

Allergic contact dermatitis caused by dimethylthiocarbamylbenzothiazole sulfide (DMTBS) in canvas shoes: in search of the culprit allergen

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Summary

Background. During rubber vulcanization, new compounds can be formed.

Objectives. To report a case of allergic shoe dermatitis in which the search for the allergen ultimately led to the identification of dimethylthiocarbamylbenzothiazole sulfide (DMTBS).

Methods. A female presented with eczema on her feet after wearing Sperry Top Sider[®] canvas sneakers. Patch testing was performed with the European baseline series, additional series, shoe materials, and extracts of shoe materials. Thin-layer chromatography (TLC) was performed for additional patch testing, and high-performance liquid chromatography and gas chromatography–mass spectrometry were used for chemical analysis.

Results. Positive reactions were found to thiuram mix (+), tetramethylthiuram monosulfide (TMTM) (+), shoe material (+), and shoe extracts in eth. (++) and acetone (+). The extracts did not contain TMTM or other components of thiuram mix. TLC strips yielded a positive reaction (+) to one spot, whereas chemical analysis gave a negative result. Thereafter, a similar sneaker from another patient with shoe dermatitis was analysed, and DMBTS was identified. New extracts of the shoe of our first patient were then also shown to contain DMTBS. DMTBS as culprit allergen was confirmed by positive patch testing with a dilution series with DMTBS.

Conclusion. DMBTS was identified as the culprit allergen in shoe dermatitis, giving rise to compound allergy. The positive reaction to TMTM was considered to represent cross-reactivity.

Key words: allergic contact dermatitis; CAS no. 3432-25-5; case report; dimethylthiocarbamylbenzothiazole sulfide; DMTBS; gas chromatography–mass spectrometry; shoe dermatitis; thin-layer chromatography; thiurams; ultrasonic bath extract.

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Thiurams, dithiocarbamates and mercaptobenzothiazoles are the three main groups of allergenic rubber accelerators. Because of the chemical reactivity of these substances, new compounds can be formed when different compounds are mixed, and especially as a result of the elevated temperatures used in vulcanization (1). In the following case report regarding a patient with shoe dermatitis, we describe the first case of allergic contact dermatitis caused by dimethylthiocarbamylbenzothiazole sulfide (DMTBS) (CAS no. 3432-25-5, molecular weight 254), a substance triggering compound allergy (1).

Case Report

An 18-year-old non-atopic Dutch female presented with the third episode of eczema on her feet in a period of 6 months. During this third episode, she recognized that her eczema was apparently caused by wearing Sperry Top Sider® canvas sneakers. The eczema was bilateral and symmetrical on the toes, spreading to the dorsal side of the feet (Fig. 1). She had no eczema on the hands or any other part of the body.

Methods

Patch testing, thin-layer chromatography (TLC) and chemical analysis

Patch testing was performed with our extended European baseline series (TRUE Test® panels 1 and 2 supplemented with investigator-loaded allergens), and shoe, rubber additives, textile, and plastic and glues series (Smart-Practice Europe, Reinbek, Germany). Moreover, thin pieces of the patient's own shoe materials moistened with physiological saline (Fig. 1) were tested in Van der Bend chambers (van der Bend, Brielle, The Netherlands). The patch tests were applied on the back and occluded for 48 h; readings were performed on day (D) 3 and D7, according to ESCD guidelines (2). Positive patch test reactions were found to thiuram mix 27 µg/cm² (+) on D3, tetramethylthiuram monosulfide (TMTM) 1% pet. (+) on D3 and D7, and five pieces of shoe material of different parts of the shoe (+) on D3 and D7 (Table 1).

As we suspected contact allergy to a thiuram compound, extracts from the fabric and from the inner sole of the shoe were prepared in acetone and in ethanol. Extraction was performed with an ultrasonic bath; subsequently, the extract was concentrated with a vacuum evaporator (3). Positive patch test reactions were seen to the extracts of the shoe fabric (++) and the shoe sole (+). Eleven controls were tested with the extracts, and this yielded

Table 1. Test results from patch testing of patch test series, shoe materials, extracts, substances, thin-layer chromatography strips, and dimethylthiocarbamylbenzothiazole sulfide (DMTBS) dilution series

