

Reaction profile in patch testing with allergens formed during vulcanization of rubber

Christer Hansson¹, Ann Pontén², Cecilia Svedman² and Ola Bergendorff¹

¹Department of Dermatology, Skåne University Hospital, SE-221 85 Lund, Sweden and ²Department of Occupational and Environmental Dermatology, Skåne University Hospital, SE-205 02 Malmö, Sweden

doi:10.1111/cod.12168

Summary

Background. Vulcanization of rubber changes its allergen pattern.

Objectives. To estimate the contact allergic reactivity profile of users of finished rubber products.

Methods. Twenty-four patients with known contact allergy to rubber accelerators were patch tested with 21 compounds found in chemical analyses of vulcanized rubber products. No diphenylguanidine, *p*-phenylenediamine antioxidants or thioureas were included in the study.

Results. Thiuram monosulfides formed during vulcanization showed generally stronger test reactions than the corresponding thiuram disulfides. We also obtained more positive thiuram reactions to the monosulfides than to the disulfides. A positive reaction to a dithiocarbamate was accompanied by a positive reaction to the corresponding thiuram, except for 1 patient. The nitrogen substituents showed only minor differences between the methyl, ethyl and pentamethylene groups, but the butyl derivatives gave, in most cases, a negative response. Dialkylthiocarbamyl benzothiazole sulfides, formed between thiurams and mercaptobenzothiazoles during vulcanization, showed strong test reactions in almost all patients who were sensitive to dithiocarbamates, thiurams, or mercaptobenzothiazoles.

Conclusions. We found thiuram monosulfides to be better markers of thiuram sensitivity than the corresponding disulfides or dithiocarbamates. Surprisingly, the dialkylthiocarbamyl benzothiazole sulfides were good markers of both thiuram and mercaptobenzothiazole sensitivity. This is an unexpected finding that needs to be confirmed in a larger study.

Key words: accelerator; allergy; contact dermatitis; dithiocarbamate; mercaptobenzothiazole; monosulfide; rubber; thiuram; vulcanization.

Contact dermatitis caused by rubber products is one of the most common types of allergic skin reaction. Handling of rubber material occurs daily in most workplaces, and also daily during leisure time. Investigation of suspected rubber allergy is based on information obtained from the producers of rubber objects. From them comes

information on which accelerators are added to the rubber bulk before vulcanization. For most types of rubber material, both natural rubber and synthetic rubber, several different types of accelerator are added to the batch before vulcanization. These compounds constitute the information supplied by the producers, and this information is adequate for investigation of rubber allergy of workers in the rubber factories. However, in most countries, the number of workers in the rubber industry has declined, owing to increased automation of the process, but the number of users of rubber products, such as rubber gloves, is increasing. Furthermore, users

Correspondence: Ola Bergendorff, Department of Dermatology, Skåne University Hospital, SE-221 85 Lund, Sweden. Tel: +46 46 173154; Fax: +46 46 172114. E-mail: ola.bergendorff@skane.se

Accepted for publication 25 September 2013

of finished rubber products are exposed to allergens other than those affecting workers in rubber factories.

In a recent study, we analysed the concentrations of different allergens in the rubber material at different stages during vulcanization in a pilot-plant process in a rubber factory (1). These chemical analyses showed that the concentrations changed for essentially all allergens. Several compounds disappeared completely, and new compounds were formed by reactions between thiurams/dithiocarbamates and/or mercaptobenzothiazoles, giving, for example, dimethylthiocarbamyl benzothiazole sulfide (DMTBS). Later, we found some of these newly formed compounds to be important allergens regarding rubber allergy, but they are not tested in the rubber series that are commonly used today (2). These molecules consist of one part of the dithiocarbamate linked by a monosulfide bond to the mercaptobenzothiazole. In the present study, we included two derivatives of this mixed type of accelerator, one with nitrogen methyl substituents, DMTBS, and one with ethyl substituents, diethylthiocarbamyl benzothiazole sulfide (DETBS).

For 20 years, we have been analysing haptens in finished rubber products, and today we can analyse essentially all important rubber haptens (3–5). On the basis of these chemical investigations on finished rubber materials, we have designed a series for patch testing of rubber accelerators, and have tested 24 patients with known allergy to rubber accelerators, to evaluate the importance of the different haptens in finished rubber products (Table 1).

