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January 21, 2000

Dockets Management Branch (HFA-305)
Food and Drug Administration
5630 Fishers Lane
Room 1061
Rockville, MD 20852

Re: **FDA Proposed Rule: Surgeon's and Patient Examination Gloves; Reclassification.**
Docket No. 98N-0313

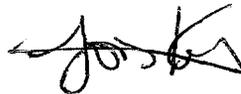
Dear Sir or Madam:

National Starch and Chemical Company appreciates the opportunity to provide comments on the FDA proposed rule to reclassify surgeon's and patient examination gloves. National Starch and Chemical Company is a manufacturer of absorbable dusting powder, USP.

As shown in the attached comments, National Starch and Chemical Company supports the reclassification of medical gloves to Class II devices with special controls. Several items of concern are discussed in the attached comments, such as powder and protein limits and the continued need for powdered medical gloves.

Thank you again for the opportunity to provide comments on the proposed rule. If you have any questions, you may contact me at the telephone number listed above.

Sincerely yours,



Lois A. Kotkoskie, Ph.D., D.A.B.T.
Senior Toxicologist
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LAK/jlm
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National Starch and Chemical Company (NSC) Comments

**FDA Proposed Rule: Surgeon's and Patient Examination Gloves;
Reclassification. 64 FR 41709-41743, July 30, 1999.
Docket No. 98N-0313**

Summary of Comments:

In general, NSC supports the reclassification of medical gloves to Class II devices with special controls. However, NSC does not agree with the following items in the proposed rule as summarized below:

| FDA Proposed Rule | NSC Proposal |
|---|--|
| 4 categories of medical gloves | 2 categories of medical gloves |
| 1200 µg/glove protein limit (recommended) | 300 µg/dm ² protein limit (required) |
| 120 mg/glove powder limit (recommended) | 150-200 mg/glove powder limit (required-natural latex gloves only) |
| powder limit on synthetic gloves | no powder limit on synthetic gloves |

We request FDA to consider the barrier to trade that could arise if the proposed protein limit of 1200 µg/glove was implemented. It is estimated that 70-80% of the glove manufacturers in Southeast Asia would be unable to comply with the proposed protein limit on gloves. A more reasonable extractable protein limit should be proposed, such as 300 µg/dm².

There is a need for powdered medical gloves to exist because they have the best performance properties of all glove types. If the FDA reclassifies medical gloves as Class II medical devices with special controls such as powder and protein limits, then it will not be necessary to take further regulatory action to ban or limit the use of powdered medical gloves.

NSC Comments on Proposed Protein Limit of 1200 µg/glove

NSC believes it is appropriate to limit the amount of water extractable protein content on natural rubber latex (NRL) medical gloves, because natural latex (NL) proteins are the cause of Type I hypersensitivity reactions. However, NSC does not agree with the proposed protein limit of 1200 µg/glove, regardless of glove size, and the sensitivity limit of 300 µg/glove. As discussed in more detail below, NSC proposes an extractable protein limit based on per unit area of 300 µg/dm².

FDA does not provide any scientific justification for the proposed protein limit of 1200 $\mu\text{g}/\text{glove}$. Therefore, it is not known if this protein limit will protect the public health from latex allergies. In addition, in order to adequately protect the public health, the extractable protein limits should be required limits and not recommended limits.

The protein from corn starch is not allergenic (Tomazic et al., 1994) and therefore, the allergenic NL protein in gloves should be measured as extractable protein. Extractable protein should be measured by the newest version of the ASTM Lowry Method D5712 and the limit of sensitivity of this method should be re-evaluated. FDA states the limit of sensitivity is 300 $\mu\text{g}/\text{glove}$ (based on a 6 gram glove), but this limit seems to be too high to determine the extractable protein (Beezhold et al., 1996).

The unit of measurement of micrograms of protein per glove is not appropriate because gloves have various sizes, weight and thickness. Therefore, extractable protein should be measured based on surface area, in the units of micrograms per unit area ($\mu\text{g}/\text{dm}^2$).

NSC proposes an extractable protein limit of 300 $\mu\text{g}/\text{dm}^2$ per glove, which is approximately equal to 2400 micrograms of protein in a 7 gram glove. Most Asian glove manufacturers can comply with this extractable protein limit.

