

Investigation of diethylthiourea and ethyl isothiocyanate as potent skin allergens in chloroprene rubber

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Summary

Background. Exposure to chloroprene rubber has resulted in numerous cases of allergic contact dermatitis, attributed to organic thiourea compounds used as vulcanization accelerators. However, thiourea compounds are not considered to be strong haptens.

Objectives. To analyse common commercial chloroprene materials for their contents of diethylthiourea (DETU), dibutylthiourea (DBTU), diphenylthiourea (DPTU), and their degradation products, isothiocyanates; and to investigate the sensitization potencies of possible degradation products of the mentioned thiourea compounds.

Methods. Liquid chromatography/mass spectrometry (MS) was used for quantification of organic thiourea compounds in chloroprene products, such as medical, sports and diving gear; isothiocyanates were measured by solid-phase microextraction/gas chromatography/MS. Sensitization potencies were determined with the murine local lymph node assay (LLNA).

Results. DETU was identified at concentrations of 2.7–9.4 µg/cm² in all samples, whereas neither DBTU nor DPTU was detected. At 37°C, degradation of DETU in the materials to ethyl isothiocyanate (EITC) was detected. EITC and ethyl isocyanate showed extreme and strong sensitization potencies, respectively, in the LLNA.

Conclusions. DETU can act as a prehapten, being degraded to EITC when subjected to body temperature upon skin contact. EITC could thus be the culprit behind allergic contact dermatitis caused by chloroprene rubber.

Key words: chloroprene; contact dermatitis; diethylthiourea; ethyl isothiocyanate; exposure analysis; local lymph node assay; neoprene; rubber chemicals.

Chloroprene is a rubber material that is widely used because of its stretchable, waterproof, chemical-resistant and abrasion-resistant properties. These properties make the rubber ideal for use in products such as wet suits,

orthopaedic devices, and sports gloves. Chloroprene rubber is manufactured from 2-chloro-1,3-butadiene with the addition of accelerators such as thiourea compounds (1). Those compounds also function as antioxidants in the finished product (1). The extensive use of chloroprene materials has resulted in many cases of allergic contact dermatitis, considered to be caused by thiourea compounds present in the rubber (2–9). Today, three different thiourea compounds – diphenylthiourea (DPTU), diethylthiourea (DETU), and dibutylthiourea (DBTU) – are used in clinics as diagnostic markers for chloroprene rubber allergy (Fig. 1). Although DETU and DBTU have been detected in case reports of allergic contact dermatitis caused by chloroprene rubber (10–12),

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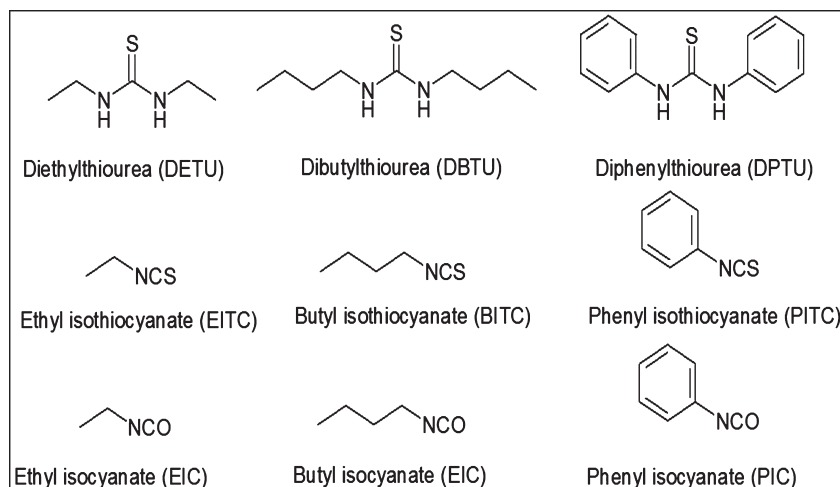


Fig. 1. Chemical structures of thioureas, isothiocyanates and isocyanates discussed in this article.

no studies have been performed to determine their occurrence and concentrations in common chloroprene products intended for prolonged use in contact with the skin.

Allergic contact dermatitis is a common chemical-induced health problem. The majority of haptens are of low molecular weight and are able to react with nucleophilic moieties of skin proteins, forming an immunogenic complex that can be recognized as foreign by dendritic cells in the skin and induce an immune response. In experimental studies, thiourea compounds have been shown to be weak sensitizers or non-sensitizers (13–15). DPTU has been identified as a sensitizer in the guinea-pig maximization test; however, in the murine local lymph node assay (LLNA), it was identified as a non-sensitizer (13, 15). Both DETU and DBTU have been identified as non-sensitizers in the LLNA (13, 14).