In the European baseline series, rubber allergy is investigated by using the thiuram mix, mercaptobenzothiazole mix, 2-mercaptobenzothiazole (MBT), and *N*-isopropyl-*N*-phenyl-4-phenylenediamine (IPPD). IPPD is an antioxidant that is chemically different from the accelerators and is for this reason not included in this study. Carba mix is excluded from the European baseline series, but is included in the TRUE Test® panel and in the North American baseline series. In the thiuram mix, there is thiuram monosulfide as well as disulfide for the tetramethyl derivatives – tetramethylthiuram disulfide (TMTD) and tetramethylthiuram monosulfide (TMTM) – but no monosulfides for the ethyl and pentamethylene derivatives, as only the disulfides tetraethylthiuram disulfide (TETD) and dipentamethylenethiuram disulfide (DPTD) are included. We think that there is a lack of knowledge concerning testing with the ethyl and pentamethylenethiuram monosulfides, especially as the monosulfide test (TMTM) is usually stronger and more often positive than the corresponding disulfide test (TMTD) (6, 7). We and others have observed, in chemical analyses, the presence of different monosulfides in finished vulcanized rubber

products such as rubber gloves. For that reason, we included the monosulfides tetraethylthiuram monosulfide (TETM), tetrabutylthiuram monosulfide (TBTM) and dipentamethylenethiuram monosulfide (DPTM) in the present series.

The real haptens of the thiurams/dithiocarbamates are unknown. The metabolism of TETD in the liver has been carefully investigated, owing to its use as an anti-alcoholic drug, disulfiram (Antabus®) (8). The *S*-methylation of the dithiocarbamate is a key reaction in this metabolism. We synthesized and included the *S*-methylated zinc dimethyldithiocarbamate (ZDMC) and zinc diethyldithiocarbamate (ZDEC), that is, methyl *N,N*-dimethyldithiocarbamate (MeDMC) and methyl *N,N*-diethyldithiocarbamate (MeDEC), at the same concentrations as used for thiurams in our test series. *S*-methylation of MBT is the corresponding reaction of the mercaptobenzothiazole compounds (9). We have observed some patients with positive reactions to this substance, and we therefore also included *S*-methylated MBT in our study series.

The aim of the study was to obtain information on the reactivity profile of patients allergic to finished rubber. With this knowledge, we can propose changes in the rubber series to make it more consistent with the allergens in the vulcanized, finished rubber products used in occupational and non-occupational settings.

Materials and Methods

Patients

Patients who previously tested positive with at least a ++ reaction to one of the compounds in the three groups of rubber compounds – thiurams, dithiocarbamates, or mercaptobenzothiazoles – were recruited from the register of contact allergy at the Department of Dermatology, Lund, and the Department of Occupational and Environmental Dermatology, Malmö. Twenty-four patients accepted the invitation, and were tested with a series composed of thiurams, dithiocarbamates, mercaptobenzothiazoles, and substances formed from these groups during vulcanization and metabolism (Table 1). No workers in rubber factories were included. In routine investigations with the baseline series, the Lund department uses TRUE Test® including carba mix, and the Malmö department uses Chemotechnique without any dithiocarbamate in the base series. Consequently, there may be some differences between the patients from the two departments, especially as false-negative results for contact allergies to thiuram mix and carbamix in TRUE Test® have been shown in a multicentre study performed

Table 1. The study patch test series

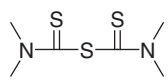
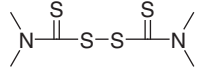
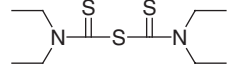
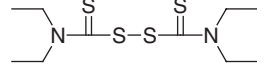
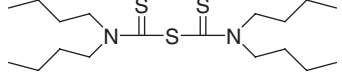
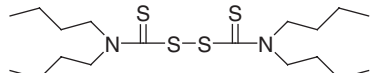
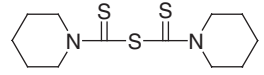
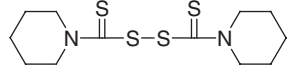
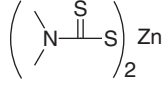
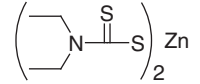
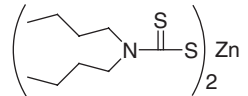
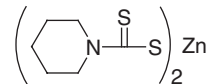
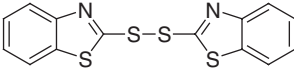
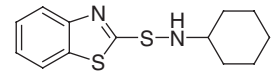
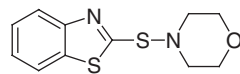
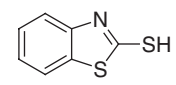
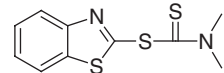
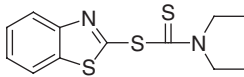
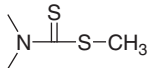
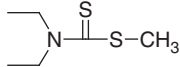
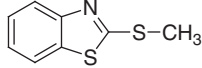
Chemical name	Abbreviation	Structure	CAS	MW
Tetramethylthiuram monosulfide ^a	TMTM		97-74-5	208.37
Tetramethylthiuram disulfide ^a	TMTD		137-26-8	240.43
Tetraethylthiuram monosulfide	TETM		95-05-6	264.47
Tetraethylthiuram disulfide ^a	TETD		97-77-8	296.54
Tetrabutylthiuram monosulfide	TBTM		93-73-2	376.69
Tetrabutylthiuram disulfide	TBTD		1634-02-2	408.75
Dipentamethylenethiuram monosulfide	DPTM		725-32-6	288.50
Dipentamethylenethiuram disulfide ^a	DPTD		94-37-1	320.56
Zinc dimethyldithiocarbamate	ZDMC		137-30-4	305.82
Zinc diethyldithiocarbamate ^b	ZDEC		14324-55-1	361.91
Zinc dibutyldithiocarbamate ^b	ZDBC		136-23-2	474.14
Zinc pentamethylenedithiocarbamate	ZPD		13878-54-1	385.95
2,2'-Dibenzothiazyl disulfide ^c	MBTS		120-78-5	332.49
N-Cyclohexyl-2-benzothiazolsulfenamide ^c	CBS		95-33-0	264.41
2-(4-Morpholinylmercapto)benzothiazole ^c	MMBT		102-77-2	252.36
2-Mercaptobenzothiazole ^c	MBT		149-30-4	167.25
Dimethylthiocarbamylbenzothiazole sulfide	DMTBS		3432-25-5	254.39

