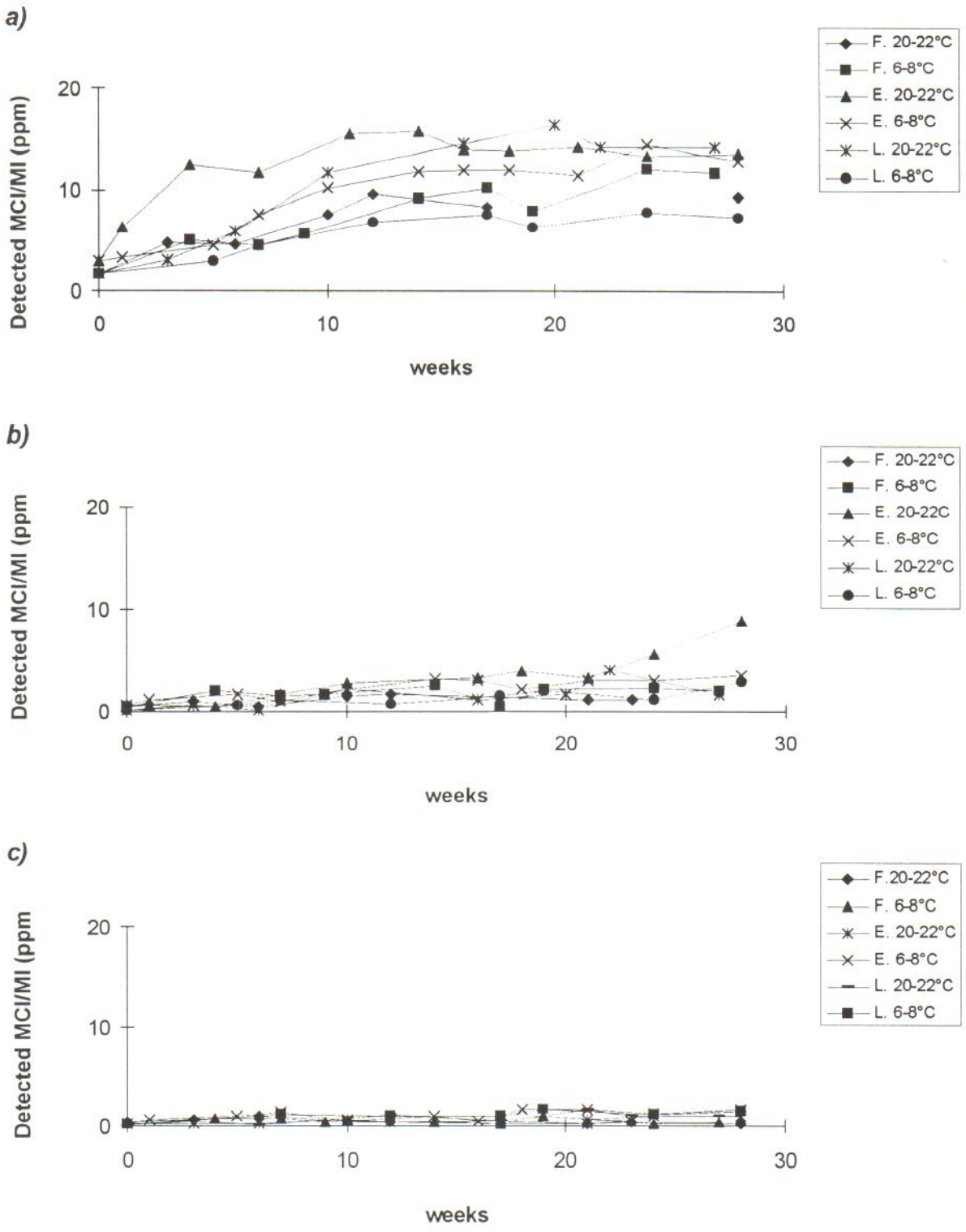

Fig. 5. The inactivation of 15 ppm MCI and MI added to samples of GSH-containing Fenuril (F), Essex (E), and Locobase (L) kept in room temperature (20-22°C) or refrigerated (6-8°C); a) 0.10 % GSH in F, E, and L, respectively; b) 0.50 % GSH and c) 2.0 % GSH, respectively.