Tested series, substances	Concentration, vehicle	D3	D7
European baseline series			
Thiuram mix	27 µg/cm ²	+	–
Shoe series		–	–
Rubber series*			
Tetramethylthiuram monosulfide	1% pet.	+	+
Plastic and glues series			
Own shoe materials, 'as is':			
1. Fabric front of shoe	'as is'	+	+
2. Fabric border side ankle	'as is'	+	+
3. Fabric shoe upper	'as is'	+	+
4. Fabric + glue inner layer	'as is'	+	+
5. Fabric inner layer shoe upper	'as is'	+	+
6. Shoelace	'as is'	–	–
Shoe extracts			
Shoe fabric extract	ac.	+	+
	eth.	++	+
Shoe inner sole	ac.	+	+
	eth.	?	?
Substances in shoe extracts			
Zinc dibutylthiocarbamate	2% pet.	–	–
Dibenzothiazyl disulfide	2% pet.	–	–
Octyl methoxycinnamate	10% pet.	–	–
TLC			
TLC extract fabric/inner sole	eth.	+ Spot 4 [†]	+
TLC extract fabric/inner sole	ac. [‡]	+ Spot 4 [†]	+
DMTBS dilution series			
	0.1% to 0.0000032% ac. [§]		
DMTBS	0.1% ac.	+	++
DMTBS	0.032% ac.	+	+
DMTBS	0.01% ac.	+	+
DMTBS	0.0032% ac.	+	+
DMTBS	0.001% ac.	?+	–
DETBS	0.1% ac.	–	–
DETBS	0.01% ac.	–	–

ac., acetone; DETBS, Diethylthiocarbamylbenzothiazole sulfide; eth., ethanol; TLC, thin-layer chromatography.

*Among others negative: dipentamethylenethiuram disulfide, tetraethylthiuram disulfide, and tetramethylthiuram disulfide.

[†]TLC spots 1–3 and 5–6 negative.

[‡]Used for chemical analysis.

[§]DMBTS series: only concentrations with positive and doubtful results are shown.

nine negative reactions, one doubtful reaction, and one irritant reaction. High-performance liquid chromatography (HPLC) was used to analyse the extracts. The results showed no presence of TMTM or other components of thiuram mix. However, zinc dibutylthiocarbamate (ZDBC), dibenzothiazyl disulfide (MBTS) and octyl methoxycinnamate were present at low concentrations. Patch testing



Fig. 1. Left: symmetrical eczema on the toes, spreading to the dorsal side of the feet. Right: several tested pieces of shoe materials corresponding to the localization of eczema.

with ZDBC and MBTS at higher concentrations (2% pet.) than in the rubber series and of octyl methoxycinnamate 10% pet. gave negative results.

Thereafter, new extracts were made from the fabric and the inner shoe material. TLC was performed to separate the components of the extracts of the shoe materials in order to obtain TLC strips for patch testing. Several spots each containing 10 µl of the acetone extract, and several spots each containing 10 µl of the eth. extract, were applied to a sheet of thin-layer material with silica gel bound to a plastic carrier (TLC plastic roll, Silica Gel 60F 254; Merck, Darmstadt, Germany). Elution was performed with a mobile phase consisting of heptane and ethyl acetate (50:50, vol/vol) (4). The patient was tested with TLC strips, and reacted positively to spot no. 4 (+) (Fig. 2). The area corresponding to the positive reaction on the TLC strip to which the acetone extract had been applied was analysed by scraping off the silica gel from the duplicate of the tested TLC strip and extracting the gel with a minimal amount of ethyl acetate. The obtained solution was analysed by gas chromatography–mass spectrometry (GCMS), which did not give any conclusive results (5).

At that time, we could not identify the culprit allergen. However, several months later, a number of Belgian patients presented with shoe dermatitis caused by similar Sperry Top Sider® canvas sneakers. HPLC analysis of a shoe of one of these patients showed high amounts of DMTBS in the water-repellent inner layer of the shoe (E. Hulstaert, in preparation 2017). Consequently, a new extract was made of the water-repellent inner layer of the shoe of the Dutch patient, with the same method as before. The presence of DMTBS could now be shown. When DMTBS was used as a reference substance in TLC, the retention factor corresponded to TLC spot no. 4, to which the patient had reacted. To confirm that DMTBS was the culprit allergen in our patient, a dilution series of DMTBS in acetone was tested, as was another closely related rubber substance, namely, diethylthiocarbamyl-benzothiazole sulfide. Positive reactions were observed to various concentrations of DMTBS in acetone (Fig. 3, Table 1). Eventually, we concluded that our patient suffered from shoe dermatitis caused by contact allergy to DMTBS in Sperry Top Sider® canvas sneakers; the positive patch test reaction to TMTM was considered to represent cross-reactivity.

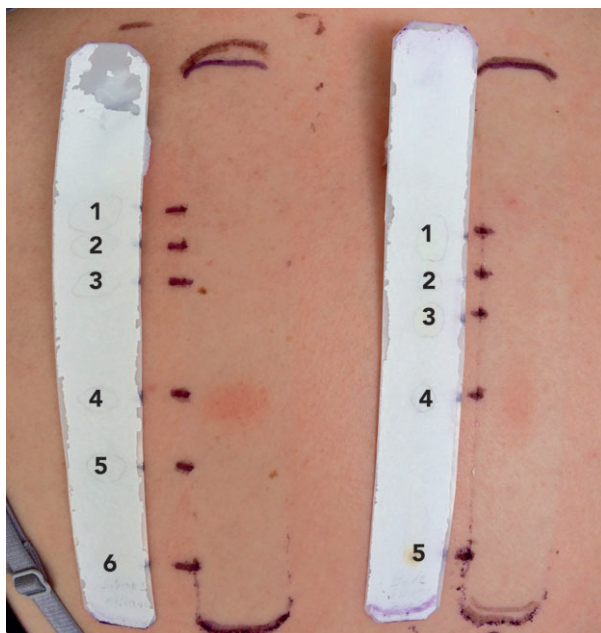


Fig. 2. The patient reacted to spot no. 4 of the thin-layer chromatography strip (day 3), strip. Left strip: ethanol. Right strip: acetone.

