

Chemical changes in rubber allergens during vulcanization

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Allergic contact dermatitis to rubber is caused by residues of chemicals used in manufacturing a rubber product. Several different additives are used to achieve a final product of the desired characteristics. Accelerators such as thiurams, dithiocarbamates, and mercaptobenzothiazoles are often among the additives responsible for allergic reactions recognized by dermatologists. The chemistry of the vulcanization process is complicated; as it occurs at an elevated temperature with a mixture of reactive chemicals, the compositions of the initial and final products differ. This paper investigates the changes in composition of common allergens during vulcanization, doing so by chemically analysing various rubber formulations at different stages of the process. Major changes were found in which added chemicals were consumed and new ones produced. An important observation is that thiuram disulfides rarely appear in the final rubber although they may have been used as additives. Instead, thiurams are often converted to dithiocarbamates or to products formed by addition to mercaptobenzothiazole structures, if these have been used together with thiurams as accelerators.

Key words: allergic contact dermatitis; dithiocarbamate; mercaptobenzothiazole; rubber chemicals; thiuram; vulcanization. © Blackwell Munksgaard, 2007.

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Allergic contact dermatitis to rubber additives is a serious occupational health problem, more often seen among users of rubber products than among rubber industry personnel. Occupations at risk include health care workers using disposable gloves and car factory workers assembling rubber details. When patients allergic to rubber are investigated at dermatological clinics, information regarding the allergens to which these individuals are exposed is often based on data from the manufacturer regarding the raw materials used. Also, when the patient's allergy has been confirmed, alternate products free of the chemical additives responsible for the allergic reactions can only be recommended if the actual composition of the product is known. These additives are reactive chemicals used in vulcanization, which is performed at an elevated temperature for a considerable time. This process results in chemical changes in the rubber constituents, giving rise to a material of unknown composition. This fact is, of course, not unfamiliar to rubber scientists and has been thoroughly investigated (1). However, in the industry, development efforts have focused on the technical aspects of the rubber and not on

the levels or nature of the residual chemicals in the final product.

At the Department of Dermatology at Lund University Hospital, we have for many years worked on the analysis of rubber items (2–5) as well as studying the chemistry of various rubber additives (6, 7). As the question of chemical changes occurring during vulcanization often arose, it was natural to collaborate with a Swedish rubber factory (Trelleborg AB, Trelleborg, Sweden) to investigate the chemistry of allergenic rubber additives. We wanted to examine why thiuram disulfides are seldom found in rubber and also whether new allergens are produced during vulcanization.

At the Trelleborg site, different rubbers were chosen from a range of industrial products and prepared at an experimental scale. The rubbers were made from well-defined ingredients, including common accelerators known to cause allergic reactions. In one experiment, samples were taken at different stages during the process and deep frozen to stop the reaction. The samples were then analysed and the concentrations of the different additives monitored. We also analysed several other

vulcanized rubber samples for which the additives used were known. The analytical approach used is based on a high-performance liquid chromatography (HPLC) method developed by Mathieu et al. (8) that lets us identify and quantify in a single analysis almost all of the known allergenic rubber compounds, including dithiocarbamates, in a rubber extract. The method is based on the principle that the contact of dithiocarbamates with metal surfaces in the chromatographic system should be eliminated. Therefore, the injector loop, capillaries to the column, and the column itself are made of metal-free polyether ether ketone (PEEK). Furthermore, the mobile phase is supplemented with zinc sulfate to outnumber possible free metal ions other than zinc ions. The sample is then subjected to a gradient elution and peaks are detected by ultraviolet (UV) detection. Because of the high number of UV-absorbing substances present in rubber extracts and eluting from the column, it is necessary to use a diode-array detector that presents a UV spectrum for the compounds appearing on the chromatogram.

Materials and Methods

Chemicals

2-Mercaptobenzothiazol (MBT), 2,2¹-dibenzothiazyl disulfide (MBTS), and *N*-cyclohexyl-2-benzothiazyl sulfenamide (CBS) were obtained from Bayer (Antwerp, Belgium). Tetramethylthiuram monosulfide (TMTM), 97%, and tetramethylthiuram disulfide (TMTD) were obtained from Janssen Chimica (Geel, Belgium). Zinc dimethyldithiocarbamate (ZDMC) was obtained from Fluka (Buchs, Switzerland). *N*-Cyclohexylthiophthalimide (CTP) was obtained from Monsanto (Brussels, Belgium). Dimethylthiocarbamoyl chloride 97% was obtained from Aldrich-Chemie (Steinheim, Germany). Acetone of analytical grade and acetonitrile of HPLC grade were obtained from Labscan Ltd (Dublin, Ireland). Zinc sulfate (analytical grade) was obtained from Merck (Darmstadt, Germany). The MBT, MBTS, CBS, TMTD, ZDMC, and CTP used were all of technical grade.

