STUDY

Allergen Content of Patient Problem and Nonproblem Gloves: Relationship to Allergen-Specific Patch-Test Findings

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Background: **Identification of putative contact allergen and source material is often done by a combination of patch testing and manufacturer-supplied product information. The accuracy of the identification of allergen-source material and level of allergen in that allergen-source material is not known.**

Objective: **The objectives of the study were to survey the chemical allergen content of glove allergic contact dermatitis (ACD) patient-identified problem and nonproblem gloves and to evaluate the ability of the patient to discriminate between problem and nonproblem gloves.**

Methods: **Gloves from patch-tested rubber allergen–positive ACD patients were analyzed for species and amount of rubber allergen.**

Results: **Approximately half the subjects were able to correctly identify their problem and nonproblem gloves. Correct association of a glove with ACD was directly related to patch-test reaction severity and inversely related to the number of glove brands being used by the patient. Of note, thiurams were not detected in any of the gloves examined.**

Conclusions: **Although patch testing is invaluable in identifying individual allergen sensitivities, the identification of the ACD-causative specific chemical allergen and source material remains problematic. All glove brands used within days prior to and during an ACD episode should be considered potential sources of the contact allergen.**

ADVERSE REACTIONS to rubber gloves worn for personal protection are a common problem. The three types of adverse reactions that can occur with glove use are irritation, type IV hypersensitivity (allergic contact dermatitis [ACD]), and immediate type I hypersensitivity reactions (rhinitis, asthma, contact urticaria). The most serious of these reactions to gloves, immediate type I reactions, are unique to natural rubber latex gloves and are caused by residual proteins found in the natural rubber. Type I reactions are in decline owing to (1) recent manufacturing control measures that have resulted in a dramatic reduction of residual protein in the gloves and (2) the use of nonpowdered gloves.[1] Rubber chemical additives can cause type I reactions, but these are rare. ACD (type IV reaction) is caused by chemical accelerators (such as carbamates, thiurams, and mercaptobenzothiazoles) that are present both in latex gloves and in most synthetic rubber gloves. These chemical allergens are present in other rubber and synthetic rubber products as well. Measures taken to reduce protein levels in gloves do not necessarily reduce chemical accelerator levels.

Rubber glove allergies are commonly identified in workers who use rubber gloves; these include health care workers,[2,3] food processing workers,[4] and construction workers.[5] The prevalence of reactivity to specific etiologic agents of ACD in the worker population is difficult to assess. Thiurams are consistently identified as the most common cause of rubber ACD in these populations but are no longer identified in surveys of accelerator content in surgical and examination gloves.[6,7] Thiocarbamates and mercaptobenzothiazoles are also commonly noted sensitizing agents, found by patch testing.[2,8,9] In addition, alkyl thioureas, diphenylguanidine, and dianilines may also be used in some rubber products.[10]

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ACDS

Exposure to these or closely related chemicals may occur in ways other than by the wearing of gloves. For example, exposure to rubber accelerators can occur through contact with other rubber items, such as condoms or condom catheters,[11,12] footwear,[13] earplugs,[14] surgical drains,[15] and sports equipment.[8] Thiocarbamate pesticides are another potential source of exposure.[16] The initial sensitizing contact allergen exposure is termed "induction," and subsequent exposure that produces ACD is termed "elicitation." Thus, sensitization may occur through glove usage or via an alternative exposure. In general, the dose of a chemical required to elicit ACD is much less than that required to produce sensitization. Prevention of the elicitation of ACD would therefore also protect workers from becoming sensitized to accelerators during glove use.

ACD manifests during the elicitation phase following exposure and generally peaks 48 to 72 hours after exposure. The induction and elicitation of ACD are influenced by the type and concentration of chemical allergen in the glove, by contact time and number of gloves used, by skin condition, and (possibly) by coexposure to other chemicals. Glove use (exposure) can vary drastically among health care professionals. Surgeons may use fewer gloves but have longer contact times during extended surgical procedures. In contrast, phlebotomists often use two boxes of examination gloves per day, but exposure to each individual glove lasts only a few minutes. Rubber gloves are also used in many nonmedical fields (such as construction, food service, and hairdressing) and by homemakers. In some situations, the worker may wear the glove for extended periods and may come into contact with a variety of solvents (eg, paint thinners).