Chemicals that are not able to cause sensitization on their own, but that can be activated to reactive sensitizing compounds, are called either prohaptens or prehaptens. Activation of prohaptens occurs in the skin through the action of oxidative enzymes such as the flavin-containing mono-oxygenases and the cytochrome P450 (CYP450) enzyme system (16). A number of CYP450 enzymes have been identified in skin (17). Prehaptens are activated outside the body by chemical reactions, such as oxidation or thermal degradation of the parent compounds (18). In some cases, the same hapten can be formed in either pathway, as we have shown in the case of the fragrance terpene geraniol, from which the same sensitizing aldehyde is formed via both autoxidation and cutaneous metabolism (19, 20).

Recently, DPTU has been shown to be bioactivated to an array of compounds, among which the metabolites most important for sensitization were considered to be phenyl isothiocyanate (PITC) and phenyl isocyanate (PIC)

(15). Both of these metabolites were shown to be strong sensitizers (15). In that study, degradation of DPTU in the absence of enzymes was observed in buffer at 37°C, and it was suggested that exposure to PITC could occur from degradation of DPTU during the manufacture of chloroprene at high temperature.

In a case of contact allergy to PVC tape, DPTU was identified in the tape along with the strong sensitizer PITC (21), the latter being considered to be a decomposition product. Thiourea has also been shown to decompose at elevated temperatures, although the decomposition products were not identified (22). The vulcanization process for rubber has been shown to yield a large number of detectable degradation products, many of which were considered to be skin sensitizers (23).

The overall purpose of this study was to gain more insight into the numerous cases of contact allergic reactions to chloroprene. Thus, the occurrence of both thiourea compounds and isothiocyanates in common chloroprene products was investigated. Also, the sensitization potencies of isothiocyanates and isocyanates, which are possible degradation products of DETU and DBTU, were determined *in vivo*.

Materials and Methods

Chemicals

Dichloromethane, methanol, methyl-*tert*-butyl ether (MTBE) and acetonitrile were purchased from Rathburn Chemicals Ltd (Walkerburn, UK), and toluene was purchased from Scharlau Chemie SA (Mas d'en Cisa, Spain). Pentane was purchased from Lab Scan Ltd (Dublin, Ireland). Ammonium hydroxide (aqua, 25%) and solid sodium hydroxide (purity of > 99%) were

purchased from Merck (Darmstadt, Germany). DETU (> 98%), DBTU (> 97%), DPTU (> 99%), tetrahydrofuran (THF), carbon disulfide (> 99%), *p*-toluenesulfonyl chloride (tosyl chloride, > 99%) and triethylamine (> 99%) were all purchased from Sigma-Aldrich (St Louis, MO, USA). Ethyl-d₅-amine hydrochloride (purity 99.5%) was purchased from Qmx Laboratories (Quebec, Canada). Ethyl isocyanate (EIC) (> 98%), ethyl isothiocyanate (EITC) (> 97%), butyl isocyanate (BIC) (> 98%) and butyl isothiocyanate (BITC) (> 99%) were all purchased from Sigma-Aldrich. The purity of the chemicals was investigated with nuclear magnetic resonance (NMR) spectroscopy.

Synthesis of deuterium-labelled internal standards

DETU-d₅ was synthesized in the laboratory by refluxing ethylamine-d₅-hydrochloride (50 mg, 0.6 mmol), EITC (80 mg, 0.9 mmol) and sodium hydroxide (36 mg, 0.9 mmol) in 15 ml of methanol for 2 hr. The crude product was extracted with 15 ml of ethyl acetate, and then recrystallized from toluene/heptane 1:1 (vol/vol).

EITC-d₅ was synthesized by slowly adding carbon disulfide to a solution of ethyl-d₅-amine hydrochloride and triethylamine in THF/H₂O 9:1 (vol/vol) in a water ice bath, after which the mixture was left at room temperature for 1 hr. It was then again transferred to an ice bath, and tosyl chloride was added. The crude product was then left at room temperature for 0.5 hr, and this was followed by extraction with MTBE. EITC-d₅ was purified by Isolera One flash chromatography (Biotag, Uppsala, Sweden) on silica gel by elution with pentane and monitoring at 240 nm (24).

For comparison, DETU and EITC were synthesized with the same protocols as used for the corresponding deuterated compounds, but with non-deuterated ethylamine hydrochloride. ¹H-NMR spectra were acquired with CDCl₃ as solvent on a JEOL Eclipse+ 400-MHz spectrometer: DETU, δ5.78 (t, H), δ3.45 (m, 4H), and δ1.23 (t, 3H); EITC, δ1.37 (t, 3H) and δ3.56 (q, 2H). The purities were > 95% for both compounds according to NMR. For EITC-d₅, the purity was > 98% according to gas chromatography (GC)/electron ionization (EI)-mass spectrometry (MS) analysis in full scan.

Sensitization experiments in mice

The sensitizing potencies were investigated with the LLNA, as previously described (19). All animal procedures were approved by the local ethics committee. Compounds were tested at five different concentrations, with mice in groups of 3. Results are expressed as mean

disintegrations/min/lymph node for each experimental group, and as the stimulation index (SI), that is, test group/control group ratio. Test materials that, at one or more concentrations, cause an SI of > 3 are considered to be positive in the LLNA. EC3 values (the estimated concentration required to induce an SI of 3) are calculated by linear interpolation and used to compare the sensitizing capacities of the different test materials (25). We classified the sensitization potencies according to the proposal by Kimber et al.: EC3 < 0.1%, extreme; EC3 ≥ 0.1% to < 1%, strong; EC3 ≥ 1% to < 10%, moderate; and EC3 ≥ 10% to < 100%, weak (26).