The most important reason why the FDA should reconsider the proposed protein limit of 1200 $\mu\text{g}/\text{glove}$ is the potential barrier to trade. We have been in contact with several trade associations and glove manufacturers in Southeast Asia; approximately 70-80% of the glove manufacturers in this region cannot comply with the recommended protein limit of 1200 $\mu\text{g}/\text{glove}$. If the protein limit of 1200 $\mu\text{g}/\text{glove}$ is implemented, these glove manufacturers will either have to do extra leaching, manufacture powder-free gloves, or go out of business.

NSC Comments on Proposed Powder Limit of 120 mg/glove

The 120 mg/glove powder limit is not based on science and there is no justification for this choice. There has been no comprehensive study on the amount of absorbable dusting powder on medical gloves to justify the choice of 120 mg/glove. If a less than optimal amount of powder is used on a medical glove, then certain performance characteristics will be affected such as donning properties, increased chance of glove stickiness, durability, shelf life, etc. NSC suggests a required powder limit of not more than 150-200 mg/glove, in order to provide an optimal amount of powder for different sized gloves and to adequately protect the public health.

Absorbable dusting powder is not, by itself, an allergen. It is hypothesized that the powder adsorbs latex proteins during the manufacturing process. The airborne powder-NL protein moiety is hypothesized to produce latex allergies. Therefore, if the protein

limit and the manufacturing process can be controlled, then it will not be necessary to limit powder on NL medical gloves. Powder limits should be required secondary to protein limits and manufacturing process controls for NL protein. Recommendations for protein limits and manufacturing process controls are found elsewhere in this document.

The value of 120 mg/glove seems to be an arbitrary value that was not chosen using appropriate risk assessment procedures. Therefore, it is quite surprising that FDA would be able to predict the reduction in expected allergic reactions as shown in the proposed rule, because, as FDA states, "the scientific data to define the quantitative relationship between respiratory allergic reactions and powder level on NL gloves are not available at this time" (p. 41712, FDA proposed rule).

There is no justification whatsoever for a powder limit of 120 mg/glove for synthetic gloves. Synthetic gloves do not contain NL proteins and therefore, are not associated with NL allergies.

FDA states in the proposed rule that it is concerned about foreign body reactions caused by glove powder, either on a NL glove or synthetic glove. This issue of foreign body reactions caused by glove powder has been previously reviewed by FDA in the September 1997 Medical Glove Powder Report, which discusses foreign body reactions and concludes that foreign body reactions are most likely caused by sutures and not glove powder. In fact, the overall conclusion of the 1997 Medical Glove Powder Report is: "the major adverse impact of glove powder appears to be its contributing role in natural rubber latex allergies" (U.S. FDA, 1997, p. 2) and the issue of foreign body reactions is not mentioned. Therefore, there is insufficient scientific evidence to demonstrate that powder plays a role in foreign body reactions. If the FDA has new evidence to show that powder does cause foreign body reactions, then it should make these data available to the public for review and comment.

The ability of cornstarch to be a growth source for bacteria and a carrier for endotoxin is not well supported by scientific evidence. One paper by Williams and Halsey (1997) addresses the endotoxin issue. These investigators found low levels of endotoxin in dry powder; these results have been confirmed by NSC for ABSORBO[®] HP absorbable dusting powder, USP (NSC, 1999a). Williams and Halsey hypothesized that endotoxin was being introduced into the glove manufacturing process in the starch slurry tank; endotoxin levels in the starch slurry tank tested were 64000 ng/g, compared with 0.32 ng/g for dry starch powder (Williams and Halsey, 1997). The September 1997 Medical Glove Powder Report reviewed the issue of bioburden and powder and found no evidence that powder and bioburden are related (U.S. FDA, 1997). ABSORBO[®] HP absorbable dusting powder, USP contains low microbial levels at less than 1000 Total Plate Count per gram of powder (NSC, 1999b). If adequate manufacturing controls are placed on the manufacturing process at the starch slurry tank, then the levels of endotoxin and presumably microbial contamination will also be controlled.