Table 1. continued

Chemical name	Abbreviation	Structure	CAS	MW
Diethylthiocarbamylbenzothiazole sulfide	DETBS		95-30-7	282.45
Methyl <i>N,N</i> -dimethyldithiocarbamate	MeDMC		3735-92-0	135.25
Methyl <i>N,N</i> -diethyldithiocarbamate	MeDEC		686-07-7	163.30
2-(Methylmercapto)benzothiazole	MeMBT		615-22-5	181.28

CAS, chemical abstract number; MW, molecular weight.

^aComponent in thiuram mix.

^bComponent in carba mix.

^cComponent in mercapto mix.

All substances were tested at 1% pet., except for 2-mercaptobenzothiazole, which was tested at 2% pet.

by the North American Contact Dermatitis Group (10). The study design was approved by the ethics committee of the University of Lund, and all participants gave their written informed consent to participate in the study.

Chemicals

TMTM, TMTD, TETD, DPTD, ZDMC, ZDEC, zinc dibutyldithiocarbamate (ZDBC), 2,2'-dibenzothiazyl disulfide (MBTS), *N*-cyclohexyl-2-benzothiazolsulfenamide, 2-(4-morpholinylmercapto)benzothiazole and MBT were all purchased from Chemotechnique Diagnostics (Vellinge, Sweden) as petrolatum preparations. TETM was obtained from Apoteksbolaget (Umeå, Sweden). Tetrabutylthiuram disulfide (TBTd) was obtained from TCI Europe (Zwijndrecht, The Netherlands). In chemical analysis this product showed high levels of impurities, and was therefore subjected to purification by flash chromatography on silica gel (30–70 µm, eluted with 1% ethyl acetate in heptane). Zinc pentamethylenedithiocarbamate (ZPD) was obtained from Robinson Brothers (West Bromwich, UK). DETBS (EthylacTM) was obtained as a gift from Trelleborg AB (Trelleborg, Sweden). 2-(Methylmercapto)benzothiazole (MeMBT) (97%) was purchased from Aldrich (Steinheim, Germany). DPTM was obtained from MP Biomedicals (Illkirch, France), and was purified by recrystallization in ethanol (99%).

Synthesis

TBTM was synthesized by reacting TBTd with potassium cyanide in ethanol (95%) at room temperature for

60 min (11). The reaction mixture was dried under a stream of nitrogen, and the residue was dissolved in heptane. After being washed several times with water, the heptane solution was injected onto a preparative high-performance liquid chromatography (HPLC) column (Econosphere 10 µm, 250 × 22 mm) eluted with 1% ethyl acetate in heptane (10 ml/min). The eluent was monitored with an ultraviolet (UV) detector at 254 nm. DMTBS was synthesized according to a method published previously (1). MeDMC was synthesized by reacting sodium dimethyldithiocarbamate trihydrate (Aldrich) with methyl iodide (Merck, Schuchardt, Germany) in ethanol (99%) at room temperature for 30 min. The reaction mixture was poured into a separatory funnel containing water and methyl-tert-butyl ether (MTBE). After vigorous shaking, the ether phase was separated, and the water/ethanol phase was extracted with a new portion of MTBE. The MTBE phases were pooled, dried with sodium sulfate, and finally evaporated to dryness. The product was then purified by flash chromatography on silica gel (30–70 µm, eluted with 10% ethyl acetate in heptane). MeDEC was synthesized in a similar way, with sodium diethyldithiocarbamate (Aldrich). Pure MeDEC was obtained after extraction with water and MTBE, and the product was crystallized in MTBE after drying with sodium sulfate. The purity of all substances used for preparation of patch tests was checked by HPLC with a previously published method (5), and was determined to be at least 97%. The structures of TBTM, DMTBS, DETBS, MeDMC and MeDEC were confirmed by nuclear magnetic resonance spectroscopy, mass spectroscopy, and UV spectroscopy.

