

Allergologically relevant rubber accelerators in single-use medical gloves

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To prevent contact with specific rubber accelerators, sensitized patients have to know in which glove brands these accelerators are present. Additionally, quantitative measurements of the bioavailable amounts of accelerators are needed to assist consumers in selecting gloves with the lowest possible amount of residual rubber accelerators. The aim of the study was to develop an analytical method by which residuals of rubber accelerators in single-use medical gloves could be determined qualitatively and quantitatively. 19 different glove brands were analysed for content of accelerators, and the results were compared to manufacturers' ingredient claims of the identical gloves. ZDEC, ZDBC, ZMBT and ZPC were the most frequently detected chemicals. In 9 of 15 gloves discrepancies were found, usually minor, between content of accelerators declared by manufacturers to be present and accelerators detected by analysis. Both phosphate buffer and acetone were tested as extraction media. No accelerators were detectable with the described chemical analysis in phosphate extracts, whereas acetone was demonstrated to be a technically suitable medium for extraction. However, more kinetic studies of the extraction procedure and studies of skin penetration are needed to document that the extraction procedure simulates the clinical situation.

Key words: natural rubber latex; medical gloves; rubber accelerators; chemical analysis; extraction procedures; ingredient information; clinical relevance. © Munksgaard, 2000.

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Rubber gloves, mainly used in the medical health care sector, are major contributors to allergy to rubber accelerators (1–7). To reduce sensitization to rubber accelerators, the use especially of thiurams (THs) in glove production has declined during recent years, according to the manufacturers. Furthermore, efforts have been made to reduce the total amounts of residual chemicals in natural rubber latex gloves (NRL gloves) (8). Dermatologists have for many years tried to obtain information about the rubber accelerators actually used during production, in order to advise sensitized patients about possible exposures (2). Many manufacturers have claimed, however, that it is but of little interest to consumers which chemicals have been used during production, as the important question is which chemicals are bioavailable from the final products. The aim of this study was to compare the results of an analytical determination of the

accelerator content in selected medical gloves with the manufacturers' claims as to the accelerators used during the production of these gloves. An analytical method needed to be developed which allowed the determination of relevant residual accelerators in gloves, quantitatively as well as qualitatively. The method needed to be relevant in relation to the amounts of chemicals causing sensitization and dermatitis in sensitized individuals. An appropriate extraction procedure needed to be established. This procedure needed to be comparable to the biological conditions present during glove use, with extraction of bioavailable accelerator residues only.

It is not known whether the penetration of hydrophobic accelerator molecules into the skin takes place via the sweat, directly via skin lipids, or if both routes are relevant. Theoretically, both aqueous media as well as lipophilic media could therefore be of interest. When extraction of rubber items for analysis is intended to be total, chloroform is commonly used as extraction solvent. However, this solvent al-

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ters the glove material by an excessive swelling, and this procedure was considered to be very different from biological conditions. Alcohols might be eligible for extraction, but the most important accelerators were found to have a higher solubility in acetone than in alcohol (9). As acetone was found not to cause visible changes to the rubber-film within 1/2 h, this medium was selected as a compromise between strong apolar solvents such as halogenated hydrocarbons and polar media as synthetic sweat (9). Preliminary studies showed that the stability of THs in acetone was limited but still high enough to allow quantitative analysis, if this was carried out without delay. The stability of carbamate (C) solutions in acetone was high (9). A few experiments were additionally carried out using 20 mM phosphate-buffer, pH 6.5, a medium discussed by the American Society for Testing and Materials (ASTM) for this purpose (Perella, 1995 [private communication]).