Rubber vulcanization

Samples of 5 different types of rubber were prepared at an experimental scale at the research laboratory of Trelleborg AB. The rubber based on a styrene-butadiene polymer was called SBR-1, while the pure natural rubbers were denoted NR-1 to NR-4. The rubbers were made in a pilot process mimicking the full-scale industrial process from well-defined ingredients in combination with

zinc oxide and stearic acid, including common accelerators known to cause allergic reactions. These ingredients were mixed mechanically and then vulcanized at 160° under pressure to generate a rubber sheet. Parallel experiments were performed and stopped at different stages of the SBR-1 process; samples were taken and deep frozen to stop the reaction. The samples were then analysed using HPLC and the concentrations of the different allergens were measured.

In addition, the final compositions of 4 samples based on natural rubber latex, referred to as NR-1, NR-2, NR-3, and NR-4, were analysed.

High-performance liquid chromatography

Chromatographic analyses were performed using HPLC on a reversed-phase column (Alltima C18, 4 µm, 150 × 4.6 mm, PEEK lined; Alltech Associates, Deerfield, IL, USA), eluted with acetonitrile–aqueous zinc sulfate (10⁻⁵ mol/l), 50:50, for 5 min, followed by a linear gradient to 100% acetonitrile for 35 min. Eluent was pumped using a Waters 600 pump (Waters Chromatography Division, Milford, MA, USA) at a flow rate of 1 ml/min and monitored at 280 and 325 nm using a 1100 Series diode-array detector (Hewlett-Packard Co., Palo Alto, CA, USA). All devices in contact with the mobile phase after the injector were made of PEEK. Peak area was used to determine the concentration, and identification was done by comparing the retention times and UV spectra recorded by the diode-array detector.

Analysis of the rubber

1 sample (0.5 g) of each rubber was cut into small pieces with a pair of scissors and placed in a 10-ml test tube covered with a Teflon-lined screw cap (ScherfChroma, Meiningen-Dreissigacker, Germany) containing 5 ml of acetone. The test tube was placed on a shaker, and after 10 min of extraction at room temperature, the extract was pipetted into a round-bottomed flask and carefully evaporated under vacuum. The residue was then dissolved in 5 ml of acetonitrile and filtered before injection into the HPLC column. The chromatogram was compared with analyses of reference compounds for quantification. Duplicate analyses were performed for each piece of rubber; and the mean values are presented. This method has been validated by us in an earlier publication (5) where the detection limit was determined to be 10 µg/g and the recovery at extraction 73%. These figures are established for analysis of zinc dithiocarbamates, but we have found them to be valid for other rubber components as well. However, it is

important to note that the concentrations presented are not corrected for the extraction yield.

Spectroscopy

The mass spectra (FAB ionization, positive mode) were recorded using a Jeol SX102 spectrometer (Tokyo, Japan); ^1H -NMR (nuclear magnetic resonance) spectra were recorded using a Bruker DRX-400 spectrometer at 400 MHz.

Synthesis of *N,N*-dimethylthiocarbamylbenzothiazole sulfide (DMTBS)

First, 0.5 g (3 mmol) MBT was dissolved in 13 ml of 1.0 M NaOH. To this solution, 3 mmol dimethylthiocarbamoyl chloride was slowly added while stirring. After 15 min, the precipitate formed was centrifuged off and the upper solution was discarded. The precipitate was washed twice with distilled water and left to dry overnight on a glass plate. Analysing this product by means of HPLC gave a peak with a retention time corresponding to the peak of rubber extracts with identical UV spectra recorded by the diode-array detector. The purity was estimated to be >97% and the yield 0.2 g (0.78 mmol, 26%).