Clinically, the etiologic agent (allergen) responsible for a patient's glove-induced ACD is determined by epicutaneous patch tests. Although the tests are generally accurate, false-negative patch-test findings may occur because exposure to the back during a patch test (48 hours of exposure on an 8 × 8 mm spot) may be less capable of producing a response than the patient's wearing of gloves for prolonged durations. ACD sensitivity differs between the hand and the back or other anatomic areas. Frequent hand washing may disrupt the stratum corneum barrier. Gloves may be worn during cleaning and disinfection procedures where additional chemicals may potentially alter the form of the accelerator or change its leach rate. Emmett and colleagues reported that, although 2-mercaptobenzothiazole (MBT) was a more potent sensitizer, its oxidation product, 2,2-dithio(bis)benzothiazole (MBTS), had a greater rate of leach from latex.[17] Many gloves contain multiple accelerators or chemicals that may interact in either a chemical way or a physiologic or pathologic way to alter the apparent effective dose. Holness and Mace reported that 11% of health care workers presenting with skin care problems had both ACD from thiuram and contact urticaria from latex.[18] Permeation of nonglove-related chemicals through the glove onto the skin may potentially produce ACD, making identification of the specific etiologic agent difficult.

The purpose of our study was to (1) survey the chemical allergen content (chemical species and concentration) of ACD problem and nonproblem gloves identified by allergen patch test–positive patients presenting to the dermatology clinic with glove dermatitis and (2) evaluate the ability of the patient (using numerous gloves) to discriminate between problem and nonproblem gloves.

Methods

Subject Selection

Subjects were selected from patients reporting to participating clinics of members of the North American Contact Dermatitis Group (NACDG). Criteria for inclusion in the study included (1) a clinical diagnosis of ACD from latex or synthetic rubber gloves; (2) a positive patch-test reaction to one or more latex accelerators or to the glove material; (3) a patch test with the NACDG standard series of 65 known allergens; and (4) the patients' ability to provide samples of the gloves they thought caused their ACD (and the gloves they believe did not cause ACD, if any). The gloves patients identified as causing their ACD are herein referred to as "problem gloves," and those they believe did not elicit their ACD are referred to as "nonproblem gloves." In addition, at the discretion of the investigator, certain patients could be enrolled if they did not meet the criteria noted above. These could include patients with negative patch-test results but strong suspicion of glove allergy and subjects with a positive patch-test reaction to one or more gloves but negative patch-test results for latex contact allergens. Patient history that included occupational history and glove use (the duration and number of glove brands then being used by the patient) was recorded.

Exclusion criteria included the use of medications that interfere with the interpretation of the patch tests and the presence of any other dermatologic conditions that might prevent adequate evaluation of hand dermatitis due to gloves. Individuals with a history of type I (immunoglobulin E–mediated) latex allergy were not excluded but were not

exposed to any glove material containing natural rubber latex during the course of the study.

Glove Analyses

A 55 mm^2 rectangular piece was cut from each glove with a standard form and weighed to obtain its density. The chemical accelerator or allergen content of each glove was determined by a modification of the method of Depree and colleagues.[6] In short, material from each glove was cut into small pieces and was extracted in acetonitrile (1 g per 10 mL) for 2 hours at room temperature. Extracts were centrifuged at 1,000 g for 10 minutes at room temperature to remove residual material or powder. Extracts were initially screened in the presence of cobalt for the presence of carbamates, thiurams, and MBT at ultraviolet wavelengths equaling 320 nm and 370 nm (American Society for Testing and Materials D7558).