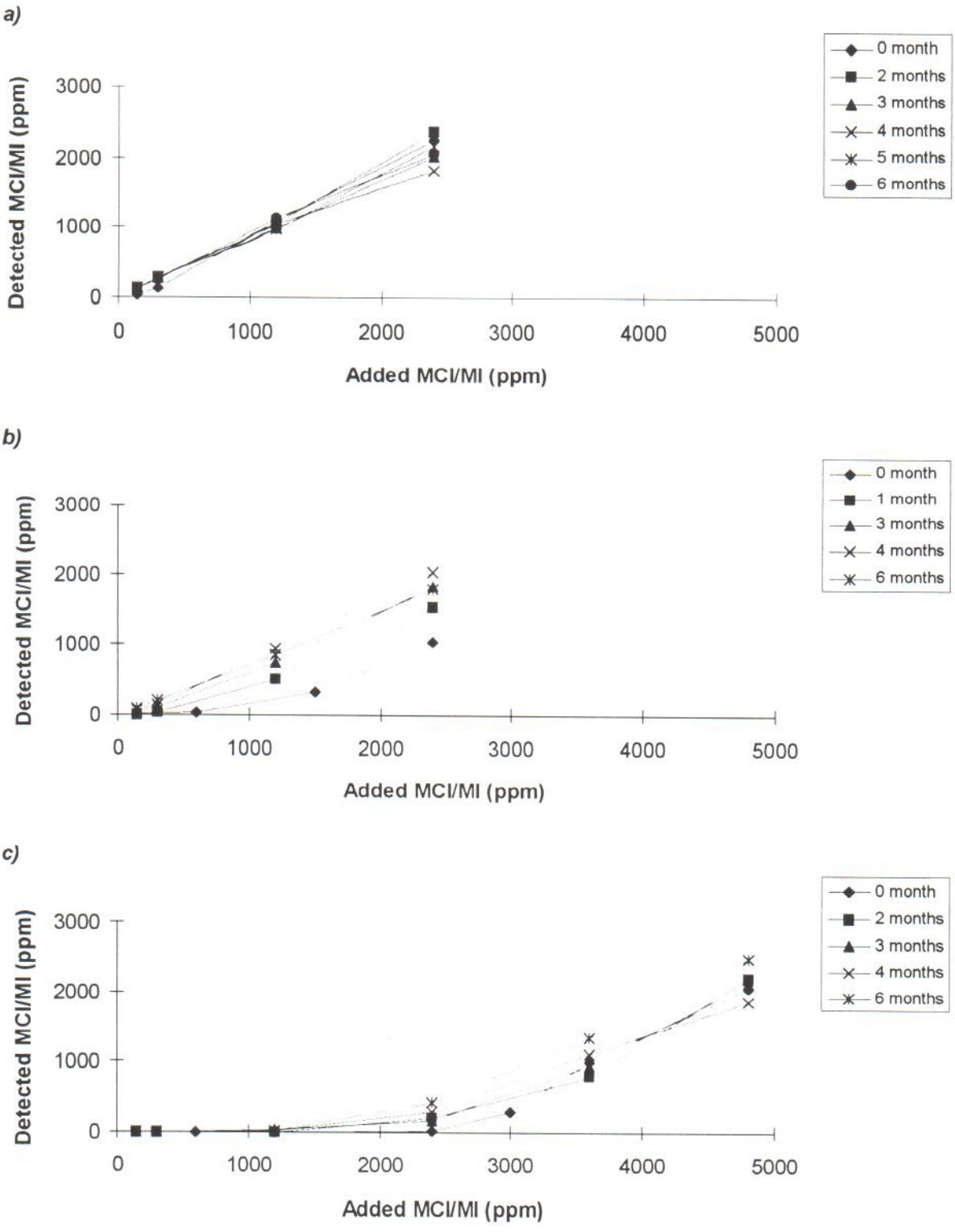

Fig. 6. The inactivating capacity of GSH in F, E, L, kept in 20-22° C, during a period of 6 months a) 0.10 % GSH in F, b) 0.50 % GSH in E, and c) 2.0 % GSH in L.

DISCUSSION

The present study started just after K-CG had been demonstrated to be a sensitizer (7, 17-19). Our first patients with a hypersensitivity to a methylisothiazolinone-containing preservative had been exposed to such a preservative when using cutting fluids containing K-886. In most of such occupational products the information on preservatives are given on the safety data sheets.