Selection of chloroprene rubber objects for analysis

Three product categories were identified in which the products are in prolonged contact with the skin, namely diving gear, sports support products, and medical devices. Although chloroprene rubber can be found in many other types of products, those product categories represent items that are intended for repeated and prolonged contact with the skin. Four medical devices and five sports products were obtained, representing the main brands on the European market. Owing to the high cost, only one wet suit (for children) was analysed. Instead, six samples of other diving gear were analysed, including gloves, socks, and mending pieces for wet suits. All products were declared to be made from neoprene, the trademark for chloroprene.

Analysis

Chloroprene samples (n = 16) were screened for their contents of DETU, DBTU and DPTU with liquid chromatography (LC)/ESI-MS in positive mode on a Finnigan LTQ instrument (Thermo Electron Corp., San Jose, CA, USA) equipped with an Agilent G1312A binary pump (1100 series; Agilent Technologies, Santa Clara, CA, USA) and a Symmetry[®] C₁₈ column (3.5 μm, 2.1 mm i.d. × 150 mm; Waters Corp., Milford MA, USA). Isocratic LC separations of samples and standard solutions were performed at a flow rate of 0.3 ml/min with acetonitrile/50 mM ammonium hydroxide (aqua) 7:3 (vol/vol) as the mobile phase. The heated capillary inlet temperature was set to 275°C. The auxiliary gas flow was set to 34, and the sheath gas to 25 (arbitrary units). The electrospray voltage was 4.5 kV. Quantification was performed by using selected ion monitoring (SIM) of the [M + H]⁺ ions within 2-*m/z* windows. To increase the identification strength, the analyses were also performed on a Luna NH₂ column (5 μm, 3 mm i.d. × 150 mm; Phenomenex, Torrance CA, USA) with isocratic elution at 0.2 ml/min and acetonitrile/50 mM

ammonium hydroxide (aqua) 95:5 (vol/vol) as the mobile phase. The identity of DETU was further verified by running both chromatographic systems for two of the samples (diving gear D1 and D2) and standard in MS/MS product ion scan mode ($n = 3$). The collision energy was set to 28 (arbitrary units), and the scan range was m/z 50–140.

Determination of isothiocyanates in chloroprene samples was carried out by solid-phase microextraction (SPME) followed by GC/MS on a Varian 4000 ion trap instrument (Varian Inc., Walnut Creek, CA, USA). Separation was performed on an Agilent J&W DB-35ms column (30 m $\times$ 0.250 mm internal diameter, 0.25- μ m film; Agilent Technologies). The oven temperature program was as follows: 50°C for 0.5 min, then an increase of 15°C/min to 200°C, with the final temperature held for 1 min. The chromatographic run was complete in 12 min. A split/splitless injector (Varian 1177) was used, with a splitless time of 2 min. The injection temperature was set to 250°C. The analysis was performed in EI mode at 70 eV, and acquisition was performed in full scan within the m/z range 45–120. The emission current was set to 25 μ A, the number of microscans to 3, and the automatic gain control to 20 000 counts.

Extraction of thioureas from chloroprene

An amount of 100 mg of each chloroprene material was cut into small pieces and then spiked with internal standard (15.0 μ g of DETU- d_5 in 50 μ l of dichloromethane), after which it was left to dry for 5 min. The sample was ultrasonicated in 10 ml of acetonitrile/dichloromethane 1:4 (vol/vol) for 30 min. An aliquot of 1 ml was taken from the extract. This procedure was repeated three times per sample, and the three extracts were pooled and then evaporated under a gentle stream of N_2 to almost complete dryness, after which the sample was redissolved in 1 ml of acetonitrile:water 1:1 (vol/vol). The sample was finally filtered with a Titan 2 PP Membrane syringe filter (17 mm, pore size 0.45 μ m; Titan Filtration Systems, Rockwood, TN, USA). A volume of 5 μ l was injected into the LC/MS apparatus.

Extraction of EITC from chloroprene

Different amounts of chloroprene material were cut into small pieces and spiked with EITC- d_5 (16 ng in 25 μ l of pentane) after being placed in a 20-ml vial. The vials were immediately sealed with a septum and then placed in an oven at 37°C. The headspace of each vial was extracted after 2 and 6 hr, respectively, with SPME. In parallel, 25 μ l of a standard solution was sampled in a similar way after 4 hr in a separate vial. The standard solution contained

0.25 ng/ μ l EITC and 0.65 ng/ μ l EITC- d_5 . Sampling by SPME was carried out with a 75- μ m Carboxen-PDMS fibre (Supelco, Bellefonte, PA, USA). Each extraction was performed for 20 min. After sampling, the fibre was immediately desorbed in the injection port of the GC/MS apparatus at 250°C for 2 min.

