Occupational allergic contact dermatitis caused by sterile non-latex protective gloves: clinical investigation and chemical analyses

Ann Pontén1, Nils Hamnerius1, Magnus Bruze1, Christer Hansson2, Christina Persson2, Cecilia Svedman1, Kirsten Thörneby Andersson3 and Ola Bergendorff2

1Department of Occupational and Environmental Dermatology, Lund University, Skåne University Hospital, SE-205 02 Malmö, Sweden, 2Department of Dermatology, Skåne University Hospital, SE-221 85 Lund, Sweden, and 3Department of Dermatology, Central Hospital, SE-291 85 Kristianstad, Sweden

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Summary

Background. An increased frequency of occupational contact hand dermatitis among surgical operating theatre personnel has been noticed.

Objectives. To evaluate patients with occupational contact dermatitis caused by their rubber gloves, and to describe a method for analysing the content of the allergens in the gloves.

Materials and methods. Patch tests were performed with the baseline series, a rubber chemical series, and the patients’ own gloves. A method for analysing 1,3-diphenylguanidine (DPG) and cetylpyridinium chloride in the gloves was developed.

Results. Contact allergy to thiuram mix was found in 8 of 16 patients, whereas 12 of 16 patients reacted to DPG. In 7 of 8 patients, contact allergy to cetylpyridinium chloride was found. In the patients’ gloves, cetylpyridinium chloride and DPG were detected at higher concentrations on the inside of the gloves than on the outside. Most patients had worked for decades in their present occupations, but their hand dermatitis had only been present for months.

Conclusion. Contact allergy to DPG in gloves has been disputed, but, in this study, we were able to confirm the presence of DPG and cetylpyridinium chloride in the causative gloves by using a modified method for the analysis. The presence of these chemicals in gloves caused an increase in occupational contact dermatitis in surgical operating theatre personnel.

Key words: allergic contact dermatitis; biocides; cetylpyridinium chloride; diphenylguanidine; occupational; protective gloves; rubber chemicals.

When investigating a patient for occupational contact dermatitis, it is necessary to patch test with the baseline series of contact allergens, occupation-specific series and allergens, and relevant materials from the patient. The relevance of patch test reactions found can be difficult to establish, as analysis of the possible offending components of the patient’s own material may be complicated, and the development of appropriate analytical methods is therefore essential.

For several years, the chemistry of rubber additives has been studied and chemical analyses of rubber items have been performed as part of our investigations of rubber-allergic patients (1–3). Recently, clinical experience in hospitals in our region in Sweden, as well as elsewhere, has suggested that occupational contact dermatitis caused by synthetic rubber gloves and rubber chemicals has
increased among surgical operating theatre personnel, that is, surgeons and surgical nurses. For healthcare personnel in general, hand hygiene and the wearing of appropriate protective gloves are mandatory. In a Danish study, the 1-year prevalence of self-reported hand eczema in an unselected hospital population was 23%, varying between 8% and 32% for different occupational groups. Assistant nurses reported the highest frequency. With respect to departments, the highest frequencies were reported in medical and surgical wards (4). The available qualities and brands of protective gloves used at the hospital departments are dependent on large-scale purchases decided by central purchasing departments, and change over time, owing to costs and occupational and environmental requirements.

It has been judged necessary to replace natural rubber latex (NRL) gloves with synthetic rubber gloves in healthcare, owing to the risk of type I allergy caused by latex proteins. The use of powder in gloves has been replaced by other techniques for facilitating donning (5). In the baseline series, thiuram mix is considered to be an important marker of contact allergy to accelerators in rubber gloves (6). In addition to rubber chemicals, patients do react to other constituents of the rubber gloves, such as cetylpyridinium chloride (Fig. 1). Cetylpyridinium chloride has antibacterial properties and might act both as an irritant and an allergen (7, 8), and may be added as a lubricant for powder-free gloves. In recent years, there have been several changes with respect to the composition of the materials used for protective gloves, which have to be worn on a daily basis in healthcare work.