In our region, the frequency of contact allergy to K-CG in patients with suspected contact dermatitis was as high as 4 - 5 % when they were patch tested with our standard test series (7). However, we did not know how these patients had been exposed to MCI and MI. Occupational exposure to preservatives based on MCI and MI could not be demonstrated. According to the manufacturer of K-CG methylisothiazolinone-containing preservatives were recommended to be added to products such as toiletries and cosmetics. However, at that time labelling of ingredients of cosmetics and toiletries was not required by law in Sweden and other European countries. Thus, it was necessary to find a method to demonstrate MCI and MI in various products.

Demonstration of MCI and MI

To demonstrate MCI and MI in products HPLC can be used (I, 20). The 2 peaks in the chromatogram, corresponding to MI and MCI, are required for the identification of preservatives containing the 2 methylisothiazolinones. The chemical investigation demonstrated MCI and MI to be common in the analyzed products and were as common in leave-on products as in rinse-off products (I). Another important finding was that every individual hypersensitive to K-CG had been or was exposed to at least one product containing MCI and MI. Thus, skin exposure to toiletries and cosmetics containing MCI and MI at recommended concentrations could induce sensitization.

The HPLC method (I) is used routinely at our department to detect MCI and MI in various types of products. Sometimes modifications of the mobile phase or addition of K-CG to the sample have to be done to demonstrate or rule out the presence of MCI and MI (I). Such modifications including other mobile phases and a gradient HPLC system have later on also been emphasized (21). Some products may contain ingredients which can disturb the analysis. Amines, mercaptans, and ammonia are examples of substances which can cause degradation of particularly MCI and they may be found in cosmetics and toiletries (22). In K-CG the concentration ratio between MCI and MI is normally 3:1. When MCI and MI are degraded the ratio will probably be lower than 3:1 since MCI is most prone to react with other substances. Such a lower ratio has also been noticed by other authors (21, 23) and whether a disturbed MCI/MI ratio in a product would constitute a different risk factor has been discussed (21, 22, 24-26). A lower ratio means that there is proportionally less MCI, which is the stronger sensitizer (II, IV) and should therefore imply a lower risk of sensitization. However, the sensitizing and eliciting capacities of metabolites from MCI and MI have also to be considered. N-methylmalonamic acid, malonamic acid, and malonic acid are known degradation products (3, 4) but individuals hypersensitive to K-CG did not react to them when patch tested at equimolar concentrations (27). Other test solutions containing degradation products of MCI and MI have caused both negative and positive test reactions in individuals hypersensitive to K-CG (VIII).

Sometimes, when products preserved with MCI and MI belonging to patients allergic to K-CG are analyzed, MCI is not detected in the HPLC chromatogram but only the peak corresponding

to MI. It was earlier therefore concluded (10) that the individual allergic to K-CG was not exposed to the sensitizer. Initially, the patient probably had been exposed to both MCI and MI. Nevertheless, the remaining MI is a sensitizer although of a lower potency (II, IV). Therefore, when the chromatogram demonstrates only the peak corresponding to MI the patient is today recommended to avoid this product.

Contact allergens in Kathon® CG

Three chemically defined sensitizers, MCI, MI and 45243-K-CG, were demonstrated in K-CG (II-V). A positive test reaction was also demonstrated to each of the other 2 fractions (II). However, one fraction was not pure and the other fraction consisted of a too small amount of substance so further chemical investigations were not performed. Pilger et al (28) could also demonstrate MCI and MI to be sensitizers after isolation and patch testing in individuals allergic to K-CG. 45243-K-CG, demonstrated to be a sensitizer by us (III, V), is a dichlorinated methylisothiazolinone. Only 1 individual hypersensitive to K-CG reacted to 45243-K-CG when patch tested with concentrations corresponding to the concentration in K-CG 300 ppm a.i (II). Since the proportions of MCI, MI and 45243-K-CG were found to be approximately 45:15:1, the test concentration of 45243-K-CG was only 5 ppm. Furthermore, when patients hypersensitive to K-CG were tested with the 3 sensitizers at equimolar concentrations the strength of the reactivity for MCI and 45243-K-CG was similar (III).