Blank samples were analysed in triplicate for both DETU and EITC, with the same analytical protocols as used for chloroprene samples, but without chloroprene. Neither DETU or EITC could be detected in any of the blank samples.

Linearity, detection limits, and accuracy

Statistical evaluation was performed with WINSTAT (version 3.24, Anders Colmsjö, copyright 1996, Analytical Chemistry, Stockholm University).

Results

Screening of thioureas in chloroprene products

First, as a preliminary investigation of 16 different commercial products, including diving gear, sports support products, and medical devices, possible traces of DETU, DBTU and DPTU were investigated. After extraction and solvent change, the samples were analysed by LC/ESI-MS in positive mode. All three compounds were shown to be efficiently ionized and could be chromatographically separated in reversed-phase mode on a C_{18} LC column. To achieve as low a detection limit as possible, data were acquired in SIM mode, with monitoring of the protonated quasi-molecular ions.

The analyses showed that, among the selected thiourea compounds, only DETU could be detected. This compound was detected in all 16 samples. Thus, further analyses were focused on eight different samples, regarding their content of DETU and its possible degradation product EITC. Quantification of DETU was performed with SIM of only one ion. For this reason, the analyses were repeated with an NH_2 LC column in HILIC mode, to increase the identification strength. The chromatograms for a diving suit sample (D2) are shown in Figure S1.

The identity of DETU was further verified for two of the diving gear samples (D1 and D2) by MS/MS product ion scanning. The spectra both for samples and standards are included in Figure S2. The intensity ratio of m/z 133 $[M + H]^+$ and m/z 88 $[M + H - NH_2CH_2CH_3]^+$ was not significantly different between standard and samples. The mean ion ratio (133/88) for the standard compound was 0.91 [relative standard deviation (RSD) 2%, $n = 3$], 0.91 for sample D1 (RSD 1%, $N = 3$), and 0.96 for sample D2 (RSD 8%, $n = 3$).

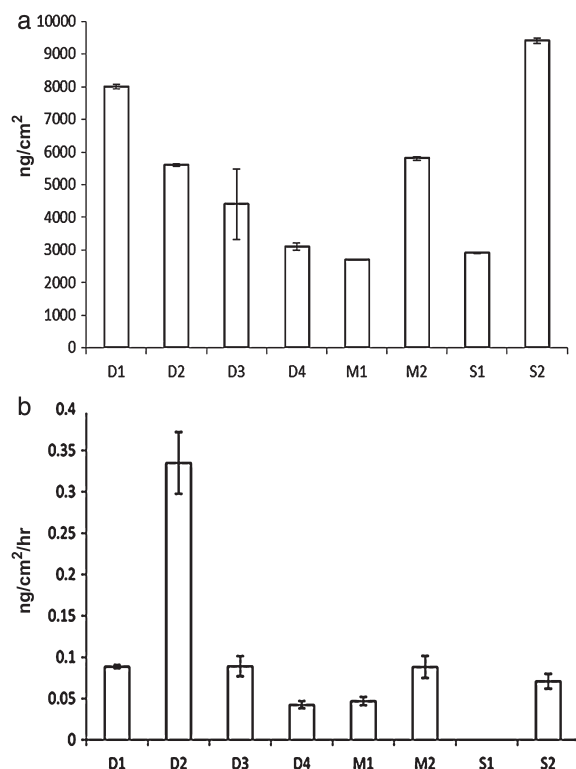


Fig. 2. Levels of diethylthiourea (DETU) (a) and rate of ethyl isothiocyanate (EITC) production (b) in the studied chloroprene products ($n = 3$). D, diving gear; M, medical brace; S, sports product. EITC was detected in all DETU-containing products. However, the increase in EITC (formation from heating) was not significant for sample S1.

Quantification of DETU and EITC in chloroprene products

Samples from wet socks (D1), a junior wet suit (D2), mending pieces for wet suits (D3 and D4), a medical thumb wrap (M1), a medical wrist wrap (M2) and two knee supports for sports (S1 and S2) were analysed for their contents of DETU and EITC. The different chloroprene samples showed concentrations of DETU varying between 2.7 and $9.4 \mu\text{g}/\text{cm}^2$, as shown in Fig. 2a. The analysis was found to be highly reproducible, with RSD values from 1% to 3% ($n = 3$), except for the diving gear D3. The reason for the low precision for this sample is unknown, although the physical properties could be affected by a different material composition, thus affecting the extraction repeatability. However, this was not further investigated. The method was found to be linear up to an extract concentration of $1 \mu\text{g}/\text{ml}$, and the detection limit was determined to $46 \text{ ng}/\text{ml}$ from the calibration curve. The mean accuracy for spiked samples was found to be 108% ($n = 3$). The concentration of DETU expressed as $\mu\text{g}/\text{g}$ of product is shown in Figure S3.