In this study, contact allergies to chemicals present in rubber gloves used by surgeons and surgical nurses with hand eczema caused by their rubber gloves are described. Different inner coatings are used to facilitate donning and as preservatives. Therefore, it is important to develop methods to evaluate the actual skin exposure from these substances. Previously, we have analysed extracts from disintegrated pieces of rubber with respect to the presence of residues of rubber chemicals such as vulcanizers and antioxidants. Now, we present methods for the detection of 1,3-diphenylguanidine (DPG) (Fig. 1), a chemical present in some rubber gloves, and of cetylpyridinium chloride in rubber gloves. Furthermore, the insides and the outsides of the gloves were analysed separately; thereby, the inner surface of the glove, which is in immediate contact with the skin, could be analysed.

**Materials and Methods**

**Patients and patch testing**

The patients were investigated according to the procedures for establishing occupational contact dermatitis at the Department of Occupational and Environmental Dermatology in Malmö and at the Department of Dermatology in Kristianstad. They were patch tested with a baseline series and, according to the individual history, appropriate additional series, as well as their own material, including their own rubber gloves. The rubber chemical test preparations present in the baseline series in Malmö and Kristianstad are thiuram mix 1.0% pet. (containing tetramethylthiuran monosulphide 0.25%, tetramethylthiuran disulphide 0.25%, tetraethylthiuran disulphide 0.25%, and dipentamethylenethiuran disulphide 0.25%), black rubber mix 0.6 pet. (containing N-isopropyl-N-phenyl-4-phenylenediamine 0.1%, N-cyclohexyl-N-phenyl-4-phenylenediamine 0.25%, and N,N′-diphenyl-p-phenylenediamine 0.25%), and mercapto mix 2.0% [containing N-cyclohexyl-2-benzothiazylsulphenamide 0.5%, 2-mercaptobenzothiazole 0.5%, dibenzothiazyl disulphide 0.5%, and 2-(4-morpholinylmercapto) benzothiazole 0.5%]; in Malmö, mercaptobenzothiazole 2.0% pet. is also part of the baseline series. DPG is not present in the baseline series, but is included in the rubber chemical series used. All of these patch test preparations were obtained from Chemotechnique Diagnostics AB (Vellinge, Sweden). Finn Chambers® with a diameter of 8 mm (Epitest OY, Tuusula, Finland) on Scanpor® tape (Norgeplaster AS, Vennesla, Norway) were used to apply the allergens to the upper back. Cetylpyridinium chloride was obtained from Sigma-Aldrich (Steinheim, Germany), and was patch tested at 0.1% (wt/wt) in water (8). In Malmö, cetylpyridinium chloride was not available for testing until February 2010, whereas all reported patients from Kristianstad were tested with cetylpyridinium chloride. Besides testing all of the patients’ gloves ‘as is’, in Malmö all patients except for 1 were patch tested with ultrasound.

![Fig. 1. Chemical structures of 1,3-diphenylguanidine (DPG) and cetylpyridinium chloride (CPC).](image-url)
extracts of the gloves made according to a standardized procedure (9). For patch testing, 20 mg of pet. test preparations and 15 μl of test solutions were used (10, 11). This corresponds to concentrations per area of 30 μg/cm² for cetylpyridinium chloride and 400 μg/cm² for DPG. In all patients, the tests were applied to the back, left on for 48 hr, and read on D3 or D4 and D7. The patch test results were scored according to International Contact Dermatitis Research Group guidelines (12). Patch testing with cetylpyridinium chloride 0.1% (wt/wt) was also carried out in 20 controls.

Chemical analysis

Chemicals. Cetylpyridinium chloride (99%) and DPG (97%) were obtained from Sigma-Aldrich. Acetone of analytical grade and methyl-tert-butyl ether, acetonitrile and methanol of high-performance liquid chromatography (HPLC) grade were obtained from Labscan Ltd (Dublin, Ireland). Ethanol (95%) was obtained from Kemetyl AB (Haninge, Sweden).