It is recommended that the method to be developed by FDA to measure glove powder should be specific for absorbable dusting powder.

Response to FDA's Specific Request for Comments

- 1. FDA requests comments on the timeframe for implementation of the proposed rule considering the need for change in production, technology, and labeling, as well as the immediate need to address adverse health concerns associated with medical gloves. Although FDA prefers a 1-year effective date, FDA is proposing a 2-year effective date based on indications from industry that the necessary changes could not be made in 1 year and that a shortage of medical gloves could result.*

NSC Response: We support the 2-year timeline for implementation of the proposed rule.

- 2. In the proposed guidance document, FDA recommends a limit of no more than 120 mg powder per powdered glove, regardless of size, as the maximum level in order to reduce exposure to particulates and airborne allergens. FDA requests comments on the recommended limit with regard to the minimum level of powder needed for adequate donning of gloves.*

NSC Response: As discussed above in more detail, NSC does not agree with the recommended powder limit of 120 mg/glove. There is no scientific evidence that 120 mg powder per glove will reduce the incidence rate of adverse reactions from gloves. Most manufacturers use 150-200 mg powder per glove. Therefore, if manufacturers reduce the amount of powder in the glove to a less than optimal level, quality problems may result such as: less donnability, higher reject rate, decreased shelf life due to gloves sticking together, and higher cost to the consumer for extra processing of gloves to meet powder limit.

There is no scientific justification whatsoever for a powder limit on synthetic medical gloves. There is no known interaction between the powder and synthetic gloves and no known adverse effects from the use of powder on synthetic medical gloves. The powder limit should apply only to NRL medical gloves.

- 3. FDA requests comments on the feasibility and desirability of additional labeling requiring manufacturers to state the primary ingredients in glove powder in the product labeling.*

NSC Response: In order to provide accurate information to the consumer, the label should clearly state "contains absorbable dusting powder, USP" or the chemical name of the glove powder used in medical gloves.

4. *In the proposed guidance document, FDA is recommending no more than 2 mg powder per glove, regardless of size, as the recommended powder level for those surgeon's and patient examination gloves labeled "powder-free". FDA requests comments on the proposed limit. FDA is also seeking comments on the possible impact of this powder limit on barrier properties and shelf-life of NL gloves.*

NSC Response: We propose to increase the powder limit for powder-free gloves to 4 mg/glove and then reduce in stages by 1 mg/glove within a two-year period to 2 mg/glove. Glove manufacturers are requesting this additional time to make the necessary processing changes to comply with the recommended powder level for powder-free gloves.

5. *FDA is also considering a future requirement that all surgeon's and patient examination gloves marketed in the United States be powder-free. FDA requests comments as to whether a continued need for powdered gloves exists, and, if so, the reason for this need.*

NSC Response: Overall, there is substantial support from the market that there is a need for powdered medical gloves. Powdered medical gloves are the most economical and technically, the best glove in terms of donnability, durability, shelf life, appearance, tactile properties. If FDA and the glove manufacturers can control the amount of protein and powder on a medical glove, then the incidence rate of adverse reactions should decrease.

6. *FDA considered restrictions on the sale (advertising), distribution, and use of powdered surgeon's and patient examination gloves. FDA is seeking comments on the feasibility of such restrictions.*

NSC Response: There is still a need for powdered gloves due to their excellent technical properties. Therefore, there should not be any restriction on the sales, advertisement, and distribution of powdered gloves. The proposed warning labels and required powder and protein levels will be sufficient to protect the public health.

7. *In the proposed guidance document, FDA is recommending an upper limit of no more than 1,200 µg protein per NL glove, regardless of size, as the maximum level for NL surgeon's and patient examination gloves. FDA is seeking comments on the proposed recommended limit.*

NSC Response: As stated above in our detailed comments on the protein limit, NSC proposes a required protein limit of 300 µg/dm². The FDA proposed protein limit does not take into account the size or thickness of the glove, which can vary significantly. In addition, we believe the protein limit should be required rather than recommended in order to adequately protect the public health.