Because of its high volatility and inefficient ionization in LC/MS, the possible contents of EITC in the materials were investigated with headspace SPME followed by GC/EI-MS. For the standard compound, equilibrium between headspace and fibre was achieved after 10 min. The EITC formation rates for the different products measured between 2 and 6 hr are shown in Fig. 2b. The detection limit corresponded to an initial amount of 0.62 ng of EITC in the sampled material. The response of EITC in GC/MS was found to be linear within the investigated range (up to 10 ng added to the sealed vial). However, sampling of the chloroprene products showed that a steady state was never achieved up to 10 hr of sampling, indicating production of EITC, most likely owing to degradation from DETU. An SPME/GC/MS chromatogram of a diving suit sample (D2) is shown in Figure S4.

DETU degradation to EITC at 37°C

Degradation of DETU to EITC was first investigated with headspace SPME of DETU standard ($3.0 \mu\text{g}$ added to the

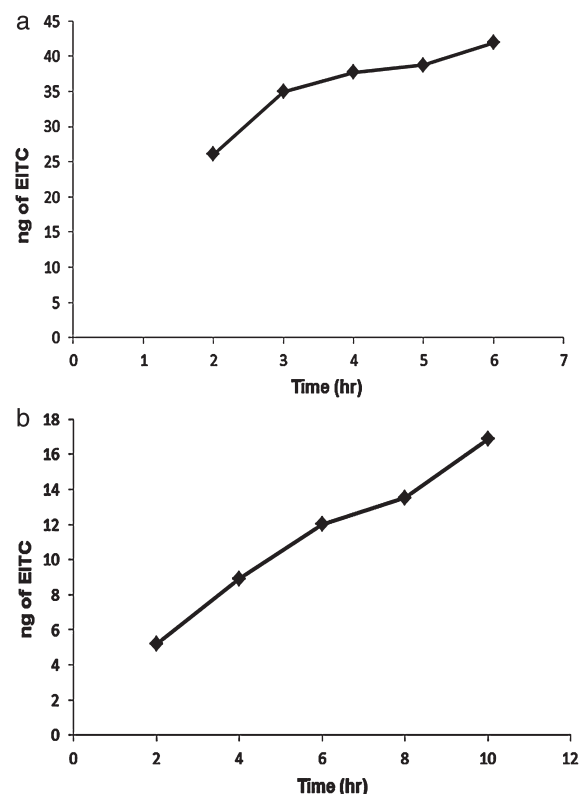


Fig. 3. Amount of ethyl isothiocyanate (EITC) formed at 37°C from (a) $3.0 \mu\text{g}$ of diethylthiourea and (b) a 200-mg chloroprene sample (diving suit, D2). Sampling was performed with solid phase microextraction (see Materials and Methods).

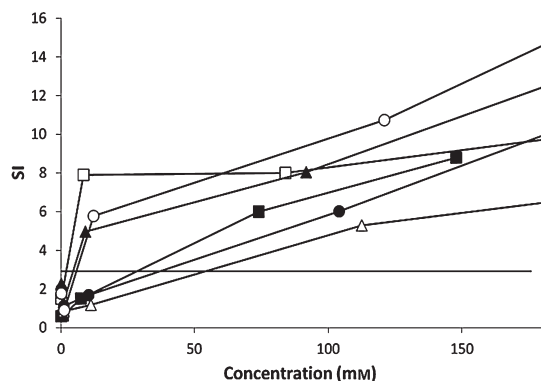


Fig. 4. Local lymph node assay (LLNA) responses for isothiocyanates and isocyanates of thioureas used in the diagnosis of contact allergy to chloroprene rubber. □, phenyl isocyanate (PIC); ○, butyl isocyanate; ▲, ethyl isothiocyanate; ■, phenyl isothiocyanate (PITC); ●, butyl isothiocyanate; △, ethyl isocyanate. The horizontal line marks a stimulation index (SI) of 3, the minimum response for a positive result in the LLNA. The results for PIC and PITC have been published previously (15).

vial). Interestingly, DETU was shown to already degrade to measurable quantities of EITC at 37°C. The chloroprene materials were then analysed accordingly. The EITC concentration in the headspace was shown to continuously increase for all investigated products, except for a knee support sample (S1). For the latter, there was no significant increase in emission. The EITC emissions with time from pure DETU and a chloroprene sample, respectively, are shown in Fig. 3.

Sensitization potencies of potential degradation products

The sensitization potencies of possible degradation products of DETU and DBTU, that is, EITC, EIC, BITC, and BIC, were investigated with the LLNA. The results from the animal experiments are shown in Fig. 4 and Table 1. All four compounds were found to be highly sensitizing, with potencies comparable to those previously reported for PITC and PIC, potential degradation products of DPTU. Figure 4 shows a diagram of SI versus concentration for the different compounds, with previously published data for PITC and PIC included for comparison (15). In general, isocyanates were stronger sensitizers than the corresponding isothiocyanates; however, this situation was reversed for EIC and EITC, whereby EITC exhibited higher sensitization potency.