High performance liquid chromatography. HPLC analyses of thiurams, dithiocarbamates and mercaptobenzothiazole derivatives were performed according to a previously described method (2). Analyses of DPG and cetylpyridinium chloride were performed by HPLC on a cyano column (Genesis CN, 4 μm, 150 × 4.6 mm; Grace, Deerfield, IL, USA), eluted with a linear gradient consisting of solvent A [20% (vol/vol) acetonitrile, 10% (vol/vol) methanol, and 70% (vol/vol) sodium acetate buffer (50 × 10⁻³ mol/l, pH 5.0)] to solvent B [60% (vol/vol) acetonitrile, 30% (vol/vol) methanol, and 10% (vol/vol) sodium acetate buffer (50 × 10⁻³ mol/l, pH 5.0)] for 5 min. The eluent was pumped with a Waters 600 pump (Waters Chromatography Division, Milford, MA, USA) at a flow rate of 1.5 ml/min, and monitored at 254 nm with a 1100 Series diode-array detector (Hewlett-Packard Co., Palo Alto, CA, USA). Peak area was used to determine the concentration, and the identifications were made by comparison of retention times and ultraviolet (UV) spectra recorded by the diode-array detector.

Analysis of the gloves

Different extraction solvents were compared by using acetone, methyl-tert-butyl ether, ethanol (95%), water, or solvent A (used for HPLC). Analysis of the inside of the glove was performed according to the following procedure. A finger was cut from the glove, and extraction was carried out by pouring 2 ml of a solvent inside, sealing the open end with a bag clip, and agitating with a rocking motion for 10 min at room temperature. Another finger from the same glove turned inside out was used for analysis of the outside. The extracts were filtered before injection onto the HPLC column. When extracts were made with organic solvents, they were evaporated and dissolved in solvent A before filtering and injection. Integrated peak areas in the chromatograms were compared with analyses of reference compounds for quantification. Duplicate analyses were performed with two different fingers from each glove. The concentrations of cetylpyridinium chloride and DPG were determined as μg/cm² after measuring the area of the extracted finger with a ruler. Concentrations of individual mercaptobenzothiazoles, thiurams and zinc dithiocarbamates were determined according to a method described previously (2), whereby the gloves are cut into smaller pieces before extraction and the components are therefore determined relative to the weight of the glove (mg/g).

Method validation

Linearity of the standard curve was determined in the 10–200 μg/ml range, with triplicate standard preparations of DPG and cetylpyridinium chloride at five concentration levels for each substance. The precision of the standard solution was determined after analysis of five standard solutions of DPG and cetylpyridinium chloride at 100 μg/ml.

The extraction recovery was determined by repeating the extraction described above several times with fresh solvent. The solvent was pipetted off and analysed, another portion of solvent was added, and extraction of the same sample was performed for another 10 min. After four extraction cycles, almost no DPG or cetylpyridinium chloride was left in the sample, and by dividing the amount of analyte from the first extraction by the total amount, a yield could be calculated.

Results

Patients

A total of 16 patients are reported (5 patients in Kristianstad and 11 patients in Malmö). Among these, 4 of 16 had a history of atopic dermatitis. The majority of the investigated patients had worked for decades in their present occupations, whereas their hand dermatitis had only lasted for months.

The contact allergies to cetylpyridinium chloride, rubber chemicals and test preparations of their rubber gloves are shown in Table 1. All were tested with thiuram mix and DPG, but only 8 were tested with cetylpyridinium
chloride. Twelve of the patients reacted to DPG, 8 patients had contact allergy to thiuram mix, and 2 patients had contact allergy to zinc diethyl dithiocarbamate. Of those reacting to thiuram mix, 6 were positive to tetraethylthiuram disulfide, and 1 was negative when tested with the individual thiurams, in spite of a strong reaction to thiuram mix, whereas 1 had no reaction to the rubber chemicals tested.