Sensitizing capacities

Testing in guinea pigs with equimolar concentrations of MI, MCI and 45243-K-CG demonstrated MCI and 45243-K-CG to be strong sensitizers and MI to be a weak sensitizer (IV,V) and this result was in accordance with the result from patch testing in humans (II, III).

Cross reactivity

Simultaneous positive test reactions to MCI and MI and chemically related substances are reported in humans (17, 29). When the substances giving positive test reactions are chemically related cross reactivity is likely, but simultaneous allergies can not be excluded. Since preservatives are used in most water-based products/systems exposure to many preservatives are common. Therefore, positive test reactions to several preservatives may depend on multiple sensitization. Actually, to demonstrate cross reactivity between substances, studies on animals, has to be performed. In our animal studies cross reactivity between the 3 methylisothiazolinones on the one hand and BIT and octylisothiazolinone on the other could not be demonstrated (IV, V). In animals sensitized to 45243-K-CG more animals reacted to MCI than to 45243-K-CG at rechallenge (V). It could not be explained by contamination of MCI in 45243-K-CG. On the other hand, the results may indicate that these compounds are not immunologically distinguishable. This hypothesis, however, was not supported by the results of the corresponding tests in animals sensitized to MCI. Less than half the number of animals reacted to 45243-K-CG as compared to MCI. Another interpretation is conversion of 45243-K-CG to MCI in the skin by a metabolic process.

Contact allergy

Since the first reports on hypersensitivity to K-CG (17-19) there have been many reports on contact allergy, mainly from European countries but also from USA (7, 30-69). Since MCI and

MI are not commercially available patch testing is performed with K-CG. The hypersensitivity frequency among dermatitis patients varies between 0 and 10.2 % and a recent Swedish multicenter study demonstrated a frequency of 3.1 % (70). The frequency figures from different countries are difficult to compare since factors such as test concentrations and methods may vary. Since the dose-response curve for K-CG is very steep the test concentration and volume applied are crucial (7, 67). Internationally, an aqueous test preparation of K-CG 100 ppm a.i. has been recommended (71). The main reason for this recommendation seems to be the result of a 21 day cumulative irritancy assay where aqueous K-CG preparations at various concentrations were repeatedly applied on the upper arm in healthy volunteers (72). The study showed that K-CG 100 ppm a.i. appeared to have low irritancy potential (72). The limiting factor, however, for the routine patch test concentration is patch test sensitization rather than irritancy. The true patch test sensitization rates for various concentrations are not known and are difficult to ascertain (73-76). Approximately 1 % of the tested dermatitis patients became patch test sensitized when 300 and 250 ppm MCI and MI were used (7). In Sweden, aqueous K-CG 200 ppm a.i. has been recommended for routine patch testing and no active sensitization has been noticed (personal communication with members of the Swedish Contact Dermatitis Group, 1997).

Clinical relevance

It can be difficult to assess whether the hypersensitivity to K-CG in a patient is clinically relevant. After establishing the contact allergy, demonstration of a present exposure to MCI and MI as well as assessment of whether this type of exposure explains the clinical picture and course of dermatitis, have to be done. Demonstration of a present exposure to MCI and MI may be particularly difficult since both occupational and non-occupational exposure to MCI and MI are common and many products such as toiletries and cosmetics still lack labelling of ingredients. If such unlabelled products are patch tested the concentration of MCI and MI, when present, is usually too low to give a positive test reaction. Thus, many times chemical investigations (I) are needed to demonstrate MCI and MI in non-labelled products. However, even if a product is labelled the information given on the packages may be erroneous (VI). Consequently, to ascertain the presence of MCI and MI in a product chemical analyses are required.

Even if it is known that an individual hypersensitive to K-CG is exposed to products containing MCI and MI it may still be difficult to ascertain the clinical relevance of the exposure to these products. Repeated open application test (ROAT) can be carried out with the product applied to the flexor aspect of the patient's forearm near the cubital fossa or to the outer upper arm twice daily for a week (77, 31). A negative use test does not prove that the MCI- and MI-containing product is not relevant for the patient's skin disease since these tests are performed on normal skin and many individuals have used the product on damaged skin during a longer period than one week. Previously, exposure to MCI and MI in rinse-off products were considered to be non-relevant for a hand ezcema since the products remain on the skin for only a short time before washed off (78, 79). However, when use tests were performed in an individual hypersensitive to K-CG with his cleansing cream containing MCI and MI and the same cleansing cream containing another preservative (in a way corresponding to his ordinary working conditions), only the product containing MCI and MI caused a dermatitis (80). Without the use test with his rinse-off product the significance of this exposure would have been overlooked.