Chemical speciation and quantifications were performed with a high-performance liquid chromatograph coupled to a photodiode array detector (Shimadzu Instruments Inc., Columbia, MD). Chemical separation was performed with a 250 × 4.6 mm Supelco Discovery C18 column (Supelco Inc., Bellefonte, PA).

Three different gradient elution methods were used. A solvent flow of 1 mL per minute was used for all high-performance liquid chromatography (HPLC) elutions. Zinc dithiocarbamates were eluted from the HPLC column by use of a linear gradient starting with 85% acetonitrile (ACN) in water and progressing to 100% ACN over 3 minutes and then maintained at 100% ACN for 12 minutes. Prior to the injection of samples and standards and intermittently thereafter, injections of zinc dimethyldithiocarbamate (ZDMC) were made to bind nickel from the HPLC stainless steel components. MBT and MBTS were assayed by use of a step gradient starting with 100% water to 50% ACN in water over 5 minutes, progressing to 80% ACN in water over the next 5 minutes and to 100% ACN over the next 9 minutes, and then held at 100% ACN for 2 minutes. All other species were determined by use of a linear gradient from 100% water to 100% ACN over 60 minutes. The following chemicals could be detected by the above method: MBT, MBTS, tetramethylthiuram disulfide, tetraethylthiuram disulfide, ZDMC, zinc diethyldithiocarbamate (ZDEC), zinc dibutyldithiocarbamate (ZDBC), zinc pentamethylenedithiocarbamate, 1-3-diethylguanidine, 1-3-diphenylguanidine, 1-3-diethyl-2-thiourea, 1-3-dibutyl-2-thiourea, N-isopropyl-N′-phenyl-p-phenylenediamine, phenylenediamine, diethylamine, and 4-4-dithiodimorpholine. In addition, both direct-probe and gas chromatographic mass spectrometry were used on select extracts to confirm the chemical species present and to screen for other potential chemical species for which standards were not available.

Results

Subject Demographics

Patients who met the criteria were selected from those reporting to a participating NACDG clinic between August 2006 and September 2009. Eighty-eight gloves from 30 subjects were assessed for their accelerator content. Fourteen of the subjects were male (16 of the 30 subjects were female). Only 12 of the 30 subjects were either presently employed or retired health care workers. Among subjects who were not health care workers, rubber glove exposure resulted from housework, a hobby, or an occupation (machinist, factory worker, food industry worker, etc).

Patch-Test Reactions

Thirty subjects were patch-tested with thiurams, MBT, mercapto mix, thioureas, and carbamates, including ZDEC and ZDBC, except for one subject for whom the carbamate patch test was not available. Patient dermal test reactions were evaluated at 48 hours and again between 2 and 5 days after patch application; 21, 24, 5, and 2 positive patch-test reactions were observed for carbamates, thiurams, MBT, and mercapto mix, respectively, and 1 positive reaction to thioureas was observed. One subject had negative reactions to all the above allergens but had a positive reaction to N-(cyclohexylthio)phthalimide. N-(Cyclohexylthio)phthalimide could not be confirmed in gas chromatographic mass spectrometric analyses of the extract from this subject's glove because an authentic analytic standard was not available for proper analytic assessment; as a result, this subject and glove set were not used for subsequent comparisons of glove content versus ACD patch-test results. Other positive patch-test reactions noted in this population included reactions to diphenylguanidine (in two subjects) and diethylamine.

Women constituted 4 of 6, 7 of 12, and 5 of 12 of the subjects with a maximal skin test reaction graded as extreme (+++), strong (++), and mild (+), respectively. Half the female subjects were retired from or presently working in the health care industry. Female subjects who

did not work in health care included homemakers and blue- and white-collar workers.