Only a few papers deal with quantitative considerations of allergologically relevant accelerator residues in rubber products (10–15). In a previous study, 1/3 of patch tested TH-sensitive individuals reacted to TH mix in aqueous solution at dilutions containing 1 μg TH mix per cm^2 skin-test-area (16). TH mix is a mixture of equal parts of tetraethyl-thiuramdisulfide (TETD), dipentamethylene-thiuramdisulfide (PTD), tetramethyl-thiurammonosulfide (TMTM) and tetramethyl-thiuramdisulfide (TMTD)). The amount of THs released from rubber gloves into an aqueous medium has been estimated to be in the range of 0.5 $\mu\text{g}/\text{cm}^2$ –20 $\mu\text{g}/\text{cm}^2$ during 24 h (13). The sensitivity of the method selected for this study had to be within the same range as the results from these studies indicated. The analytical method used in the former study was gas chromatography with mass spectrometry detection (GC-MS), using isotope-dilution. This technique is not suitable for routine analyses of common industrial products because of its complexity. Several analytical methods were considered for the present study, including high performance liquid chromatography (HPLC), gas chromatography with MS- and flame ionization (FID) detection, thin layer chromatography (TLC), and high performance TLC (HPTLC). According to results from pilot studies (9), HPTLC was found to be the method most eligible for identification of the substances and GC-FID the method most eligible for quantitative analyses.

Methods and Materials

Gloves

Single-use medical gloves (sterile and non-sterile) included in the present study were selected so that

different chemical compositions of the most commonly used derivatives of benzothiazole, Cs and THs were included. Some, in the EU, very commonly used glove brands were represented, but information about market shares in the countries participating in the study was not available. For the majority of the gloves investigated, information was available from the manufacturer concerning accelerators used in the glove production. All glove brands were investigated by means of acetone extraction followed by chemical analysis. 2 glove brands were additionally investigated by means of phosphate buffer extraction and analysed in the same way as acetone extracts. These 2 glove brands were selected because of high content of accelerator residues detected in the acetone extracts and the claimed use of ZMBT, respectively.

Extraction procedures

Extraction media were acetone, analytical grade, and 20 mM phosphate-buffer, pH 6.5. For extraction, 5 cm of the middle finger of a glove was cut off and weighed with a precision of 0.1 mg. Thereafter, the glove finger was turned inside out and placed tightly over a test tube with an outer diameter of approximately 20 mm. By this procedure, the inner side of the glove was tested. The test tube with the glove finger was placed in another test tube with an outer diameter of approximately 30 mm, containing 10 ml of extraction medium, thus covering most of the glove material but not submerging the open end. The outer test tube was sealed with a lid and submerged in an ultrasonic bath for 30 min at room temperature. This procedure did not cause visible changes to the product. Acetone extracts were evaporated without any delay in a rotary evaporator. For further analysis, the residuals were re-dissolved in 2 ml chloroform. Phosphate buffer extracts were extracted with 2×2 ml chloroform (liquid/liquid-extraction) and the volumes of resulting chloroform extracts were reduced in a nitrogen stream to 2 ml, which were used in further analysis.

Chemical analyses

HPTLC. Merck HPTLC plates 10×10 cm, silicagel 60, without fluorescence indicator, with a 10×2.5 cm concentration zone, were used without any activation at elevated temperature. The following eluents were used: n-hexane, toluene and ethyl acetate in the ratio of (V/V/V) 30:58:12. The chloroform solutions described above were used directly for analysis. Standard solutions were applied to the plate simultaneously with the

Table 1. Rubber accelerators found to be present or used during production according to manufacturers claims (15 gloves) in the 19 gloves analysed

Abbreviation	Substance	Used for testing in European standard series	Used in special rubber series	Claimed by manufacturers to be present in 1 or more of the 15 gloves, ingredient declared.	Detected by analysis in 1 or more of the 19 gloves analysed	Compound can be a conversion product
TMTM	tetramethylthiuram-monosulphide	(TH mix)	yes			
TMTD	tetramethylthiuram-disulphide	(TH mix)	yes		yes	
TETD	tetraethylthiuram-disulphide	(TH mix)	yes		yes	
PTD	dipentamethylen-thiuramdisulphide	(TH mix)	yes			
TBTD	tetrabutylthiuram-disulphide		yes		yes	
PTT	dipentamethylen-tetrasulphide			yes		
ZDMC	zincdimethyldithio-carbamate		yes			yes
ZDEC	zinc-diethyldithiocarbamate	(carba mix)	yes	yes	yes	yes
DPG	1,3-diphenylguanidine	(carba mix)		yes		
ZDBC	zinc-dibutyldithiocarbamate	(carba mix)		yes	yes	yes
ZPC	zinc-dipentamethylen-dithiocarbamate			yes	yes	yes
ZDiBC	zinc-diisobutyl-dithiocarbamate				yes	yes
PPD	piperidine-pentamethy-lene-dithiocarbamate			yes		
MBT	mercaptobenzothiazole	yes				
CBS	N-cyclohexyl-2-benzothiazolesulfenamide	(mercapto mix)				
MOR	4-morpholinyl-2-benzothiazyl disulphide	(mercapto mix)				
MBTS	2,2dibenzothiazyl-disulfide	(mercapto mix)			(yes, traces)	
ZMBT	zinc-mercaptobenzothiazole			yes	yes	yes
MBI	mercaptobenzimidazole			yes		
DMA	dimethylamine					yes
DEA	diethylamine					yes
DBA	dibutylamine					yes
PP	piperidine					yes
	thiureas		yes			