Testing procedure

The patients were patch tested with Finn Chambers® on Scanpor® tape. All allergens were diluted in pet. and patch tested at a concentration of 1%, except for MBT, which was patch tested at 2%. All tests were read by one person in Lund (C.H.) or by one of two persons in Malmö (A.P. or C.S.). The tests were applied on the upper back, removed after 2 days, and read after 3 or 4 days and after 1 week. They were scored according to the International Contact Dermatitis Research Group guidelines. More than 10 negative controls were tested with the two new allergens DMTBS and DETBS.

Results

The aim of this study was to evaluate the reactivity profile in patients allergic to thiurams, dithiocarbamates, or mercaptobenzothiazoles, and other contact allergies found in these patients are therefore not presented here. The test results of the present study are shown in Table 2.

We conclude that, when there is a reaction to a thiuram disulfide, there is always also a reaction to the corresponding monosulfide, except for two weak reactions to TETD (patients 10 and 15). On the other hand, we observed some strong reactions to a monosulfide without any reaction to the corresponding disulfide. Furthermore, several patients showed a stronger reaction to the monosulfide than to the corresponding disulfide.

Concerning the nitrogen alkyl groups, the differences in the molecular structures between the thiurams are small, such as between TMTD and TETD. In this study, most patients with a strong reaction to one of the thiurams also reacted positively to other structurally similar thiurams, independently of the nitrogen substitution. This observation concerns both monosulfides and disulfides of methyl, ethyl, and pentamethylene, but not butyl substitution. Only 1 patient reacted to the chemically similar tetrabutylthiurams TBTM and TBTD.

Six patients reacted to at least one of the dithiocarbamates, but all of these except for 1 (patient 20) also reacted to the corresponding thiuram. The reaction to ZPD in this case might be a false-positive reaction resulting from extremely strong reactions with mercapto compounds. No patient reacted to ZDBC, analogously to the absence of reactions to the tetrabutylthiurams.

We included methylated ZDMC and ZDEC, that is, MeDMC and MeDEC, at the same concentrations as used for thiurams in our test series; 6 patients reacted to methylated dithiocarbamates, but their test reactions were never stronger than those to the corresponding thiuram monosulfides.

Only 5 patients were allergic to mercaptobenzothiazole derivatives in the study. All of them reacted to all four MBT derivatives, except for 2 patients who did not react to MBTS. We also added the S-methylated mercaptobenzothiazole (MeMBT) to the test series, and found 2 patients with positive reactions to this substance, but with the same strength as the reaction to its corresponding non-methylated derivative.

In our test series, we also added two compounds formed during vulcanization by reactions between thiurams/dithiocarbamates and mercaptobenzothiazoles, DMTBS and DETBS. We found that all patients except for 1 (patient 10) who reacted to any thiuram or dithiocarbamate also reacted to these mixed substances. We also found that all patients who reacted to any MBT derivative also reacted to DMTBS.

Discussion

In our study on the chemical reactions during vulcanization, we found that thiurams react with mercaptobenzothiazoles, forming compounds with both dithiocarbamate and mercaptobenzothiazole structures, for example DMTBS (1). We also found these types of substance in finished rubber products (2). In patch testing, we found that all patients, except for 1, who reacted to any of the thiurams, dithiocarbamates or benzothiazoles also reacted to the mixed dialkylthiocarbamylbenzothiazole sulfides (DMTBS and DETBS). These results were not expected, and it is not obvious to us what the structure of the real happen is. As DMTBS is formed during vulcanization, and the patients may have been exposed to these substances, we cannot distinguish between sensitization to DMTBS and cross-reactivity to thiurams/dithiocarbamates or mercaptobenzothiazoles. In contrast to these observations, 1 patient, who attended our clinic during the study and was investigated because of his dermatitis related to the rubber in his swimming goggles, had an isolated positive test reaction to DMTBS when tested with the allergens in Table 1. This patient was not included in our study. Chemical analysis of his swimming goggles showed them to contain DMTBS, analogously to the case mentioned above (2), where the goggles contained DETBS.