Several reports on use tests with creams and lotions on groups of patients have been published

(7, 31, 32, 41, 44-46, 51, 54, 60, 66, 77). These results are of great importance for the risk assessment concerning exposure to MCI and MI in leave-on products. When the relationship between the use test results and threshold patch test concentrations in individuals hypersensitive to K-CG was investigated it was demonstrated that the patients who reacted to the lowest patch test concentrations (25 ppm) had statistically more positive use tests than those who only reacted to 100 ppm (81).

Allergic contact dermatitis

MCI and MI in cosmetics and toiletries such as moisturizers, shampoos, and sunscreens have been the most common causes of allergic contact dermatitis from MCI and MI (17-19, 31, 38, 39, 45, 46, 54, 55). Most of these patients have been exposed to products containing MCI and MI at recommended concentrations, but exposure to higher concentrations have been reported (31, 54). Another source of allergic contact dermatitis from MCI and MI is moistened toilet paper containing MCI and MI (82-85).

Occupational allergic contact dermatitis

Several reports on occupational allergic contact dermatitis from MCI and MI have been published. It has been described in a radiology technician (55, 86), in a truck driver (55) in chemists (28, 87), in construction painters (88) and in workers in a starch factory (89), in a textile industry producing nylon (90) as well as in other workers after exposure to MCI and MI in lubricating oil (91), coolant oil/cutting fluids (7, 28, 92, 93), milk sample pots (33), fountain solutions (94, 95), paints (96), and products for skin and hair care (31, 54, 55, 80).

MCI and MI in paints have also been reported to cause airborne contact dermatitis (23, 96, 97).

MCI and MI at a plant manufacturing binders

High concentrations of MCI and MI are corrosive to skin and cause chemical burns at skin exposure (87, 98-102). In all reported cases except one the chemical burn was followed by allergic contact dermatitis which in some cases persisted for a long time (87, 98-101).

In the company where binders for paints and glues are manufactured the workers handled MCI and MI at high concentrations (VII). The employer is aware of the risks when handling preservatives containing high concentrations of MCI and MI as well as other hazardous products (VII). Efforts and measures to prevent skin exposure are therefore continuously being carried out mainly by making the processes more closed. However, in spite of this, workers have recently become sensitized to MCI and MI. Also in the investigated workers the opinion that a chemical burn from MCI and MI predisposes to sensitization to MCI and MI is strongly supported since all workers with a chemical burn were sensitized to MCI and MI (VII). Furthermore, of all workers with hypersensitivity to MCI and MI 45 % had suffered from a chemical burn (VII). Therefore, since a chemical burn from MCI and MI seems to induce sensitization to MCI and MI, patch testing with the incriminated preservative or K-CG, unless MCI and MI hypersensitivity is already known, is recommended in all individuals with chemical burns caused by preservatives containing MCI and MI.

When visiting the plant we found that besides the possible accidental exposure to high

concentrations of MCI and MI there was another way of exposure which was overlooked as a possible cause of sensitization by the company (VII). At different stations in the process samples from the production of the binders were taken frequently and at the end of the process where the binders were filtered. The emulsion usually contains 15 ppm but may contain up to 30 ppm MCI and MI. Therefore, the exposure implies a risk of sensitization to MCI and MI, particularly as the clothes often were contaminated with the emulsion and not changed until the end of the day. Another observation in the production was a frequent and inappropriate use of a concentrated and undiluted washing-up liquid instead of a soap, which caused irritant contact dermatitis in many workers with a subsequent risk of sensitization to MCI and MI on continuous exposure.

This study (VII) also emphasizes the necessity of visiting the work sites to get knowledge of occupational hazards and occupational dermatosis (103).