8. *FDA's objectives in this proposed rulemaking are to reduce adverse health effects from allergic reactions and foreign body reactions by controlling the levels of water-extractable*

protein and glove powder on NL gloves. FDA requests comments as to whether there are feasible alternative approaches to achieve these objectives. If other alternatives or data submitted present feasible methods to protect the public health or suggest that different powder or protein levels are adequate to protect the public health, FDA may incorporate such data or approaches in a final rule.

NSC Response: The manufacturing process for powdered gloves could be better controlled in order to reduce the NL protein level in finished gloves. One suggestion is to require process control of the starch slurry tank. Some manufacturers do not change the starch slurry tank on a regular basis, and as a result, NL protein concentration rises in the tank over time. Therefore, finished gloves produced from the 'old' starch slurry would theoretically have higher NL protein levels than gloves produced from 'fresh' starch slurry. It may be necessary to require manufacturers to change the tank on a regular basis, such as every day or twice a week. Also, regular changes to the starch slurry tank may reduce other possible problems such as endotoxin and bioburden contamination on finished gloves.

In addition, as stated previously, a required extractable protein limit of 300 $\mu\text{g}/\text{dm}^2$ and a required powder limit of 150-200 mg/glove would be predicted to adequately protect the public health.

9. *FDA also invites comments on the issue of whether the recommended limits on powder and protein proposed in this rule should be recommended limits or required limits.*

NSC Response: The powder and protein limits should be required limits in order to protect the public health. However, the required limits should be based on scientific judgment and glove manufacturing capabilities. Most glove manufacturers in Southeast Asia could meet the NSC proposed extractable protein limit of 300 $\mu\text{g}/\text{dm}^2$ and powder limit of 150-200 mg/glove.

10. *FDA considered allowing manufacturers to establish an initial tentative shelf-life up to a certain duration based on accelerated aging data, provided that manufacturers initiate concurrent real-time shelf-life studies to confirm and extend the tentative shelf-life. FDA has been unable, however, to determine whether any validated stability study protocols exist employing accelerated aging methodologies. The agency invites comments or information on the availability of accelerated aging stability study protocols which are predictive of glove shelf-life. If convincing information concerning such protocols is available, FDA may incorporate such an approach in a final rule.*

NSC Response: The minimum duration for shelf life should be 2 years or more. The use of accelerated aging stability studies has been used for pharmaceutical products but the protocols would not be applicable for this purpose. The use of accelerated aging studies should not be permitted until validated methods are available.

11. FDA considered requiring the use of a special air handling system at the point of use for those facilities using powdered surgeon's and patient examination gloves with powder levels over 120 mg per glove, regardless of glove size. FDA is seeking comments on the appropriateness of this restriction.

NSC Response: There is no need for a special air handling system if special controls are implemented for medical gloves. The required powder and protein limits will reduce the amount of airborne latex protein. The cost of such a system will be prohibitive and therefore, inappropriate. It also not practical and is inconvenient to the surgeons and healthworkers

References

Beezhold D, Pugh B, Liss G, Sussman G. Correlation of protein levels with skin prick test reactions in patients allergic to latex. *J Allergy Clin Immunol* 98:1097-1102, 1996.

National Starch and Chemical Company (NSC). Central Analytical Group Analysis Report. Microbiology Laboratory. Endotoxin Results for ABSORBO HP GJB 127/6. 1999a.

National Starch and Chemical Company (NSC). ABSORBO® HP Absorbable Dusting Powder, USP. Total Plate Count (1996-1999). 1999b.

Tomazic VJ, Shampaine EL, Lamanna A, Withrow TJ, Adkinson NF, Hamilton RG. Cornstarch powder on latex products is an allergen carrier. *J Allergy Clin Immunol* 93:751-758, 1994.

U.S. FDA, Center for Devices and Radiological Health. Medical Glove Powder Report. September 1997.

Williams PB, Halsey JF. Endotoxin as a factor in adverse reactions to latex gloves. *Ann Allergy Asthma Immunol* 79:303-310, 1997.

Correlation of protein levels with skin prick test reactions in patients allergic to latex

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Background: Natural rubber latex (NRL) gloves are the major source of proteins that cause latex allergic reactions in sensitized health care workers and patients.

Objective: This study evaluated the effect of manufacturing changes on reducing protein, antigen, and allergen levels of latex medical gloves.