Thiuram disulfides and dithiocarbamates constitute a redox pair, which means that, during oxidation of a dithiocarbamate, the corresponding thiuram disulfide is formed. Reduction of the thiuram disulfide restored the dithiocarbamate. In such reactions, ferric ions may act as the oxidant, and glutathione as the reductant (12, 13). Thus, there is a redox equilibrium between the dithiocarbamate and the corresponding thiuram disulfide. Before 1988, carba mix and thiuram mix were both

Table 2. Patch test results

Previously found contact allergies		Allergen																			
Patient no.		TMTD ^a	TMTM ^a	ZDMC	MeDMC	TETD ^a	TETM	ZDEC ^b	MeDEC	TBTD	TBTM	ZDBC ^b	DPTD ^a	ZPD	DMTBS	DETBS	MBT ^c	MBT ^c	CBS ^c	MMBT ^c	MeMBT
1	Thiuram mix	-	++ ^d	-	++	+	+	-	-	-	-	-	-	-	++	-	-	-	-	-	-
2	Thiuram mix	-	+++	-	++	-	-	-	-	-	-	-	-	-	+++	++	-	-	-	-	-
3	Thiuram mix	-	-	-	-	-	-	-	-	-	-	-	-	-	+++	-	-	-	-	-	-
4	Thiuram mix	+++	+++	+++	+++	++	+++	-	-	-	-	-	++	-	+++	++	-	-	-	-	-
5	Thiuram mix	-	-	-	-	-	-	-	-	-	-	-	++	-	(+)	-	-	-	-	-	-
6	Thiuram mix	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-
7	Thiuram mix	+	+++	++	-	++	+++	+++	-	-	-	-	++	(+)	+	+	-	-	-	-	-
8	Thiuram mix	-	++	-	-	++	+++	-	++	-	-	-	++	-	+++	+++	-	-	-	-	-
9	Thiuram mix	-	+	-	-	-	-	-	-	-	-	-	-	-	++	-	-	-	-	-	-
10	Thiuram mix	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	Thiuram mix	+	+	-	-	++	++	-	-	-	-	-	+	-	+	+	-	-	-	-	-
12	Thiuram mix	-	(+)	-	-	++	++	-	-	-	-	-	++	-	+++	++	-	-	-	-	-
13	Thiuram mix, carba mix	++	++	-	-	+++	+++	++	-	-	-	-	++	-	+	+	-	-	-	-	-
14	Thiuram mix, carba mix	++	+++	++	++	+++	++	++	++	-	-	-	+++	++	+++	+++	-	-	-	-	-
15	TETD, carba mix	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
16	Thiuram mix, carba mix, mercapto mix	-	+++	-	-	++	+++	-	+++	-	+	-	-	-	+++	+++	-	-	-	-	-
17	Thiuram mix, carba mix, mercapto mix	+++	++	++	-	+++	+++	++	-	-	-	-	+++	++	+++	++	++	+	+++	++	++
18	Thiuram mix, carba mix, mercapto mix	-	-	-	-	-	-	-	-	-	-	-	-	-	++	-	++	-	++	++	-
19	Mercapto mix	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	++	-	+	+	-
20	Mercapto mix	-	-	-	-	-	-	-	-	-	-	-	-	+++	+++	+++	+++	+++	+++	+++	+++
21	Mercapto mix	(+)	-	-	-	-	-	-	-	-	-	-	-	-	++	++(+)	++(+)	++(+)	++(+)	++(+)	(+)

TMTD, tetramethythyuram disulfide; TMTM, tetramethythyuram monosulfide; ZDMC, zinc dimethyldithiocarbamate; MeDMC, methyl N,N-dimethyldithiocarbamate; TETD, tetraethythyuram disulfide; TETM, tetraethythyuram monosulfide; ZDEC, zinc diethyldithiocarbamate; MeDEC, methyl N,N-diethyldithiocarbamate; TBTD, tetrabutylthiuram monosulfide; TBTM, tetrabutylthiuram monosulfide; ZDBC, zinc dibutyldithiocarbamate; DPTD, dipentamethylenethiuram disulfide; DPTM, dipentamethylenethiuram monosulfide; ZPD, zinc pentamethylenethiuram monosulfide; DMTBS, dimethylthiocarbamylbenzothiazole sulfide; DETBS, dimethylthiocarbamylbenzothiazole sulfide; MBT, 2-mercaptobenzothiazole; MBTS, 2,2'-dibenzothiazyl disulfide; CBS, N-cyclohexyl-2-benzothiazolsulfenamide; MMBT, 2-(4-morpholinylmercapto)benzothiazole; MeMBT, 2-(methylmercapto)benzothiazole.