Seven of 16 patients reacted to DPG but not to thiuram mix, whereas 3 of 16 reacted to thiuram mix without reacting to DPG, and 5 of 16 reacted to both of these patch test preparations. All reactions to DPG fulfilled the criteria for an allergic reaction, and were strong positive to extreme positive reactions (+++ to ++++). All patients with contact allergy to DPG wore sterile synthetic polyisoprene rubber gloves at work. Ten patients among the 15 patients who were patch tested with their own glove 'as is' reacted positively to the glove. Among the 9 patients tested with extracts of their own gloves, 8 reacted positively. Seven of 8 tested patients had positive test reactions to cetylpyridinium chloride, all of them showing a strong positive to extreme positive reaction (+++ to ++++), fulfilling the criteria for an allergic reaction, whereas 20 controls tested with cetylpyridinium chloride were negative ($p < 0.001$, Fisher’s exact test). Six of these patients showed concomitant contact allergy to DPG, and 2 also had a reaction to thiuram mix, whereas 1 had no reaction to the rubber chemicals tested.

### Analysis of glove extracts

**Analysis of glove extracts.** No thiurams were detected in any of the gloves. The results of the chemical analysis of the other rubber chemicals and cetylpyridinium chloride are shown in Table 2. The concentrations of DPG and cetylpyridinium chloride were determined by extraction of the inside and outside of the gloves separately, and are therefore presented relative to area ($\mu g/cm^2$) of the gloves in Table 2. For two of the gloves (Esteem® SMT and Esteem® Micro), the concentrations of DPG per area on the inside were 10-fold the concentrations on the outside. The concentrations per area of cetylpyridinium chloride were generally higher on the inside than on the outside. The chromatogram from analysis of a glove extract is presented in Fig. 2.

**Recovery.** Approximately 65% cetylpyridinium chloride and 79% DPG were recovered with the described procedure, with the use of solvent A. The performance of...
Table 2. Rubber chemicals in five different polyisoprene gloves

<table>
<thead>
<tr>
<th>Glove brand</th>
<th>DPG (μg/cm²)</th>
<th>CPC (μg/cm²)</th>
<th>MBT (mg/g)</th>
<th>MBTS (mg/g)</th>
<th>ZDEC (mg/g)</th>
<th>ZPD (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Profeel® DHD polysoprene powder-free</td>
<td>&lt;0.04 &lt;0.04</td>
<td>2.6 &lt;0.8</td>
<td>0.04</td>
<td>&lt;0.002</td>
<td>1.3</td>
<td>5.3</td>
</tr>
<tr>
<td>Profeel® DHD polysoprene powder-free Blue</td>
<td>&lt;0.04 &lt;0.04</td>
<td>6.9 &lt;0.8</td>
<td>0.03</td>
<td>&lt;0.002</td>
<td>0.6</td>
<td>5.5</td>
</tr>
<tr>
<td>Esteem® SMT</td>
<td>23.9</td>
<td>2.4</td>
<td>27</td>
<td>3.8</td>
<td>0.07</td>
<td>&lt;0.002</td>
</tr>
<tr>
<td>Esteem® Blue NT</td>
<td>4.6</td>
<td>7.1</td>
<td>13.8</td>
<td>9.1</td>
<td>0.1</td>
<td>0.05</td>
</tr>
<tr>
<td>Esteem® Micro</td>
<td>26.7</td>
<td>7.1</td>
<td>24</td>
<td>7.3</td>
<td>&lt;0.002</td>
<td>0.02</td>
</tr>
</tbody>
</table>

CPC, cetylpyridinium chloride; DPG, 1,3-diphenylguanidine; MBT, mercaptobenzothiazole; MBTS, dibenzothiazyl disulphide; ZDEC, zinc diethylthiocarbamate; ZPD, zinc pentamethylene dithiocarbamate.

Fig. 2. Example of a high-performance liquid chromatography chromatogram of a glove extract monitored at 254 nm, showing peaks corresponding to 1,3-diphenylguanidine (DPG) and cetylpyridinium chloride (CPC). mili absorbance units (mAU).

Different extraction solvents is shown in Fig. 3. Extraction with water, ethanol or solvent A proved to be approximately twice as efficient as extraction with the aprotic solvents acetone and methyl-tert-butyl ether. In all of the analyses of the patients’ gloves, solvent A was used.