For comparison are listed rubber accelerators used for routine testing or used in special rubber series. Also possible conversion products, described in the literature as sensitizers, are listed.

samples*. The eluent was evaporated at room temperature, and plates were sprayed with 0.05 % (W/W) dithizon in ethanol at room temperature to identify C and TH. Thereafter, the plates were sprayed with 0.5% (W/W) 2,6-dichloroquinone-4-chlorimide in 2-propanol and dried in a drying cabinet for 3 min at 60°C, identifying THs, Cs and mercaptobenzothiazoles according to colours and their respective R_f values. This procedure is described elsewhere (17). If confirmation of identification of THs was necessary, a new plate was prepared, and 1% copper sulfate in deionized water was used as spray reagent.

The detection limit of the HPTLC method was estimated from serial standard dilutions of the single substances, and was found to be in the range of 5–10 ppm (corresponding to 100–200 µg/g glove

material if the extraction method described above is used). If accelerator mixtures with different concentrations of some components are analysed, the detection limit is significantly higher. Also, other substances present in some glove extracts can further decrease the sensitivity of the method.

GC. A gas chromatograph equipped with a capillary column and flame ionization detector (GC-FID) was used under the following conditions: column: 25 m×0.25 mm ID; 100% methylsilicone, 0.25 µm film thickness, and temperature programme 40°C, isothermal 4 min, 20°C/min up to 280°C, run time 25 min., carrying gas: helium 70 kPa, injector: 250°C, split 1:10, detector: FID 280°C. Extracts prepared as described were derivatized in chloroform by adding TFAA (trifluoroacetic anhydride) and incubating at 75°C for 30 min. If the reaction mixture was turbid after derivatization, the suspension was filtered through a microfilter with PTFE-membrane, pore size 0.45 µm. The individual analytes were identified and quantitatively estimated by comparison of their retention times and the relevant peak areas, with standard trifluoroacetamides. Ad-

* TMTD, TETD, ZDEC, MBT, MBI (Aldrich), 97–98% purity. ZDMC, MBTS (Bayer Austria) 97–98% purity, PTT (Pharmaceuticals Corp.) ZPC, TBTD, ZDBC (Robinson Brothers), and ZMBT (Semperit), all technical grade.

ditionally, identification of derivatives was confirmed by mass-selective detection using a mass spectrometer detector in scan-mode. This method was developed on basis of a standard procedure (ISO 10398:1998) (18). A differentiation between THs and Cs with identical amino groups is not possible in this procedure.

The quantification limit varied between 10 and 20 $\mu\text{g/g}$ glove, depending on the chemical constitution of the analyte. The limits were established by a statistical procedure according to DIN 32645(19). The quantification limits of individual amines derived from accelerators present in the extract can be expected to be significantly higher.

Results

19 gloves were analysed according to the described methods, using acetone as extraction medium. 18 gloves were NRL gloves, while 1 was made of synthetic rubber. In Table 1 are listed the accelerators detected in 1 or more of the 19 gloves, together with the accelerators claimed by the manufacturers to be present. In the table are also listed the accelerators used in the standard patch test series and in the additional rubber series that may be used when allergy to rubber accelerators is suspected. Finally are listed possible conversion products, described in the literature as sensitizers. Relevant claims made by manufacturers were available for 15 of the 19 glove brands analysed.