^aComponent in thiuram mix.

^bComponent in carba mix.

^cComponent in mercapto mix.

^dThe patch test results are based on the strongest reactions on D3–4/7.

Three patients in our study are not shown in the table, as they were negative to all allergens. –, no allergic reaction was observed; (+), a reaction with a morphology corresponding to an allergic reaction but not covering the whole test area.

incorporated into the European baseline series. Logan and White then showed that testing with thiuram mix was sufficient, and that carba mix could safely be omitted (14, 15). However, carba mix was retained in the North American baseline series and at some European centres, and reports with a high relevance of testing with carba mix have been published (16, 17). The results in this study and in earlier reports have shown more and stronger reactions to the thiuram disulfide than to the corresponding dithiocarbamate (7, 18, 19). These results have been explained by the earlier preference for thiuram disulfides as accelerators in the production of, for example, rubber gloves. Today, dithiocarbamates are the predominant accelerators found in rubber gloves (5, 20–22), but positive test reactions to thiurams are still more common than positive test reactions to dithiocarbamates. Clearly, thiurams are better markers for sensitization to the dithiocarbamate/thiuram redox pair. In very recent years, we have noticed several patients with positive reactions to carba mix, but not to thiuram mix (23). When testing these patients with the rubber series, we found that most of them reacted to the non-dithiocarbamate substance diphenylguanidine (DPG) included in the carba mix patch of TRUE Test®. We have found many patients with DPG allergy and severe hand dermatitis. Upon chemical analyses of their gloves, we found high concentrations of DPG in popular protective gloves used at several hospitals in Sweden. The present study was designed to evaluate separate allergies to dithiocarbamates, thiuram monosulfides and thiuram disulfides with identical or different nitrogen substituents. The results presented in this study are in accordance with the decision that dithiocarbamates can be omitted from the base series, because, essentially, every positive reaction to any dithiocarbamate is accompanied by a positive reaction to the corresponding thiuram (14, 18, 19). However, DPG remains a substance that is worth testing in patients with suspected rubber dermatitis.

In the thiuram mix, there are methyl, ethyl and pentamethylene nitrogen substituents (TMTD, TETD, and DPTD). In this study, we also included the butyl-substituted thiurams (TBTD and TBTM), as we often find the corresponding dithiocarbamate (ZDBC) in some popular protective gloves. We have now investigated the influence of the alkyl groups on the nitrogen of the thiuram disulfides. The differences in molecular structure between the thiurams are rather small, such as between TMTD and TETD. We found that a strong reaction to one of the thiuram disulfides almost always also gave a positive reaction to the other two disulfides in the mix, but not to the butyl-substituted thiuram. We also observed

a very similar pattern for the corresponding thiuram monosulfides (TMTM, TETM, and DPTM). Whether this results from cross-reactivity or concomitant sensitization cannot be determined from our study, but similar results were reported by Rebandel and Rudzki in patients with positive reactions to TMTD after sensitization to TETD implants (24), which might indicate cross-reactivity. In this study, we found a discrepancy for TBTD, which, surprisingly, gave no positive reaction in any of our patients. Furthermore, the corresponding monosulfide, TBTM, gave only one weak reaction. The low reactivities of butyl-substituted thiurams and dithiocarbamate have been observed earlier in patch testing and in the local lymph node assay, but no explanation was given (25, 26). It is not obvious to us, either, why butyl deviates from other alkyl substituents, but the butyl group is the largest group among the substituents tested, and, in HPLC chromatography, it behaves much more lipophilically than the other studied substituents. The butyl-substituted thiurams have almost double the molecular weights of their methyl-substituted analogues. Regarding this, we should have tested the butyl derivatives at twice as high a concentration to obtain the same number of reactive haptens in the test preparation. Although the lipophilicity of these molecules usually increases with molecular weight, we have no numbers for the reactive haptens at the critical depth in the skin where the reaction between the hapten and the carrier protein takes place. This study was not intended to investigate the penetration capacity of the different rubber haptens, so we preferred to test the substances at the same concentration, but our conviction is that the inertness of the butyl-substituted haptens is not a simple question of the concentrations in the test preparations. For example, tetrabutylthiourea seems to react like other thioureas in patch tests (3, 19).