Risk assessment

Based on the results of many studies and reports from other authors and our own group including papers I-V, VII-VIII in this thesis, as well as our own experience since 1984, the risk assessment of skin exposure to MCI and MI is tentatively outlined in Table 12. Individuals may be exposed to methylisothiazolinones in the concentration range 14% (140 000 ppm) down to 0 ppm. However, for practical reasons only 2 groups representing the high concentrations of MCI and MI used professionally and the low concentrations of MCI and MI in toiletries and cosmetics are shown in Table 12. It should be noted that the different parts of the risk assessments are not equally well-grounded.

Table 12. Risk assessment of skin exposure to MCI and MI

	Chemical burn	Allergic contact dermatitis	
		Sensitization	Elicitation
High concentrations (14%) of MCI and MI	***	***	***
Low concentrations (15 ppm) of MCI and MI			
in leave-on products			
on normal skin	-	*	**
on damaged skin	-	**	***
in rinse-off products			
on normal skin	-	(*)	*
on damaged skin	-	*	**

*** = high risk, ** = moderate risk, * = low risk, (*) = minimal risk, - = no risk

It has been reported several times that spill of high concentrations of MCI and MI causes chemical burns on exposed skin (87, 98-102). It has also been pointed out (VII) that a

chemical burn "predisposes" to sensitization to MCI and MI. Furthermore, we found that all workers with a MCI and MI-induced chemical burn were sensitized to MCI and MI (VII). Thus, there is a high risk of sensitization and elicitation of allergic contact dermatitis after skin exposure to high concentrations of MCI and MI (Table 12).

In a study using various concentrations of K-CG for induction and challenge K-CG was demonstrated to be a contact sensitizer in the guinea pig (104). Based on the results of statistical calculations for the various concentrations, the authors claimed that there is a "no response concentration" zone where K-CG can be used without concern for clinically significant delayed contact dermatitis in man (104). However, the concentrations of significance in guinea pigs can not automatically be transformed to humans. Skin exposure to low concentrations of MCI and MI in toiletries and cosmetics obviously can sensitize since several investigations have shown that many individuals without occupational exposure to MCI and MI are allergic to K-CG. When the first cases of contact allergy to K-CG were reported it was considered that the sensitization was due to exposure to too high concentrations of MCI and MI (> 15 ppm) in leave-on products. However, although exposure to higher concentrations has been reported (31, 54) most patients have been exposed to products with MCI and MI at recommended concentrations (I, chemical analyses during 1984 - 1997).

There is a limited knowledge about the difference between normal and damaged skin regarding both sensitization and elicitation of allergic contact dermatitis. Use test studies with leave-on products containing MCI and MI (15 ppm or lower) have shown that repeated applications of such products on normal skin in individuals sensitized to K-CG cause dermatitis in about 30 - 50 % of tested individuals (7, 31-32, 41, 44-46, 51, 54, 60, 66, 77). Thus, there is a risk of elicitation of allergic contact dermatitis when leave-on products are used on normal skin and the risk is probably higher when they are used on damaged skin.

On normal skin the risk of both sensitization and elicitation when using rinse-off products containing MCI and MI has been considered to be almost negligible (10). However, recent use tests with rinse-off products on normal skin have shown that applications of such products followed by rinsing with water may cause allergic contact dermatitis (78, 80). Furthermore, there are reports (105, 106) which indicate that MCI and MI can accumulate within the skin. With consideration of these facts we ascribe the use of rinse-off products a low risk of elicitation of allergic contact dermatitis.

Prevention

In this part both general and individual prevention are discussed.

To prevent chemical burns from MCI and MI, preservatives containing high concentrations of MCI and MI should not be used. However, in some technical applications such preservatives may be difficult to replace. In these industries the processes then should be automatized to minimize skin contact with the concentrated preservatives. Furthermore, the workers have to use appropriate protective clothes when there is a risk of skin contact with the preservatives and always at procedures such as repairing, charging and discharging of vessels containing MCI and MI. If the skin is exposed, in spite of all these measures, immediate treatment is necessary to prevent a chemical burn. Rinsing with water has been shown to be insufficient. However, our findings that GSH is able to inactivate MCI and MI (IX) is promising and encourage further studies on the use of aqueous solutions of higher concentrations of GSH to prevent chemical burns.

Our experience is that workers with a chemical burn followed by sensitization still can continue their jobs. However, although trying to avoid exposure to MCI and MI accidental exposure to low concentrations of MCI and MI at the work site may occur (VII). Therefore, the daily use of an emollient containing GSH (IX) perhaps may prevent elicitation of allergic contact dermatitis from such an exposure.