Discussion

The present investigation indicates that DETU is by far the most common thiourea compound used in chloroprene

Table 1. Local lymph node assay^a responses for alkyl isothiocyanates and the corresponding isocyanates

Test material and concentration (% wt/vol)	[³ H] Thymidine incorporation (dpm/lymph node)	SI	EC3		EC3 (μg/cm ²)	Classification
	(%)		(mm)			
Butyl isothiocyanate	—	—	0.45	39.1	112.5	Strong
Control	226	—	—	—	—	—
0.0012	449	1.99	—	—	—	—
0.012	252	1.12	—	—	—	—
0.12	380	1.68	—	—	—	—
1.2	1359	6.02	—	—	—	—
2.4	2570	11.38	—	—	—	—
Butyl isocyanate	—	—	0.06	6.1	15	Extreme
Control	229	—	—	—	—	—
0.0012	406	1.77	—	—	—	—
0.012	206	0.90	—	—	—	—
0.12	1320	5.77	—	—	—	—
1.2	2460	10.74	—	—	—	—
2.4	4266	18.63	—	—	—	—
Ethyl isothiocyanate	—	—	0.04	4.6	10	Extreme
Control	278	—	—	—	—	—
0.0008	634	2.28	—	—	—	—
0.008	366	1.32	—	—	—	—
0.08	1380	4.97	—	—	—	—
0.8	2232	8.04	—	—	—	—
1.6	3496	12.6	—	—	—	—
Ethyl isocyanate	—	—	0.4	56.3	100	Strong
Control	1155	—	—	—	—	—
0.0008	2104	1.82	—	—	—	—
0.008	977	0.85	—	—	—	—
0.08	1368	1.18	—	—	—	—
0.8	6104	5.28	—	—	—	—
1.6	8364	7.24	—	—	—	—

EC3, estimated concentration needed to produce a stimulation index of 3; dpm, disintegrations/min; SI, stimulation index.

^aThe sensitization experiments were performed as described in Materials and Methods. The SIs correspond to the increase in thymidine incorporation of treated groups relative to vehicle-treated controls. EC3 values were calculated by the use of linear interpolation. The sensitization potencies of the test substances were classified as follows: EC3 < 0.1%, extreme; EC3 0.1% to < 1%, strong; EC3 1% to < 10%, moderate; EC3 10% to < 100%, weak (27).

production today. No large differences could be seen between different product types, as diving gear, sports braces and medical braces were shown to contain DETU within approximately the same concentration range. Previous clinical studies have shown that DETU is the most common cause of allergic contact dermatitis resulting from contact with chloroprene rubber (28). This is in concordance with the results of this study, where DETU was the only thiourea compound detected in the products.

In the present study, EITC was shown to be continuously produced from DETU in the investigated materials when they were heated to 37°C. There was no clear correlation between the emission rate of EITC and the concentration of DETU or the material thickness. Most likely, different emission rates are also attributable to different material-specific properties, such as diffusivity and density. The results indicate that remaining DETU in the products can act as a reservoir and a continuous exposure source of EITC for the wearer of chloroprene rubber products. The rate of emission into air was shown to be low. However, it is noteworthy that the products investigated are intended for prolonged use in contact with the skin. The emission rate may also be increased during skin contact. There are indications that isothiocyanates can cause severe allergic contact dermatitis through accumulation in occluding rubber materials, which could increase skin penetration of the isothiocyanate (27). Thiourea compounds are also added to function as antioxidants in the rubber material, preventing oxidation and aging of the product. Our present results have shown that isothiocyanates can be formed in this process, and that the use of excess thiourea as an antioxidant could present a risk of sensitization to the degradation products formed.

In the sensitization experiments, all investigated compounds were extreme or strong sensitizers. BIC gave an ~10-fold lower EC3 value than the corresponding isothiocyanate, confirming previous findings that isocyanates are highly sensitizing. However, the opposite was observed for EITC and EIC, as EITC was found to be the stronger sensitizer, with an EC3 value within the range of the studied isocyanates. EITC was also the main isothiocyanate detected in the chloroprene samples.

As EITC has been shown to be continuously produced in the rubber material by degradation of DETU, the total exposure dose is difficult to predict. Although the detected emission levels of EITC were low in the samples, continuous exposure to an extreme sensitizer could cause sensitization. Some of the chloroprene products investigated are used often and worn for several hours. In a previous case study, concomitant reactions to butylethylthiourea and DETU were observed. These compounds are considered to be structurally dissimilar, however, EITC can be formed by the degradation of both compounds. Previous studies of the sensitization potential of thiourea compounds have shown that they generally show low skin penetration (13, 14). In sensitization experiments in which DETU was administered topically, a negative result was obtained; however, when it was administered intradermally, the result was positive (13). These results could explain the low or moderate sensitization potential of thiourea compounds; however they do not explain the strong

eczematous reactions observed in case reports of allergic contact dermatitis caused by chloroprene products. This indicates that EITC could be the culprit behind allergic contact dermatitis caused by DETU-containing chloroprene rubber.