From our study, it is possible to validate the response to disulfides in comparison with monosulfides. There are some reports of stronger reactions to TMTM than to TMTD (6, 7). In the thiuram mix, there are three thiuram disulfides (TMTD, TETD, and DPTD), but only one methyl-substituted monosulfide (TMTM). In our study on the chemical reactions of the allergens during vulcanization, and also in chemical analysis of finished rubber products, we have observed the formation of thiuram monosulfides from all investigated thiuram disulfides during vulcanization. For the first time, we have now compared the test reactions of the monosulfides and the disulfides of all of the thiurams in the thiuram mix. We conclude that a positive reaction to a disulfide is often accompanied by a reaction to the corresponding monosulfide. There are some strong reactions to monosulfides without any reactions to the

corresponding disulfides. We observed some stronger reactions to TMTM than to TMTD, and to DPTM than to DPTD. A similar result was not obtained for TETM. We conclude that the monosulfides are probably better markers than the disulfides for investigation of thiuram sensitivity.

Metabolism of a test substance is a proposed route to obtain the real hapten that is added to the carrier protein during the formation of the complete antigen. Enzymatic S-methylation is a known metabolic reaction of thiurams, dithiocarbamates, and mercaptobenzothiazoles (8, 9). We synthesized these S-methylated metabolites, and incorporated them into our study series. We observed 4 patients who reacted to MeDMC, 3 who reacted to MeDEC, and 2 who reacted to MeMBT. These three compounds are new haptens. The strength of the test reactions was similar to that of the original thiuram/mercaptobenzothiazole, which makes it difficult to propose these metabolites as the real haptens.

Concerning the mercaptobenzothiazoles, we found only 5 patients with positive reactions. These patients reacted to all MBT derivatives, except for 2 patients who did not react to MBTS. We have shown instabilities of MBT derivatives in test preparations, and that MBT is formed from the other three MBT derivatives by reduction by glutathione (27). The results of the present study are in agreement with the formation of, and a skin reaction to, MBT. However, two separate multicentre studies have shown that tests with an MBT mix without MBT, and an additional separate MBT test, are preferable, so as to not miss MBT-sensitive patients (28, 29). However, both studies were retrospective, and no chemical analyses of the stability of the test preparations used were performed.

In conclusion, we conducted this study after realizing that vulcanization produces new allergens, and that sensitization to finished rubber products is likely to present a different spectrum of allergies than found among workers in rubber factories. We found thiuram monosulfide to be superior to thiuram disulfide as a marker of thiuram and dithiocarbamate sensitivity. We agree that dithiocarbamates are redundant in the baseline series. We found no major differences when testing with different alkyl-substituted thiurams, except for butyl-substituted thiurams, which showed remarkably low reactivity. We had too few patients who reacted to benzothiazole derivatives, but the results indicate that MBT is the essential marker of this sensitivity. Testing with S-methylated metabolites of thiurams, dithiocarbamates and mercaptobenzothiazoles showed three new allergens, but did not convince us that these are the real haptens. However, we found that DMTBS is the best marker of rubber allergy to substances of any of the three groups (thiurams, dithiocarbamates, and mercaptobenzothiazoles), missing only 1 patient with a positive reaction to any of the tested substances. We suggest that DMTBS should be evaluated as a general marker of contact allergy to thiuram, dithiocarbamate and mercaptobenzothiazole rubber chemicals in a multicentre study.

Acknowledgements

We thank Mrs Christina Persson for skilful assistance with the synthesis and purification of some of the allergens. This investigation was supported by grants from AFA Insurance, Skane County Council's research and development foundation, and the Edvard Welander Foundation for Scientific Research.

References

- Bergendorff O, Persson C, Ludtke A, Hansson C. Chemical changes in rubber allergens during vulcanization. *Contact Dermatitis* 2007; **57**: 152–157.
- Bergendorff O, Hansson C. Contact dermatitis to a rubber allergen with both dithiocarbamate and benzothiazole structure. *Contact Dermatitis* 2007; **56**: 278–280.
- Bergendorff O, Persson C M L, Hansson C. HPLC analysis of alkyl thioureas in an orthopaedic brace and patch testing with pure ethylbutylthiourea. *Contact Dermatitis* 2004; **51**: 273–277.
- Bergendorff O, Persson C, Hansson C. Extraction of haptens from solid products and their delivery to the skin, exemplified by dithiocarbamates from rubber gloves. *Contact Dermatitis* 2005; **53**: 22–26.
- Bergendorff O, Persson C, Hansson C. High-performance liquid chromatography analysis of rubber allergens in protective gloves used in health care. *Contact Dermatitis* 2006; **55**: 210–215.
- Lammintausta K, Kalimo K. Sensitivity to rubber. Study with rubber mixes and individual rubber chemicals. *Derm Beruf Umwelt* 1985; **33**: 204–208.
- Conde-Salazar L, Del-Rio E, Guimaraens D, Gonzalez D A. Type IV allergy to rubber additives: a 10-year study of 686 cases. *J Am Acad Dermatol* 1993; **29**: 176–180.
- Yourick J J, Faiman M D. Diethyldithiocarbamic acid-methyl ester a metabolite of disulfiram and its alcohol sensitizing properties in the disulfiram–ethanol reaction. *Alcohol* 1987; **4**: 463–467.
- Larsen G L, Bakke J E, Feil V J, Huwe J K. In vitro metabolism of the methylthio group of 2-methylthiobenzothiazole by rat liver. *Xenobiotica* 1988; **18**: 313–322.
- Sherertz E F, Fransway A F, Belsito D V et al. Patch testing discordance alert: false-negative findings with rubber additives and fragrances. *J Am Acad Dermatol* 2001; **45**: 313–314.
- Thorn G D, Ludwig R A. *The Dithiocarbamates and Related Compounds*. New York, Elsevier, 1962: p. 62.
- Bergendorff O, Hansson C. Spontaneous formation of thiuram disulfides in solutions of iron(III) dithiocarbamates. *J Agric Food Chem* 2002; **50**: 1092–1096.
- Stromme J H. Interactions of disulfiram and diethyldithiocarbamate with serum proteins studied by means of a