Cross-reactivity between carbamates and thiurams has been well documented. Carbamates form thiurams upon oxidation. Three subjects reacted to carbamates and not to thiurams, and four subjects reacted to thiurams and not to carbamates. Twenty-six of the 30 subjects reacted to either carbamates or thiurams (18 of 29 reacted to both). Of the 5 subjects who were patch test positive for MBT, only 2 also reacted positively to mercapto mix. This is of interest as MBT is a component of the mercapto mix and as the other components can be chemically or metabolically reduced to form MBT. Table 1 indicates the number and intensity of patch-test reactions observed in this glove allergens' subject population.

Glove Allergen Content Compared to Patch-Test Results

Subjects submitted one to four "problem gloves" and zero to four "nonproblem gloves." A total of 88 gloves were analyzed for their chemical allergen content. Included were industrial and household gloves, medical examination gloves, and surgical gloves. No identifiable allergens were detected in 29 of the submitted gloves. ZDEC, ZDBC, MBT, ZDEC plus ZDBC, and ZDBC plus MBT were found in 20, 15, 10, 11, and 2 gloves, respectively, and 4-4-dithiodimorpholine was found in one glove. No other rubber allergens of those screened (see "Methods"), including thiurams, were detected in glove extracts. Because thiurams are oxidized carbamates and because significant cross-allergic reactivity exists, positive patch-test reactions to the carbamate and thiuram mixes were combined into one group for comparison purposes. Table 2 lists the lowest rubber allergen levels measured from a problem glove associated with a respective positive patch-test result. Commercially available rubber gloves (both medical and nonmedical; latex, nitrile, and polyisoprene) associated with ACD contained one or two different chemical allergens or accelerators, with combined levels from below the limit of detection to 6,852 μg/g. (The highest per-area value [52,100 $\mu g/cm^2$] was from a thick industrial glove.)

Overall, 51% of the subjects correctly identified all the gloves they provided as problem gloves, based on the presence of the allergen for which they were patch test positive, and 54% provided nonproblem gloves that did not contain the allergen for which they were patch test positive. To better understand the ability of the ACD patient to correctly identify a problem glove, we examined the concordance between an individual's patch-test reaction and the rubber allergen composition of the gloves with respect to the severity of the patch-test reaction (Fig 1) and the number of gloves provided by the subject (Fig 2). Highly sensitized subjects, as noted by an extreme patch-test reaction, correctly identified both problem and nonproblem gloves. Those with either mild or weak patch-test reactions correctly identified problem and nonproblem gloves about 50% of the time (see Fig 1).

The other major factor in the ability of subjects to correctly identify problem and nonproblem gloves was the number of different glove brands they provided (presumably the number of glove types they were using). More than 80% of the subjects who provided a single glove (as a problem or nonproblem glove) correctly identified the glove. This ability diminished in proportion to the number of different brands of gloves provided to the dermatologist (see Fig 2).

Table 1. Glove Allergen Patch-Test Reactions

	*No. of Reactions**		
Allergen	*Mild*	*Strong*	*Extreme*
MBT	1	2	2
Mercapto mix	1	0	1
Carbamates	13	5	3
Thiurams	11	7	6
Mixed dialkyl thioureas	0	0	1

MBT = 2-mercaptobenzothiazole.

*Two strong reactions to diphenyl guanidine, one weak reaction to diethylamine, and one weak reaction to N-(cyclohexylthio)phthalimide were observed in individual subjects. Only subjects with suspected allergies to the additional agents were tested with these allergens. One subject with a strong reaction to thiurams was not tested with carbamates.

Table 2. Lowest Glove Allergen Content Associated with a Specific Patch Test–Positive Reaction

Contact Allergen	*Content** ($\mu g/g$; $\mu g/mm^2$)
ZDEC	584; 3.7
ZDBC	283; 3.1
MBT	(68)†; (0.8)†

MBT = 2-mercaptobenzothiazole; ZDBC = zinc dibutyldithiocarbamate; ZDEC = zinc diethyldithiocarbamate.

*Values are from gloves in which only one allergen type was detected. Lower specific allergen levels were detected in problem gloves that contained multiple allergens to which the subject was patch test positive.

†The concentration in the extract was less than the lowest standard used in the assay.