In estimating the risk for development of contact allergy, it is important not only to measure the exposure to a certain compound but also to consider bioactivation and other possible reactions of that compound in the skin. Isocyanates could be formed metabolically from the corresponding isothiocyanates, as has been shown with PITC and PIC (15). This is an objective for further studies. Although isocyanates are extremely reactive, their role in contact allergy to chloroprene rubber is unclear. Owing to their higher stability and their occurrence in products containing thioureas, isothiocyanates may be more important as haptens. An investigation of the pattern of reactivity to degradation products and identified metabolites among thiourea-allergic patients is an objective for further studies.

Conclusions

Altogether, our findings support previous investigations of the possible reasons behind skin reactions to thiourea compounds. More specifically, we have shown that DETU is a common prehapten in chloroprene materials, easily forming the extreme sensitizer EITC at body temperature. Also, it is possible that activation to EITC from DETU could occur by spontaneous thermal degradation in the skin. Such a pathway has, to our knowledge, previously not been considered for activation of prehapten.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. LC/ESI-MS-SIM chromatograms, monitoring $[M + H]^+$ of DETU and DETU- d_5 , respectively, of a chloroprene sample extract (diving suit D2). The sample was separated on both a C_{18} column (A and B) and an NH_2 column (C and D).

Figure S2. MS/MS product ion spectra of DETU detected in chloroprene (diving suit D2) (A) and DETU standard solution (B).

Figure S3. Levels of DETU in the studied chloroprene products expressed as µg/g.

Figure S4. GC/EI-MS full-scan chromatograms, showing a peak corresponding to EITC after SPME of chloroprene (diving suit D2). The ions at m/z 59, 72 and 87 were extracted for EITC (A), and those at m/z 60, 74 and 82 for EITC- d_5 (B).

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Fig. S1. LC/ESI-MS-SIM chromatograms, monitoring $[M+H]^+$ of DETU and DETU-d₅, respectively, of a chloroprene sample extract (diving suit D2). The sample was separated on both a C₁₈ column (A and B) and an NH₂ column (C and D).

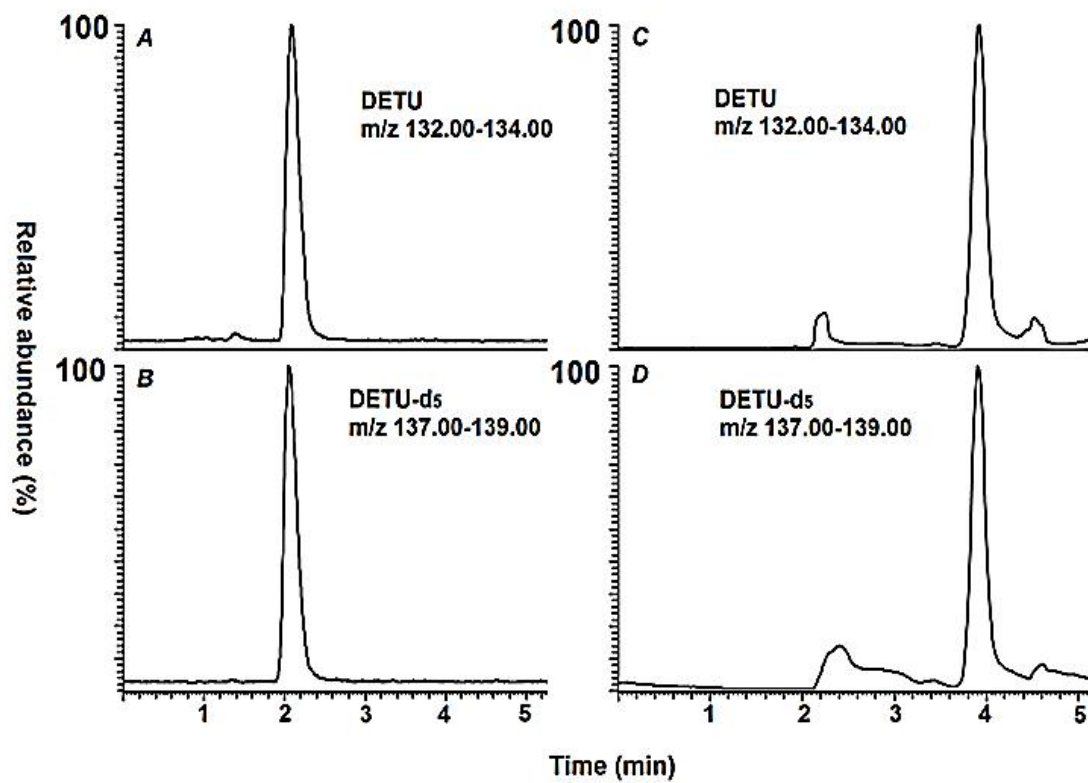


Fig. S2. MS/MS product ion spectra of DETU detected in chloroprene (diving suit D2) (A) and DETU standard solution (B)