- gel-filtration technique. *Biochem Pharmacol* 1965; **14**: 381–391.
- 14 Logan R A, White I R. Carbamix is redundant in the patch test series. *Contact Dermatitis* 1988; **18**: 303–304.
 - 15 Andersen K E, Burrows D, Cronin E, Dooms-Goossens A, Rycroft R J, White I R. Recommended changes to standard series. *Contact Dermatitis* 1988; **19**: 389–390.
 - 16 Britton J E R, Wilkinson S M, English J S C, Gawkrödger D J, Ormerod A D, Sansom J E, Shaw S, Statham B. The British standard series of contact dermatitis allergens: validation in clinical practice and value for clinical governance. *Br J Dermatol* 2003; **148**: 259–264.
 - 17 Cao L Y, Taylor J S, Sood A, Murray D, Siegel P D. Allergic contact dermatitis to synthetic rubber gloves changing trends in patch test reactions to accelerators. *Arch Dermatol* 2010; **146**: 1001–1007.
 - 18 Knudsen B B, Menné T. Contact allergy and exposure patterns to thiurams and carbamates in consecutive patients. *Contact Dermatitis* 1996; **35**: 97–99.
 - 19 Holness D L, Nethercott J R. Results of patch testing with a special series of rubber allergens. *Contact Dermatitis* 1997; **36**: 207–211.
 - 20 Knudsen B B, Hametner C, Seycek O, Heese A, Koch H U, Peters K P. Allergologically relevant rubber accelerators in single-use medical gloves. *Contact Dermatitis* 2000; **43**: 9–15.
 - 21 Brehler R, Rutter A, Kutting B. Allergenicity of natural rubber latex gloves. *Contact Dermatitis* 2002; **46**: 65–71.
 - 22 Depree G J, Bledsoe T A, Siegel P D. Survey of sulfur-containing rubber accelerator levels in latex and nitrile exam gloves. *Contact Dermatitis* 2005; **53**: 107–113.
 - 23 Pontén A, Hamnerius N, Bruze M, Hansson C, Persson C, Svedman C, Thörneby Andersson K, Bergendorff O. Occupational allergic contact dermatitis caused by sterile non-latex protective gloves: clinical investigation and chemical analysis. *Contact Dermatitis* 2013; **68**: 103–110.
 - 24 Rebandel P, Rudzki E. Secondary contact sensitivity to TMTD in patients primarily positive to TETD. *Contact Dermatitis* 1996; **35**: 48.
 - 25 Hintzenstern J, von Heese A, Koch H U, Peters K P, Hornstein O P. Frequency, spectrum and occupational relevance of type IV allergies to rubber chemicals. *Contact Dermatitis* 1991; **24**: 244–252.
 - 26 De Jong W H, Van Och F M M, Den Hartog Jager C F, Spiekstra S W, Slob W, Vandebriel R J, Van Loveren H. Ranking of allergenic potency of rubber chemicals in a modified local lymph node assay. *Toxicol Sci* 2002; **66**: 226–232.
 - 27 Hansson C, Agrup G. Stability of the mercaptobenzothiazole compounds. *Contact Dermatitis* 1993; **28**: 29–34.
 - 28 Geier J, Uter W, Schnuch A, Brasch J. Diagnostic screening for contact allergy to mercaptobenzothiazole derivatives. *Am J Contact Dermat* 2002; **13**: 66–70.
 - 29 Diepgen T L, Bruynzeel D P, Andersen K E et al. Mercaptobenzothiazole or the mercapto-mix: which should be in the standard series? *Contact Dermatitis* 2006; **55**: 36–38.