Methods: Three types of NRL gloves were manufactured with a common batch of compounded latex. The NRL gloves were analyzed for total protein by using the American Society for Testing and Materials D5712-95 Lowry method, and specifically for latex proteins by immunoassay. Allergen levels in the extracts were determined by end-point titration skin prick tests (SPTs) on patients allergic to NRL.

Results: Extracts from regular powdered gloves had detectable levels of latex proteins and allergens (62% SPT positive), whereas the powder-free gloves were low in protein content and allergenicity (5% to 8% SPT positive). No significant difference in SPT reactivity was observed between the chlorinated powder-free gloves and the polymer-coated gloves. Although the protein levels determined by the Lowry assay correlated with SPT reactivity ($r = 0.95$), the test was restricted by a high detection limit (9.3 $\mu\text{g/ml}$). Fifty-eight percent of patients allergic to latex reacted at the 50 $\mu\text{g/gm}$ detection limit allowed by the Food and Drug Administration. The ELISA had a good correlation with SPT reactivity ($r = 0.93$), and because of the greater sensitivity, gloves testing below the ELISA reporting limit (0.06 $\mu\text{g/ml}$) have a significantly lower potential for eliciting reactions in patients allergic to latex.

Conclusions: Results of protein assays are acceptable criteria with which to rate the potential allergenicity of gloves; however, the American Society for Testing and Materials D5712-95 assay may lack the sensitivity to provide clinically relevant data. (*J Allergy Clin Immunol* 1996;98:1097-1102.)

Key words: Latex allergy, latex proteins, Lowry assay, immunoassay, skin prick tests

Natural rubber latex (NRL) allergy presents as a clinical spectrum of manifestations including local contact dermatitis, allergic rhinoconjunctivitis, asthma, and life-threatening anaphylaxis.¹ It is generally understood that latex sensitization occurs as a result of repeated contact with NRL-

Abbreviations used

| | |
|-------|--|
| ASTM: | American Society for Testing and Materials |
| LEAP: | Latex ELISA for antigenic protein |
| NRL: | Natural rubber latex |
| SPT: | Skin prick test |

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containing products or by inhalation of latex aeroallergens.^{1,2} Individuals in the health care professions (5% to 15%) and children with spina bifida (40% to 68%) have the highest prevalence rates for latex allergy.³⁻⁶ Immediate hypersensitivity reactions to latex occur when individuals have specific IgE antibodies to NRL proteins. Once sensitized, certain patients experience severe sensitivity, and anaphylactic reactions have been re-

ported from NRL inhalation, skin prick testing, and inadvertent contamination in food.⁷⁻⁹

Latex proteins have been demonstrated in most medical devices containing NRL.¹⁰⁻¹⁵ The product most often implicated as causing allergic reactions is NRL gloves, especially powdered gloves. Patients allergic to latex have been reported to react less to extracts of powder-free latex gloves than to extracts of powdered gloves.¹⁶ Use of low-protein and powder-free gloves reduces the consequences of NRL occupational asthma.^{7, 17} Powdered gloves have higher latex antigen and allergen levels than powder-free gloves.^{18, 19} Recently, the Food and Drug Administration approved manufacturers' use of a modified Lowry assay to report latex protein levels on glove packages. However, it is not known what quantitative level of NRL protein is safe to prevent sensitization or allergic reactions.

Our objective in this study was to quantitatively evaluate the effect of processing changes on protein and allergen levels of NRL gloves. We measured protein, antigen, and allergen levels on regular powdered, chlorinated, and chlorinated polymer-coated gloves to correlate the modified Lowry assay and ELISA with skin test reactivity in patients with known latex allergy.

METHODS

Patient population

Thirty-nine otherwise healthy patient volunteers, diagnosed as having latex allergy, with a positive history and positive NRL skin prick test (SPT) results were recruited for this study. NRL SPTs were previously performed on these patients with a standard latex skin test reagent (Bencard, Mississauga, Ontario, Canada), and wheals 4 mm greater than the negative control were considered positive. The control group consisted of 31 volunteers not allergic to latex who were first seen consecutively in the allergy clinic (17 with allergic rhinoconjunctivitis, 5 with asthma, 4 with sinusitis, 3 with urticaria, 1 with migraine headache, and 1 with a drug reaction). The protocol was approved by the Ethical Review Committee of the Ontario Allergy Society.