Discussion

There are several reasons why DMTBS remained undetected in the first instance. Chemical substances are usually present at higher concentrations in an extract; however, the concentration of the first ultrasonic bath extract was probably too low in our patient. Patch testing with TLC strips of the positive extract is often helpful to identify allergens, but, presumably, a concentration below the detection limit was extracted from the TLC silica plate. Another reason why we could not show the presence of DMTBS in the first instance is possibly that DMTBS may break down under the high-temperature conditions used in GCMS.

Shoes are composed of many different parts and materials; some of the extracts were made from blends of these parts and materials. Chemicals may diffuse from one material into another, resulting in combinations of chemicals, and possibly chemical reactions between these substances. This might be another reason why we could not show the presence of DMTBS in the extracts in the first instance. Additionally, a probable reason why DMTBS could not be identified in our early HPLC analysis is that several components in the chromatogram interfered with the DMTBS peak. Probably, the concentration of DMTBS in the extract was higher in the shoe of the Belgian patient. Because DMTBS was shown to be present in the Belgian shoe, we could also identify DMTBS in the upper

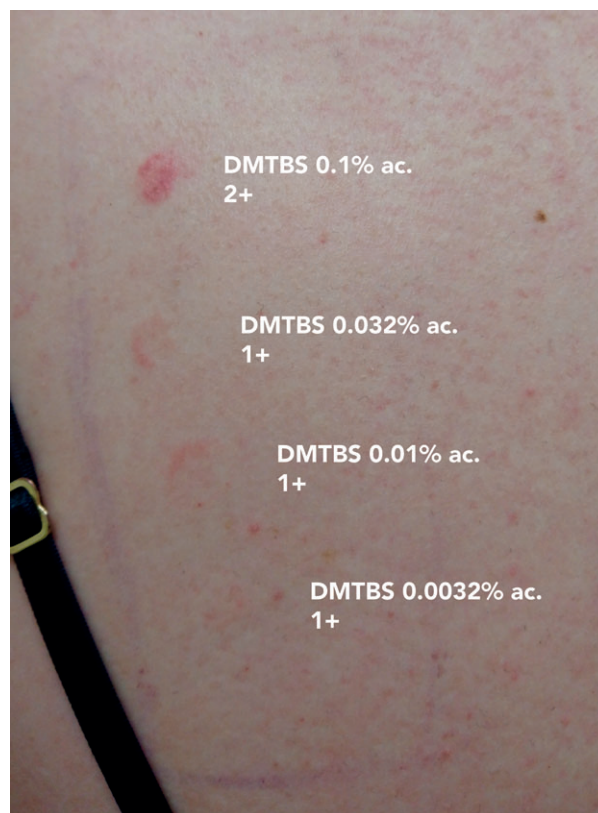


Fig. 3. Positive patch test reactions (day 7) to different concentrations of dimethylthiocarbamylbenzothiazole sulfide (DMTBS) in a dilution series. ac., acetone.

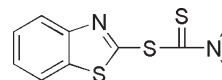


Fig. 4. Molecular structure of dimethylthiocarbamylbenzothiazole sulfide (CAS no. 3432-25-5, molecular weight 254).

water-repellent lining of the shoe of our Dutch patient, and confirmed that DMTBS was the culprit allergen by demonstrating positive patch test reactions with a dilution series of DMTBS.

During the vulcanization process, thiuram derivatives react with mercaptobenzothiazoles, forming new compounds with both dithiocarbamate and mercaptobenzothiazole structures, such as DMTBS (6) (Fig. 4). Dimethylthiocarbamylbenzothiazole disulfide (DMTBD) is formed during vulcanization, by means of the disulfide exchange reaction between MBTS and tetramethylthiuram disulfide (TMTD). Subsequently, the monosulfide DMTBS is formed from the desulfurization of DMTBD (6). Pontén et al. investigated whether DMTBS is a sensitive marker for diagnosing contact allergy to rubber

chemicals by including the allergen in the baseline series for a short period. Patch testing with DMTBS 1% showed late positive reactions, suggesting active sensitization. Retesting these subjects with DMTBS in a dilution series confirmed sensitization to DMTBS, and 1 patient reacted down to a 0.0001% dilution (7). These results suggest that DMTBS is a strong allergen. Further research on the sensitization potency of DMTBS is necessary before we can consider including this allergen in a rubber or shoe series.

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