Table 2 shows how often specified accelerators were detected in the glove products. It appears that ZDEC and ZDBC were the most common accelerators. None of these accelerators are used in the standard series for patch testing, but have been recommended in additional rubber series (20). PTT was declared to be present in several gloves, but only the conversion product ZPC was detected. None of these compounds are usually used for patch testing. TBTD may be used in special rubber test series, but is not present in the thiuram mix used for patch testing in the standard-series. Mercaptobenzothiazoles seem to be very commonly

used rubber additives as well: MBT, but not ZMBT, is used for patch testing.

The amounts of individual accelerators (μmol per g glove) determined to leach out using this experimental set up are given in Table 3. Where both the TH and the corresponding C were present, only the total amount is indicated. "*" in the table indicates that the compound was declared by the manufacturer to be present.

Identity between the qualitative chemical analysis and the latex formulation claim made by the manufacturer was found in 6 gloves (Table 3). In 2 glove brands only a C was declared, but both the C and the corresponding TH were detected by analysis. In 3 glove brands the manufacturer declared PTT, but only the corresponding C (ZPC) was found. In 5 gloves 1 or more accelerators were detected by analysis, but had not been declared by the manufacturers. In 1 glove ZMBT was claimed and seemed to be present according to the HTPLC analysis, but this result could not be confirmed by GC-MS. MBI was declared in 3 gloves, but neither MBI nor other benzimidazole derivatives were found by the analysis.

Thus, among the 19 glove brands included in the study, a substance was both declared and detected in 24 cases, declared but not detected by analysis in 7 cases, and detected by analysis but not declared in 12 cases.

Only traces of ZDEC were found in a phosphate buffer extract from a glove, where a high content of accelerator residues, among which ZDEC, had been detected in the acetone extract. Quantification by GC was impossible because of the detection limit of the method. No ZMBT was detected in a phosphate buffer extract obtained from a 2nd glove brand where a high content of ZMBT was demonstrated in the acetone extract.

Discussion

Comparison of results from analyses of acetone and phosphate-buffer extracts demonstrated that, at least with the analytical methods used here, phosphate-buffer was not a suitable medium, while acetone extracts performed acceptably in the analyses. The amounts of accelerators extracted with acetone were sufficiently high to allow qualitative and quantitative detection with the methods used. Acetone extraction alters the rubber membrane. However, gloves extracted with acetone showed no visible changes after the extraction procedure. Some alteration of the latex-membrane also takes place during normal use because of swelling of the material and influence from skin lipids, emollient and creams on the hands, etc. The

Table 2. Accelerators identified by qualitative analysis of the 19 glove brands in the study

Accelerator	No. gloves in which the accelerator was identified
ZDEC	11
ZDBC	10
ZMBT	9
ZPC	7
TBTD	4
ZDiBC	3
TETD	2
TMTD	1

Table 3. Amounts of accelerators ($\mu\text{mol/g}$ glove material) estimated in glove extracts by analysis. “*” indicates that the ingredient was claimed by the manufacturer to be present or had been used during production

Glove identification	PTT	ZPC	TETD	ZDEC	TBTD	ZDBC	ZDiBC	TMTD	ZMBT	MBI	Total $\mu\text{mol/g}$ glove
3	*)	6.7		1.4 *						*	8.1
9						0.7 *			1.0 *		1.7
25				5.4 *		5.8 *					11.2
15							0.8		< 0.15 *		<1.0
5	*	4.7		1.2 *						*	5.9
6	*	6.2		2.7 *						+ ^{a)} *	8.9
10					1.2				1.5 *		2.7
11 ^{b)}					** 3.5 ^{c)}				*		3.5
12				3.7		0.8			0.3 *		4.8
16				0.9		0.5	0.5	0.3	0.3		2.5
17	d)						0.5 *		0.3		0.8
18		0.5 *		0.6 *							1.1
19				14							14
27		4.7 *				5.7 *			0.3 *		10.7
28		3.7 *				6.6 *			*		10.3
32				**15.6	0.1				0.2 *		15.9
21				6.5		8.9					15.4
24				** 1.7		** 0.4					2.1
26		6.3 *				7.8 *			<0.15 *		<14.3

^{a)} MBI was detected qualitatively by HPTLC, but quantification by GC-FID was not possible.