^1H -NMR in CDCl_3 showed 2 singlets at δ 3.51 and 3.47 from the methyl groups. Aromatic signals appeared at 7.46 (ddd, 1.3, 7.2, 8.0 Hz), 7.52 (ddd, 0.9, 1.2, 7.3 Hz), 7.92 (dd, 1.3, 7.2 Hz), and 8.13 (dd, 0.8, 7.5 Hz). ^{13}C -NMR gave signals at δ 42.72, 45.54, 121.69, 124.16, 126.32, 126.55, 138.55, 152.99, and 191.61. High-resolution mass spectrometry gave m/z 255.0079, $\text{C}_{10}\text{H}_{11}\text{N}_2\text{S}_3$ ($\text{M}+\text{H}$) requires 255.0078 (mass error 0.3 p.p.m.).

Synthesis of *N,N*-dimethylthiocarbamylbenzothiazole disulfide (DMTBD)

Several attempts were made to synthesize and isolate the asymmetric disulfide between mercaptobenzothiazole and dimethyldithiocarbamate. All of them failed, as this disulfide is in equilibrium with MBTS and TMTD (Fig. 1). When TMTD and MBTS were mixed in equimolar amounts, a new peak emerged on the HPLC chromatogram.

When this peak was isolated, it immediately decomposed to its equilibrium state and could not be satisfactorily characterized. A similar description of this problem is given by Giuliani and McGill (9), and we believe that the peak corresponds to *N,N*-dimethylthiocarbamylbenzothiazole disulfide (DMTBD).

Results

The common rubber accelerator TMTD, together with CBS and MBTS, was added to the styrene-butadiene rubber (SBR-1) before vulcanization according to an industrial procedure. The total content of these accelerators was 30 mg/g. The relative amounts of accelerators were 3:1:1 of TMTD, MBTS, and CBS, and all of these compounds were consumed during vulcanization. Instead, other contact allergens, i.e. 3.9 mg/g ZDMC, 1.1 mg/g MBT, and traces of TMTM, were present in the final product. Furthermore, 2 other compounds appeared during the process, already during the mixing stage: *N,N*-dimethylthiocarbamylbenzothiazole sulfide (DMTBS, CAS No. 3432-25-5) and the disulfide DMTBD (CAS No. 10225-08-8) (Figs 2 and 3).

During vulcanization, DMTBD disappeared and the monosulfide DMTBS was formed, probably from the desulfuration of DMTBD (9). DMTBS was synthesized (Fig. 4), and the amount of this compound in the final product was determined to be 0.9 mg/g rubber.

Table 1 presents the qualitative results of the analysis of natural latex rubber samples NR-1, NR-2, NR-3, and NR-4. They represent different common industrially used combinations of thiuram and mercaptobenzothiazole accelerators. A characteristic pattern evident in this table is that MBT is formed in all samples, while thiurams are consumed (in SBR-1, TMTM appeared in trace amounts). Also, when both thiuram and mercaptobenzothiazole accelerators are present, DMTBS is found in the final product. The vulcanization retarder CTP was added to NR-1 and NR-2, but in none of these samples could it be found after vulcanization.

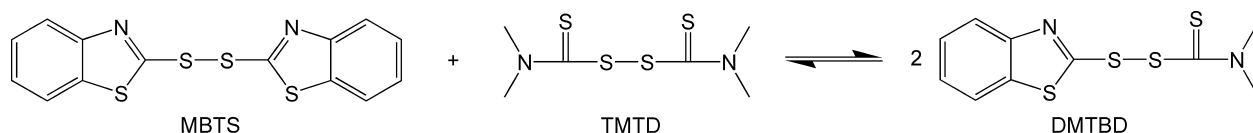


Fig. 1. DMTBD is formed during vulcanization, by means of the disulfide exchange reaction between MBTS and TMTD. DMTBD, *N,N*-dimethylthiocarbamylbenzothiazole disulfide; MBTS, 2,2'-dibenzothiazyl disulfide; TMTD, tetramethylthiuram disulfide.