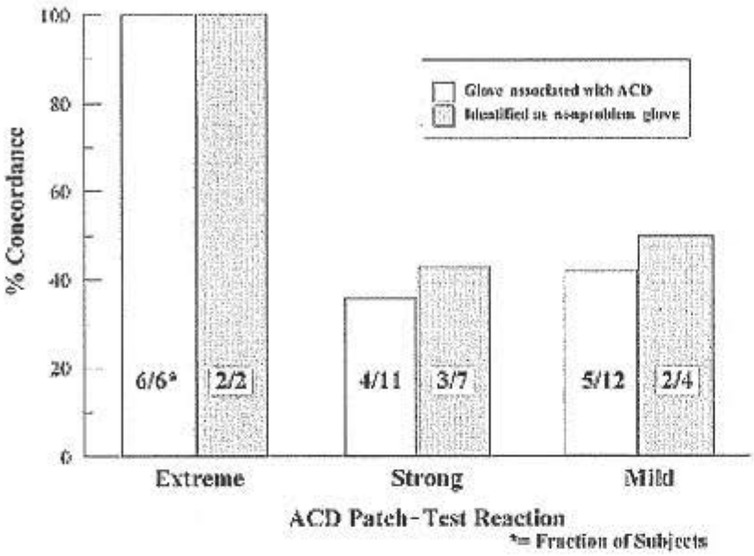

Figure 1. Influence of degree of allergic reactivity (patch-test severity) on concordance between glove allergen content and patch test–identified allergen. Subjects identified and submitted gloves associated with and (if available) gloves not associated with their allergic contact dermatitis (ACD). Only subjects displaying extreme patch-test reactions were able to consistently distinguish between gloves containing their contact allergen or free of their contact allergen.

Discussion

The accelerator content of commercially available gloves is not routinely measured, and the level that can elicit ACD under normal conditions of use is not known. In a titration patch-test study of rubber-sensitive subjects, Emmett and colleagues[17] reported that the highest concentration of MBT that did not elicit any reaction in subjects with ACD was 1.45 $\mu g/cm^2$. We found no similar study of threshold determination for carbamates in the literature. Problems concerning the applicability of patch testing for the determination of safe accelerator levels in gloves include the differences in allergic sensitivity between the back and the hands, the 48-hour occluded exposure time, the effect of the exposure matrix material, and the interpretation of a positive reaction at low doses. It is also known that the dose required for the elicitation of an ACD response is inversely related to the degree of sensitization.[19] Cross-reactivity between allergens, as between thiurams and carbamates, can also complicate the identification of the causative agent of the ACD and erroneously implicate thiurams that chemical analyses suggest are now rarely used in glove manufacturing.

The original objective of this study was to survey the contact allergen content levels in gloves that elicited ACD in workers under normal working conditions. The levels shown in Table 2 are the lowest levels of contact allergen found in a problem glove of an individual sensitized to that allergen, but they should not be viewed as elicitation threshold values. All nonproblem gloves identified by subjects with extreme patch-test reactions were free of the