Exposure to many skin irritants is common in industries where high concentrations of MCI and MI are handled. To prevent irritant contact dermatitis most employers provide barrier creams and emollients to be used ad libitum. In these industries skin exposure to low concentrations of MCI and MI is not uncommon (VII) and there is a risk of sensitization to MCI and MI after exposure to such low concentrations (I, VII). An emollient containing GSH hopefully might prevent both irritant contact dermatitis and sensitization to MCI and MI.

To prevent non-professional sensitization to MCI and MI it has been recommended not to use MCI and MI in leave-on products (10, 58). Concerning rinse-off products there is no such recommendation. Obviously, there is a low risk of skin problems (78, 80) when rinse-off products containing MCI and MI are used but if this risk is different from other preservatives is not presently known.

For individuals sensitized to MCI and MI it is important to avoid products containing MCI and MI to prevent allergic contact dermatitis. Toiletries and cosmetics, including emollients, are used daily by many people. According to legislation on ingredient labelling of cosmetics and toiletries the European consumer will in the future be able to get information on all preservatives in a product like the US consumer today. Thus, it should be possible to choose products without MCI and MI. We have, however, found that information given by the manufacturer directly or on the packages, can be erroneous and misleading (VI) and consequently, today and in the future it will be difficult to entirely avoid exposure to products containing MCI and MI if the manufacturers/suppliers will not be more careful and conscious of their responsibilities concerning the information given on preservatives. Therefore, in individuals hypersensitive to K-CG elicitation of allergic contact dermatitis from unintentional exposure to MCI and MI might be prevented by daily use of an emollient containing GSH.

GENERAL SUMMARY

Preservatives are required in water-based products/systems to prevent the growth of micro-organisms. During the eighties preservatives based on methylisothiazolinones became widely distributed. K-CG, consisting of 5-chloro-2-methyl-4-isothiazolin-3-one (MCI) and 2-methyl-4-isothiazolin-3-one (MI) as a. i., was demonstrated to be a common contact allergen. However, it was not known how the individuals had been sensitized.

A chemical method based on HPLC was developed to demonstrate the a.i. of K-CG. MCI and MI could be demonstrated in various types of products belonging to the patients and thus, the sensitization to K-CG could be explained. In patients hypersensitive to K-CG demonstration of a present exposure to MCI and MI is required to arrive at a diagnosis of allergic contact dermatitis. Still, most preservative-containing products are not sufficiently labelled, and if labelled, the information may be erroneous, so chemical analyses are needed.

Three sensitizers were isolated and identified from K-CG, MCI, MI, and 45243-K-CG, a dichlorinated methylisothiazolinone at a low concentration in K-CG.

The sensitizing capacities of the 3 allergens were determined by using the guinea pig maximization test. MCI and 45243-K-CG were demonstrated to be strong sensitizers and MI to be a weak sensitizer when the animals were tested with equimolar concentrations.

Although there are recommendations not to use MCI and MI in leave-on products, chemical analysis demonstrated MCI and MI in moisturizers presently used on the Swedish market.

MCI and MI are a.i. in many preservatives. Some preservatives contain high concentrations of MCI and MI. Skin exposure to high concentrations of MCI and MI can cause chemical burns and induce sensitization. A high frequency (17.6 %) of contact allergy to MCI and MI was demonstrated in 51 production workers in a factory handling preservatives with high concentrations of MCI and MI. Four of the 9 workers sensitized to K-CG suffered from chemical burns caused by preservatives with high corrosive concentrations of MCI and MI.

MCI and MI can be chemically inactivated by sodium bisulfite and glutathione.

COMPREHENSIVE SUMMARY IN SWEDISH

Metylisotiazolinoner. Diagnostik och prevention av allergiska kontakteksem

Konserveringsmedel är biologiskt aktiva ämnen som hindrar mikroorganismer som t.ex bakterier och svampar att föröka sig. Produkter som innehåller vatten behöver konserveringsmedel, annars kan t ex konsistens, färg och lukt förändras så att produkten blir oanvändbar. Hudvårdsprodukter, rengöringsmedel, kosmetika men även skärvätskor, målarfärg, lack och lim innehåller ofta konserveringsmedel.