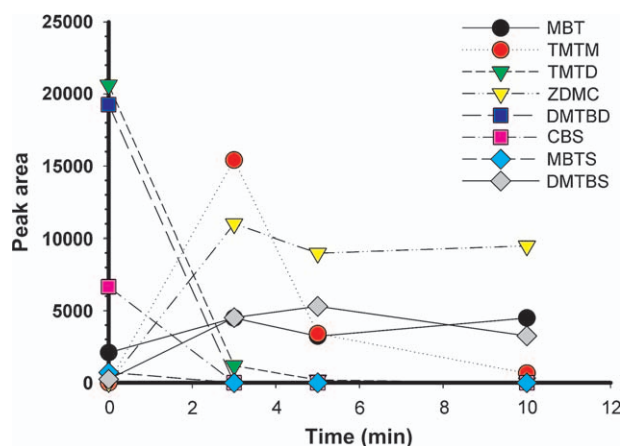


Fig. 2. Diagram indicating relative amounts of the compounds during vulcanization. At start time, '0 min', the compounds have been mixed and vulcanization starts. MBT, 2-mercaptobenzothiazol; TMTM, tetramethylthiuram monosulfide; TMTD, tetramethylthiuram disulfide; ZDMC, zinc dimethyldithiocarbamate; DMTBD, *N,N*-dimethylthiocarbamylbenzothiazole disulfide; CBS, *N*-cyclohexyl-2-benzothiazyl sulfenamide; MBTS, 2,2'-dibenzothiazyl disulfide; DMTBS, *N,N*-dimethylthiocarbamylbenzothiazole sulfide.

Discussion

This study was initiated by several patients suffering from skin reactions to rubber products whose allergies did not correlate with the ingredients of the rubber according to the producers. We thus wanted to investigate this discrepancy, by following the rubber allergens through the vulcanization process.

The aim was not to conduct a complete investigation of the chemical processes occurring during rubber manufacturing. Instead, we wanted to examine an example of the complex events the rubber allergens are subjected to during vulcanization.

Even in the mixing stage, we could already see considerable changes in the composition of the rubber, as new compounds, such as DMTBD, were formed. During this stage, all added ingredients were ground together mechanically, leading to a rise in temperature from friction heat that facilitated reactions between the chemicals. In a separate experiment, we mixed only the disulfides TMTD and MBTS, and found that even without the rubber matrix DMTBD was formed immediately (Fig. 1). Unfortunately, this asymmetric disulfide is too unstable to be isolated. We have earlier reported this problem, which occurred during the analysis of a thiuram mixture in which different thiuram disulfides in petrolatum formed asymmetric thiuram disulfides upon storage (10). This reaction was especially marked during the attempts to isolate the asymmetric products as they immediately dissociated and an equilibrium between symmetric and asymmetric disulfides appeared in organic solvents. DMTBD has been described earlier by Guiliani and McGill (9), but they did not succeed in isolating this compound.

As seen in Figs 2 and 3, disulfide compounds (TMTD, MBTS, and DMTBD) are desulfurated during the vulcanization process. This reaction is

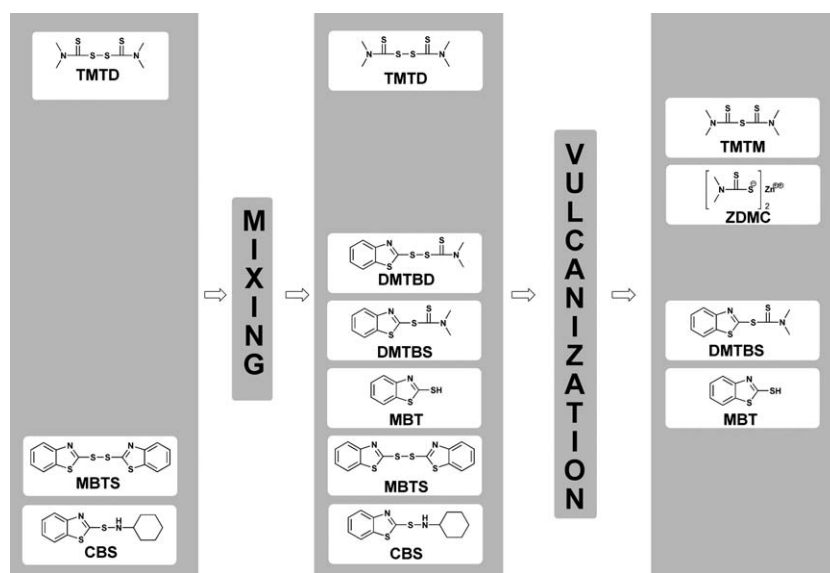


Fig. 3. An overview indicating the rubber chemicals present at different stages in the process. TMTD, tetramethylthiuram disulfide; TMTM, tetramethylthiuram monosulfide; ZDMC, zinc dimethyldithiocarbamate; DMTBD, *N,N*-dimethylthiocarbamylbenzothiazole disulfide; DMTBS, *N,N*-dimethylthiocarbamylbenzothiazole sulfide; MBT, 2-mercaptobenzothiazol; MBTS, 2,2'-dibenzothiazyl disulfide; CBS, *N*-cyclohexyl-2-benzothiazyl sulfenamide.