^{b)} Non-latex glove.

^{c)} Di-n-butylamine corresponding to ZDBC or TBTD was found by CG-FID analysis, but none of these compounds were identified by HPTLC.

^{d)} Piperidine-pentamethylenedithiocarbamate was declared. As the compound was not detected by analysis, and not available for patch testing in patients, this ingredient has not been taken into consideration.

Where both a carbamate and its corresponding thiuram was present, only the combined amount is given in the table (indicated by **).

In 2 cases, the amount of ZMBT was close to the limit of quantification, which was approximately $0.15 \mu\text{mol/g}$ glove, and the values are therefore indicated as $<0.15 \mu\text{mol/g}$.

clinical relevance of acetone as an extraction medium for qualitative and quantitative determination of the bioavailable rubber accelerator residues in rubber gloves will be further discussed in a different paper (in preparation). The solubility in acetone (and phosphate-buffer) of the compounds present in the gloves was rather low. It is possible that saturation may have occurred during the extraction procedure, at least for some of the chemicals. In that case, the extraction procedure does not fully reflect the bioavailable amount of chemical residues. Further studies of the extraction procedure are therefore desirable.

For qualitative detection, an HPTLC method

was used. This method permitted re-application of the sample, if the concentration of the compound to be studied was low. The detection limit of $100\text{--}200 \mu\text{g/g}$ glove equals an amount of accelerator of about $1\text{--}5 \mu\text{g/cm}^2$ glove, depending of glove thickness and compound studied. The detection and quantification limit of the analysis is thus comparable to the amounts of thiurams shown to cause reactions in sensitized individuals, although some patients can probably react to amounts of accelerators below the detection and quantification limits of this method. A further increase in sensitivity of the method would therefore probably be desirable.

Neither HPTLC nor GC-FID can be considered

very simple analytical methods. However, both methods are supposed to be available for larger glove manufacturers and are considered within practical possibilities. Recovery and reproducibility of GC-FID was studied and found to be more than 80% and about 90%, respectively. These results are published elsewhere (9).

It was not possible with this method to distinguish between MBT, ZMBT and MBTS, as each of these analytes may be produced from the others during vulcanization in presence of zinc. Only ZMBT was found in glove extracts. TH- and C-derivatives could be distinguished using HPLTC, while GC-FID, used for quantitative determination, did not permit any distinction between the THs and their corresponding Cs because of the initial derivatization. Although amines are expected conversion products of Cs and THs (15), no amines were detected in any of the gloves (9). Where both the TH and the corresponding C were identified by HPTLC, only the compiled amount of the 2 analytes could be determined. This may however not be pivotal in the clinical situation, as it has been demonstrated that patients sensitized to C almost always also react to TH. Patients sensitized to TH do not all react to ZDEC, but there is a strong correlation between the degree of reactivity to TH and the likelihood of reacting also to ZDEC (21). Experience has shown that in most cases patients with positive patch tests to TH, but not to C, will not be able to use gloves containing C during longer periods (22).

According to the CEN standard for medical gloves, EN 455 part 3 (23), information about sensitizing ingredients present in glove products shall be made available on users' request. Our study shows that only minor discrepancies were found between manufacturer's claims and the chemical analyses of most of the products included in this study. The discrepancies observed between declared and observed content of TH and C in the gloves may partly be due to conversion of TH to the corresponding C during production. TH present, but not declared, may originate from impurities in the technical grade purity chemicals used for production or have been added as a preservative immediately after tapping. It cannot completely be excluded that small amounts of TH may be formed during production from C in the presence of other chemicals or due to technical procedures. As discussed above, clinical significance of the distinction between C and TH may not always be present.

The chemicals detected in the products differed considerably from the compounds traditionally used for screening for rubber allergy. Since the change in use of chemicals has taken place only

recently, this change may not yet be reflected in the allergy profile of individuals using the products. It is important to observe if more patients in the future develop glove-related dermatitis that cannot be explained by sensitization to TH mix and MBT/mercapto mix (24, 25). Use of additional rubber series, including some of the recently introduced accelerators, should probably be used on wide indications, so as not to miss relevant allergens.

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