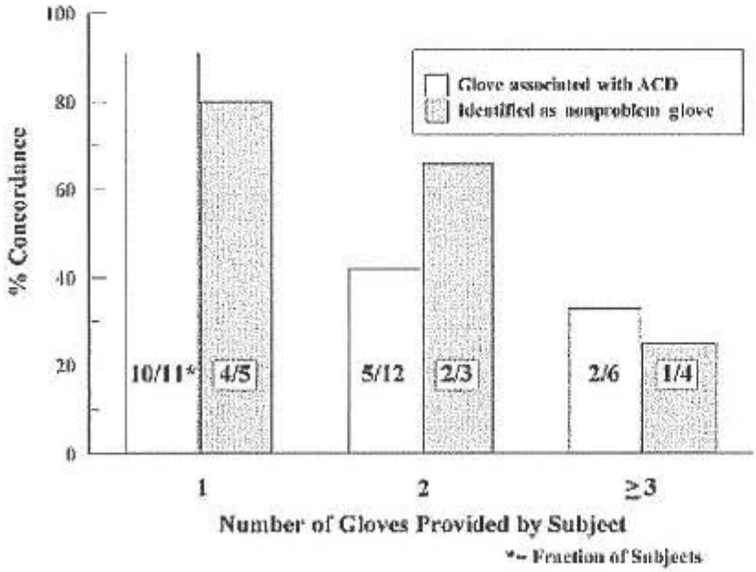

Figure 2. Influence of number of different types of gloves used by subjects on concordance between glove allergen content and patch test–identified allergen. Subjects identified and submitted gloves they associated with and (if available) gloves not associated with their allergic contact dermatitis (ACD). The correct identification of problem versus nonproblem gloves was inversely related to the number of glove types in use.

respective contact allergen. Similarly, all subjects identifying a single nonproblem glove supplied gloves that were devoid of their allergen, except for one subject who provided two nonproblem gloves: one that was free of contact allergens and one with approximately the same level of allergen (ZDBC) found in the subject-identified problem glove. It is interesting that the lowest MBT level associated with the elicitation of ACD (from a patient who had a strong patch-test reaction to MBT and who correctly identified 4 of 4 problem gloves and three-fourths of the nonproblem gloves) was less than the threshold patch-test value previously reported by Emmett and colleagues,[17] suggesting that the ACD threshold from glove contact allergens under normal conditions of use may be lower than that indicated by patch testing.

The overall discordance between specific patch-test reactivity and the allergen content of patient-identified problem and nonproblem gloves was high. The ability of a patient to identify the putative specific glove that elicited his or her ACD was directly related to the number of different rubber glove types or brands the patient used (as indicated by the number of gloves provided) and the degree of sensitization (as indicated by the severity of the patch-test reaction). The delayed reaction time between exposure and clinical symptoms may significantly impair the subject's ability to correctly associate a particular glove with his or her ACD. Use of numerous brands would confound the problem because the glove being worn at the time the subject notices the dermatitis may be misidentified as the problem glove. This time lag is greatly reduced in highly sensitized subjects, thus increasing the probability of their correctly associating a glove with ACD. Four of the six subjects who had extreme patch-test reactions identified several problem gloves (two to six), four of the six subjects who had extreme patch-test reactions provided multiple (2 to 6) problem gloves, suggesting that the ability of a highly sensitized patient to correctly identify the allergen source material is independent of the number of glove brands in use at the time of the ACD manifestation.

Of interest was the disagreement between patch-test results for MBT and mercapto mix. One subject, 2 subjects, and another 2 subjects respectively had mild, strong, and extreme patch-test reactions to MBT. Only two reactions (one mild and one extreme) to mercapto mix were observed in this group. None of the subjects had both a positive reaction to mercapto mix and a negative (no) reaction to MBT. MBT is present at 1% in petrolatum but only at 0.25% in mercapto mix (along with 0.25% each of morpholinyl mercaptobenzothiazole, MBTS, and N-cyclohexyl-2-benzothiazyl sulfenamide).

Patch testing is an important component in the diagnosis and management of ACD. Identification of the source of the allergen and other potential sources is also a critical component of disease management. This is especially true for glove-associated ACD because glove wear may be a requirement of the patient's job and the identification of proper substitute gloves may be crucial in preventing subsequent ACD episodes. The combination of chemical analysis and patch testing (as used here) is not routinely available to physicians. In the absence of chemical testing, allergens are often identified from manufacturer product information or from a published list of product content.[10] These sources often identify multiple potential allergens in a product, but all of those allergens may not be present in the product. Also, occasionally a product may contain an allergen that is not listed for that specific product. Dermatologists may opt to patch-test with the suspected glove material in addition to patch-testing with the allergen tray. The data presented in this study suggest that all gloves used by mild to strong patch-test reactors within several days of an ACD manifestation should be considered potential sources of the contact allergen.

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