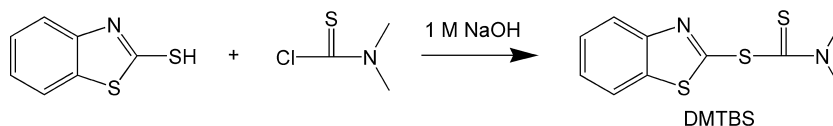


Fig. 4. Synthesis of DMTBS (*N,N*-dimethylthiocarbamylbenzothiazole sulfide).

expected and has been described in earlier studies, as well as the formation of ZDMC in ZnO/TMTD mixtures (1), which even noted the formation of tetraalkylthioureas as a result of further donation of sulfur. Thioureas were not, however, found in our samples. Although the use of thiurams in rubber glove production has been reduced (11, 12), we now know that the consumption of thiuram disulfides during vulcanization is one reason why we seldom find them in rubber extracts. Still, the principal structural element – the dialkyl thiocarbamoyl structure – is found in other compounds in the final products, as, for example, zinc dithiocarbamate, thiurammonosulfide, or other sulfides like DMTBS or DMTBD. A probable cross-reactivity between these compounds might explain the positive reactions to thiuram disulfides at patch test with the standard series, even if they would not be present in the rubber. On the other hand, the cross-reactivity among thiurams, dithiocarbamates, and their different sulfides are not definite: we have recently found *N,N*-diethylthiocarbamylbenzothiazole sulfide (DETBS) in a pair of swimming goggles where it was the reason for an allergic eczema (13). DETBS (CAS No. 95-30-7) is an accelerator sold under several trade names, for example, Nocceler 64 (Ouchi Shinko, Tokyo, Japan) and Ethylac (Pennwalt, Philadelphia, PA, USA). DETBS is chemically related to DMTBS and consists of a diethyldithiocarbamate residue instead of the dimethyl analogue found in DMTBS. The patient was positive at patch

test to DETBS and TMTM but negative to TETD (tetraethylthiuram disulfide), TMTD, ZDEC (zinc diethyldithiocarbamate), and ZDMC. In this case, the cross-reactivity is difficult to explain on the basis of recognition of the dialkyl thiocarbamoyl structure.

An assessment of exposure to contact allergens in products used in occupational activities cannot only be based on information regarding what additives were used in manufacturing the rubber product. Instead, chemical analysis of the products actually in use must be performed, to take into account the chemical changes occurring during vulcanization. Such changes will result in both the disappearance and formation of allergens and are therefore of great importance concerning insurance matters. Collaboration in this project between experts from both the rubber industry and an allergy research unit has given new insight into the chemistry of rubber allergens. The study also illustrates the usefulness of the HPLC method, which allows the whole range of substances involved to be analysed in a single run.

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Table 1. Results of analysis of different rubbers before and after vulcanization

Rubber	Additives used	Found in the vulcanized product
SBR-1	TMTD, MBTS, CBS	TMTM, ZDMC, DMTBS, MBT
NR-1	CBS, CTP	MBT
NR-2	TMTD, CBS, CTP	MBT, MBTS, CBS, DMTBS
NR-3	TMTM, MBTS	MBT, MBTS, DMTBD, DMTBS
NR-4	TMTM, CBS	MBT, DMTBD, DMTBS

TMTD, tetramethylthiuram disulfide; MBTS, 2,2'-dibenzothiazyl disulfide; CBS, *N*-cyclohexyl-2-benzothiazyl sulfenamide; CTP, *N*-cyclohexylthiophthalimide; TMTM, tetramethylthiuram monosulfide; ZDMC, zinc dimethyldithiocarbamate; DMTBS, *N,N*-dimethylthiocarbamylbenzothiazole sulfide; MBT, 2-mercaptobenzothiazol; DMTBD, *N,N*-dimethylthiocarbamylbenzothiazole disulfide.

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