Skin testing

SPTs were done on the volar aspect of the forearm with a drop of test solution, and the epidermis beneath the drop was pricked with a lancet (Miles, Warwickshire, U.K.). SPT sites were wiped clean after 15 minutes, and the wheal and flare reactions were carefully measured. Positive wheal and flare reactions (≥ 4 mm of the negative wheal) were outlined, transferred onto transparent tape, and recorded as a permanent record. Testing with the latex components was started at a 1:1,000,000 dilution and continued with 10-fold lower

dilutions (higher concentration of extracts) until a positive reaction was obtained.

Test solutions

Allergenic components used for SPTs included a standard latex skin test reagent (Bencard), a polyvalent ammoniated latex protein preparation,²⁰ four extracts prepared from commercially available latex medical gloves, and extracts of three specifically prepared test gloves. Histamine (1 mg/ml) and diluent (human serum albumin, 0.3 mg/ml in 0.9% saline solution) were used as positive and negative control reagents for the skin tests.

Test solutions were prepared by extracting gloves for 2 hours at 37° C under sterile conditions. To obtain a representative extract for skin testing, five gloves from each lot were cut into small pieces and extracted in a polypropylene container with 5 ml/gm sterile saline solution.^{20, 21} The samples were centrifuged (2000 g) to remove glove powder and particulates. To eliminate the lot-to-lot variability²² and directly observe any differences resulting from processing changes, the three test gloves were specially manufactured, starting with a common batch of compounded latex; and the same dip machine was used within an 8-hour period. The three different test gloves were processed differently in order to produce: (1) regular powdered gloves, (2) chlorinated powder-free gloves, and (3) chlorinated polymer-coated gloves (patent pending). The test gloves manufactured for this study were used within 1 month of manufacture.

Protein analysis

Total protein levels. Protein levels were determined according to the American Society for Testing and Materials (ASTM) standard test method D5712-95, which incorporates a precipitation step to reduce the interfering substances before analysis by the Lowry assay. In brief, the precipitation step was performed on 0.5 ml of each sample by first adding 50 μ l of 0.15% (wt/vol) sodium deoxycholate and incubating the sample at room temperature for 10 minutes. Next, 50 μ l of 72% (wt/vol) trichloroacetic acid and 50 μ l of 72% (wt/vol) phosphotungstic acid were added, and the samples were incubated at room temperature for an additional 20 minutes. The samples were centrifuged for 15 minutes at 6000 g, the supernatant was discarded, and the pellet was dissolved in 125 μ l of 0.1 mol/L NaOH (yielding a fourfold concentration of each sample). Protein was determined by using the Detergent Compatible protein assay (Bio-Rad Laboratories, Hercules, Calif.). Each sample was tested in duplicate by using four twofold serial dilutions. The optical density was read at 700 nm, and the concentration of protein was determined by comparing the optical density of unknowns with the ovalbumin protein standard. The detection limit was defined as three times the standard deviation at zero, and the reporting limit was defined as 10 times the standard deviation at zero.²³ The standard deviation at

zero was determined by extrapolating the standard deviations of known concentrations of ovalbumin (6 replicates of 5 protein concentrations) according to the method of Taylor.²³ The limit of detection for the modified Lowry assay was found to be 2.5 $\mu\text{g/ml}$, and the reporting limit for the method was calculated as 9.3 $\mu\text{g/ml}$ (46.5 $\mu\text{g/gm}$ with a 1:5 weight-to-volume extraction ratio).