Linearity. Triplicate injections of a standard preparation of DPG and cetylpyridinium chloride at five different levels gave a linear calibration curve in the range 10–200 μg/ml. The correlation coefficient for DPG was determined by least-square analysis to be 0.998, and the precision was determined to range between 0.8% and 3.2%. The correlation coefficient for cetylpyridinium chloride was determined to be 0.982, and the precision was determined to range between 5.6% and 12.4%.

Limit of detection. The lowest concentrations of DPG and cetylpyridinium chloride in a reference sample where a signal/noise ratio was at least 10:1 and a satisfactory UV spectrum could be achieved for identification were 0.5 and 10 μg/ml, respectively. These concentrations in the extract corresponded to concentrations of 2 μg/g DPG and 40 μg/g cetylpyridinium chloride, respectively, in the glove. We found that the weight per area of the gloves varied between 20 and 25 μg/cm². When the limit of detection is converted into area units, these are approximately 0.04 μg/cm² DPG and 0.8 μg/cm² cetylpyridinium chloride.

Precision of the standard. Five injections of standard solutions of DPG at 100 μg/ml resulted in a precision expressed as a relative standard deviation of 9.6%. The corresponding value for cetylpyridinium chloride was determined to be 17.3%.

Discussion
It has been suggested that the most of the positive reactions to DPG are probably false-positive, irritant reactions (13).
However, our study indicates that DPG is a relevant contact allergen in sterile protective polyisoprene gloves. Among the reported patients, we found a high number of positive test reactions to DPG and to cetylpyridinium chloride. The relevance of the contact allergies found is confirmed by demonstration of the substances in the suspected gloves, and by the positive test results obtained when testing with the gloves. The patients were tested with the gloves suspected and brought to the department by the patient. Several types of gloves were used by the patients, but Esteem® SMT was suspected and tested in a majority of the patients with contact allergy to DPG.

For the correct diagnosis of occupational contact dermatitis, it is necessary to evaluate the exposure to the relevant allergens. Therefore, optimized patch testing, including the patients’ own products, and chemical analysis of the offending products is necessary. This is of special importance regarding products that are not labelled. The chemical substances used in the production process often react to create other, potentially allergenic, substances. Furthermore, the combinations of substances in the products can interact to enhance the risk of sensitization.

In a recent study, 5 patients had contact allergy to DPG, but, upon analysis of six representative rubber gloves, DPG was not found (14). As previously mentioned, our aim with the chemical investigation was to analyse the contents of cetylpyridinium chloride and DPG on the surface of the gloves (Table 2). As cetylpyridinium chloride is a cationic surfactant, the usual reversed-phase chromatography on a C-18 column turned out to be unsuccessful, and a cyano stationary phase was used instead. This approach was successful, and made it possible to detect both compounds in the same run. A cetylpyridinium chloride-containing coating is applied to the inside of the glove to facilitate donning. This explains why higher amounts of this chemical were found on the inside than on the outside. However, DPG is a rubber chemical that is added to the rubber at an early stage in the process, and is therefore expected to be distributed evenly in the glove. A possible explanation for the high amounts of DPG on the inside of the Esteem® SMT and Esteem® Micro gloves could be an extraction effect of the cetylpyridinium chloride-containing lubricant. Our results show that ethanol is superior to acetone for extracting DPG (Fig. 3), and thus confirms a previous report indicating that ethanol extraction is better for the detection of contact allergy to DPG-containing rubber material (15). The amounts of cetylpyridinium chloride and DPG found in these two brands of gloves were higher than in the other gloves analysed, and it should be noted that the concentration per area of cetylpyridinium chloride found on the inside of the offending gloves was the same as the concentration per area used for patch testing (Table 2). Regarding DPG, the concentration per area used for patch testing is ∼15 times higher than the concentration found on the inside of these gloves.

As none of the controls tested reacted to cetylpyridinium chloride, an irritant reaction is unlikely with the test concentration used. We recommend patch testing with fully dissolved cetylpyridinium chloride solution at room temperature, as we noticed that, when stored in a refrigerator, cetylpyridinium chloride crystallized in the bottom of the test tube.