Eftersom konserveringsmedel är biologiskt aktiva ämnen är det inte förvånande att de vid hudkontakt även kan framkalla kontaktallergi. Flera konserveringsmedel är kända kontaktallergen. Under åttiotalet orsakade ett nytt konserveringsmedel, Kathon® CG, kontaktallergier hos många människor. Kathon® CG består av 2 aktiva substanser (metylisotiazolinoner) vars kemiska benämningar är 5-klor-2-metyl-4-isotiazolin-3-on (MCI) och 2-metyl-4-isotiazolin-3-on (MI). Eftersom många hygienartiklar och rengöringsprodukter saknade innehållsdeklarationer var det svårt att fastställa hur allergin hade uppkommit.

Syftet med avhandlingen har varit att förbättra diagnostiken av allergiska kontakteksem orsakade av metylisotiazolinoner men även att försöka finna metoder för att förhindra eksem orsakade av dessa. Avhandlingen grundar sig på 9 vetenskapliga uppsatser som alla handlar om metylisotiazolinoner.

I första delarbetet beskrivs hur metylisotiazolinonerna MCI och MI med en kemisk metod kunde påvisas i olika typer av produkter. En tredjedel av de 123 analyserade produkterna som våra Kathon® CG allergiska patienter använt innehöll metylisotiazolinoner. Dessa var ungefär lika vanligt förekommande i hygienartiklar som kvarstannar på huden under flera timmar som i produkter som snabbt sköljs av huden. Studien visade även att alla patienterna använt åtminstone en metylisotiazolinon-innehållande produkt. Med hjälp av denna metod har sambandet mellan användningen av produkter innehållande metylisotiazolinoner och kontakteksem orsakat av metylisotiazolinoner kunnat fastläggas. Eftersom många produkter saknade och fortfarande saknar innehållsdeklaration behövs den kemiska metoden för att påvisa förekomsten av metylisotiazolinoner i olika produkter.

För att kunna fastställa vilket eller vilka ämnen i Kathon® CG som orsakade kontaktallergi använde vi samma kemiska metodik för att isolera olika ämnen ur Kathon® CG (delarbete två). Av delarbetena två och tre framgår att tre kontaktallergen har isolerats och identifierats. Både MCI och MI men även en annan metylisotiazolinon, 4,5-diklor-2-metyl-4-isotiazolin-3-on (45243-K-CG) som förekommer i låg koncentration i Kathon® CG visades vara kontaktallergen.

I delarbetena fyra och fem studerades sensibiliseringskapaciteten för de tre isolerade och identifierade kontaktallergenen. De tre ämnena testades på ett likvärdigt sätt på marsvin och vi fann att MCI och 45243-K-CG var starka allergen och MI ett svagt allergen.

Konserveringsmedel som är baserade på metylisotiazolinoner innehåller alltså flera potenta kontaktallergen och det har därför rekommenderats att de inte ska användas i hygienartiklar som dröjer kvar på huden. I delarbete sex visas dock att metylisotiazolinoner fortfarande är

vanliga i sådana produkter.

Vissa industrier hanterar konserveringsmedel som innehåller höga koncentrationer av metylisotiazolinoner (delarbete sju). Om sådana konserveringsmedel spills på huden kan både kemisk etsskada och kontaktallergi för metylisotiazolinonerna uppkomma. När vi kartlade yrkeshudsjukdomar på en industri där höga koncentrationer av metylisotiazolinoner hanterades fann vi att både kemiska etsskador och allergiska kontakteksem av dessa konserveringsmedel var vanligt förekommande trots att företaget hade vidtagit åtgärder för att förhindra kontakt med konserveringsmedlen (delarbete sju).

I delarbete åtta visas att den kemiska strukturen av metylisotiazolinoner kan förstöras och härigenom minska de korrosiva och eksemframkallande egenskaperna hos konserveringsmedel som innehåller höga koncentrationer av metylisotiazolinoner.

I det nionde delarbetet visas att ett kroppseget ämne, glutation, som tillsats i hudkrämer kan inaktivera metylisotiazolinoner. Ytterligare studier får visa om det praktiskt kan användas för att förhindra allergiska kontakteksem orsakade av metylisotiazolinoner.

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