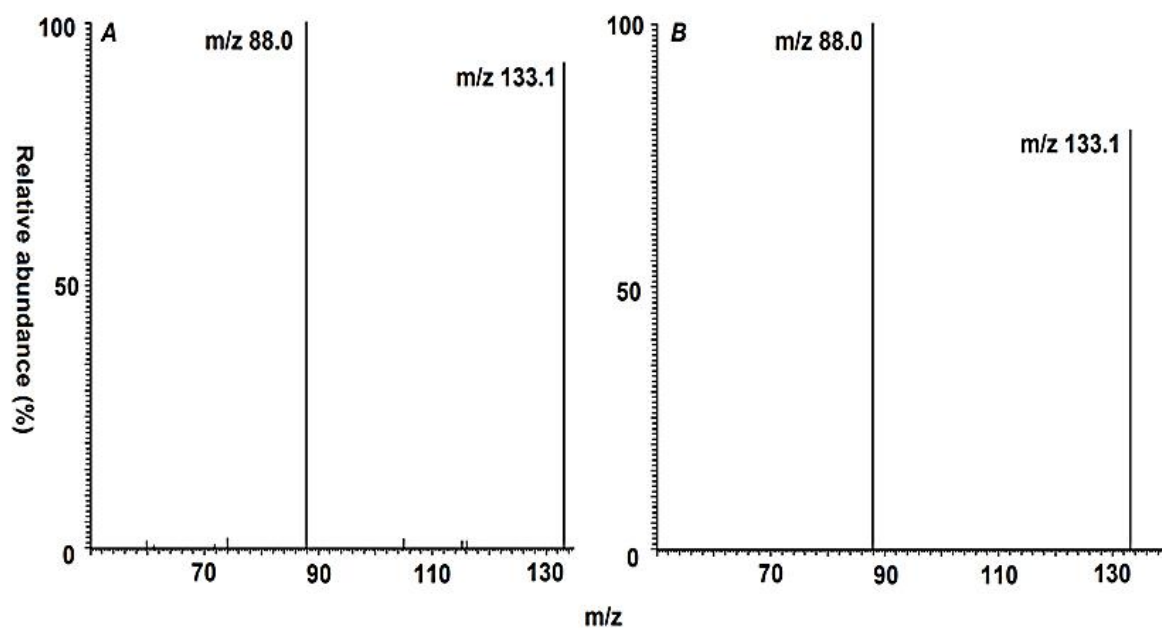


Fig. S3. Levels of DETU in the studied chloroprene products expressed as $\mu\text{g g}^{-1}$.

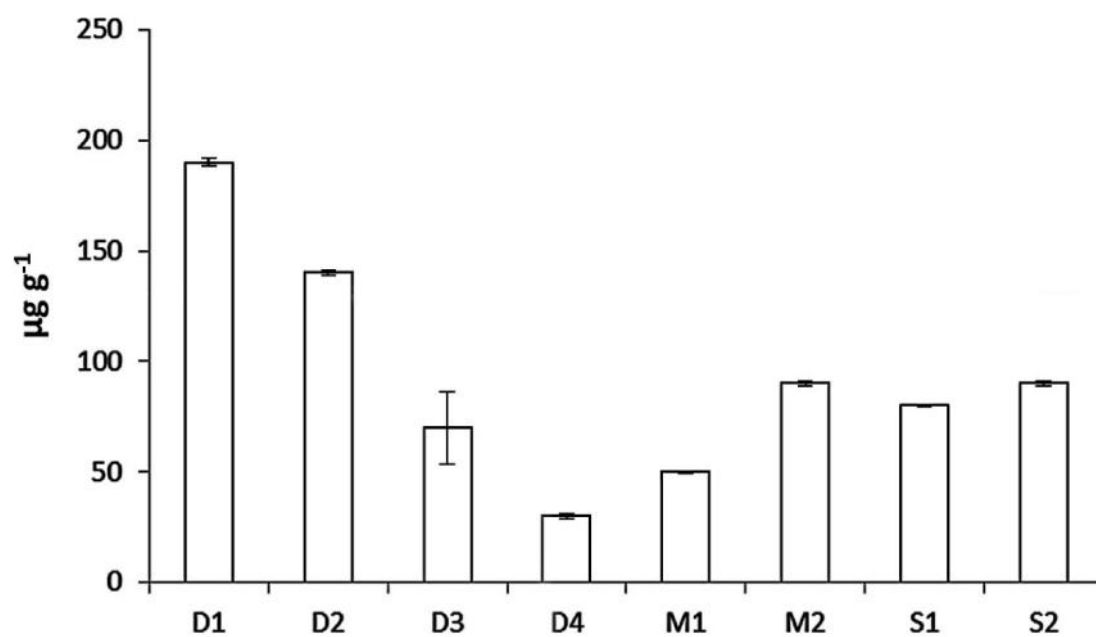


Fig. S4. GC/EI-MS fullscan chromatograms, showing a peak corresponding to EITC after SPME of chloroprene (diving suit D2). The ions at m/z 59, 72 and 87 were extracted for EITC (A), and m/z 60, 74 and 82 for EITC- d_5 (B).