In accordance with other studies, our study emphasizes that contact allergy to rubber gloves among healthcare personnel still is an urgent problem (14, 16). Hypothetically, the spectrum of contact allergies might vary with the products provided at the healthcare units. Among patients with hand dermatitis and contact allergy to thiuram mix in the baseline series, healthcare workers account for a significant proportion (6, 17, 18). From the middle of the 1980s to the middle of the 1990s, an increase in the number of contact allergies to thiuram mix has been recorded (19). The change was assumed to be associated with the more widespread use of protective gloves (6), and especially non-NRL gloves, among healthcare personnel. However, a decrease in the incidence of contact allergy to thiuram mix since the beginning of 1990s has been reported by several authors (6, 16, 19, 20). In our patch test database, there is no obvious change over the last 13 years with respect to the proportion of thiuram contact allergy among patients investigated because of suspected rubber contact allergy at the department in Malmö (Fig. 4), and nor is there any diminishing frequency of thiuram contact allergy (data not shown) among the patients tested with the baseline series. However, we noticed an increase in contact allergy to DPG between 2008 and 2010 as compared with the preceding years in patients investigated because of suspected rubber contact allergy (p = 0.004, chi-square test) (Fig. 4). Previously, we found a very low number of contact allergies to sterile rubber gloves, whereas during recent years we have noticed a substantial increase. This tendency is likely to be explained by the increased number of contact allergies to DPG.

Contact allergies to thiuram mix and carba mix have been reported to be more common in healthcare workers than in non-healthcare workers; however, they are also common in the food processing and rubber industries (14, 16, 21, 22). In many dermatology departments, DPG is patch tested as part of the carba mix, which additionally contains zinc diethyldithiocarbamate and zinc dibutyldithiocarbamate. The latter two are not chemically...
related to DPG. Unfortunately, when the frequencies of contact allergy to carba mix are reported, the ingredients of this mix are often not tested separately, and the relative importance of the carbamates and DPG is therefore not clarified. Previously, it has even been suggested that carba mix is redundant (23). False-negative results for contact allergies to thiuram mix and carba mix in the TRUE Test™ have also been reported (24).

DPG is typically used as a vulcanizer in combination with mercaptobenzothiazole. This relationship is also seen in the analyses shown in this study (Table 2). Besides DPG and mercaptobenzothiazole, zinc dithiocarbamates were detected in two gloves. However, thiurams were not seen in any of the gloves. This is in accordance with observations published previously (2, 25). Nevertheless, thiuram mix seems to be a good marker for detecting contact allergy to the dithiocarbamate group of rubber chemicals.

It is important to note that most of the operating personnel had worked for decades without any hand dermatitis, even though they had been exposed to rubber gloves for many years. The general time course of sensitization when individuals are exposed to rubber chemicals is unknown. Possibly, it is part of a general pattern that contact allergy from rubber chemicals most often occurs after many years of exposure. However, a recent report on healthcare workers indicated a temporal association of the start of hand dermatitis with a switch to latex-safe gloves (14). The comparatively large number of patients with occupational contact allergy to latex-free rubber gloves found recently in our study also suggests that the composition of the gloves and/or some other additive or synergistic factors are responsible, as discussed above. The results might even suggest that one or a few products that were, and still are, widely used caused this outbreak of occupational contact dermatitis. Whether the composition of the gloves varies in some respect between different batches is also not known.

In conclusion, this study reports on allergic contact dermatitis caused by the rubber accelerator DPG and the lubricant cetylpyridinium chloride in latex-free gloves used by healthcare workers. The relevance of contact allergy to DPG has been disputed, but in this study we were able to confirm the presence of DPG and cetylpyridinium chloride in the suspected gloves by using a modified method for the analysis. The number of cases with allergic contact dermatitis caused by sterile rubber gloves diagnosed during the last 4 years equals the number found during the 10 years preceding this period, suggesting that the change from latex to latex-free gloves has, at least, not diminished the risk of sensitization to rubber glove accelerators.
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