Antigen levels. Antigen levels in the glove extracts were quantitated by an indirect ELISA with the Latex ELISA for Antigenic Protein (LEAP) as previously described.^{12, 20-22} Latex protein antigens from the standard polyvalent ammoniated latex protein preparation or the glove extracts were adsorbed to the wells of a 96-well ELISA plate (Easy Wash; Corning Glass Works, Corning, N.Y.) by incubating eight duplicate twofold serial dilutions of the extracts (carbonate buffer, pH 9.6) in the wells for 4 hours at 37° C. Nonspecific binding sites were blocked by using 2% bovine serum albumin (Fraction V; Sigma Chemical Co., St. Louis, Mo.). A rabbit anti-latex antiserum (1:5000 dilution) was allowed to react with the plastic-bound latex proteins overnight. The specifically bound rabbit anti-latex antibodies were then reacted for 1 hour with a goat anti-rabbit IgG (1:1000 dilution) conjugated with horseradish peroxidase (Sigma). Finally, a colored reaction product was produced by incubation in 10 mg/ml o-phenylenediamine (Sigma) containing 0.001% H_2O_2 .

The LEAP uses a pooled rabbit anti-serum specific for latex protein, which has been shown to have an immunoblot profile similar to that of pooled IgE from 20 patients allergic to latex.²⁴ The LEAP has a good linear correlation, with range of sensitivity and specificity similar to those of a human IgE inhibition assay.²¹ The detection limit for the ELISA was determined as described above by extrapolating the standard deviations of known concentrations of latex protein to zero.²³ The assay has a detection limit of 0.03 $\mu\text{g/ml}$ and a reporting limit of 0.06 $\mu\text{g/ml}$ (0.3 $\mu\text{g/g}$ with a 1:5 extraction ratio). Intra-lot variability for the test gloves was determined by using four or more individual glove samples for each test condition. Samples below the detection limit were assigned a value of one half the reporting limit (0.03 $\mu\text{g/ml}$).

Statistical analysis

The proportions of patients allergic to latex reacting to glove extracts were compared by using the Mantel-Haenszel chi square test or Fisher's exact test, as appropriate.

RESULTS

Protein analysis

Several gloves for each test condition were analyzed with the modified Lowry assay (ASTM 5712-95) and the ELISA to determine intra-lot variability. The Lowry assay detected $29 \pm 8.8 \mu\text{g/ml}$ (mean \pm SD, $n = 5$) for the regular powdered

gloves, whereas all of the samples tested for both the chlorinated ($n = 6$) and the polymer-coated gloves ($n = 7$) were below the detection limit of the Lowry assay (9.3 $\mu\text{g/ml}$). With the LEAP, extracts of the regular powdered gloves had $28.1 \pm 8.2 \mu\text{g/ml}$ (mean \pm SD, $n = 4$), whereas the chlorinated gloves had $0.045 \pm 0.023 \mu\text{g/ml}$ ($n = 6$) and the polymer-coated gloves had $0.067 \pm 0.082 \mu\text{g/ml}$ ($n = 7$). Three of six chlorinated gloves and five of seven polymer-coated gloves were below the detection limit (0.06 $\mu\text{g/ml}$) of the ELISA. These data indicate that the processing changes to produce the chlorinated and polymer-coated gloves consistently lower latex protein levels.

Skin testing results

Control. Thirty-one volunteer control patients, who were not allergic to latex, were skin prick tested with all test reagents; and full-strength testing solutions were used. All control patients reacted positively to the histamine control; however, no reactions to the glove extracts were observed. It was concluded that the glove extracts did not contain substances that would cause irritation reactions.

Patients allergic to latex. The population of 39 patients with positive responses to latex was found to be highly allergic to latex. As shown in Table I, 56% of these patients reacted to a 1:10,000 or greater dilution of the Bencard standard skin test reagent. The extracts were ranked according to the percent of patients allergic to latex with positive skin test responses, and the data are summarized in Table II. Sixty-two percent (24 of 39) of the patients reacted to the extract from the regular powdered gloves. The proportion of SPT reactivity for the test powdered gloves is significantly greater than that for test powder-free gloves (2 of 39, $p = 0.0000005$) and that for test polymer-coated glove extract (3 of 39, $p = 0.0000019$). The proportion of SPT reactivity was not different between the test powder-free and the polymer-coated gloves ($p = 1.0$, two-tailed Fisher's exact test). This level of reactivity was similar to that observed for another powder-free, polymer-coated glove (4 of 39, 10%) also tested in this study. In general, the rank order of reactivity of the extracts matched with the protein levels by both the Lowry assay and the ELISA.

Correlation of protein assays to SPT reactivity

The percent of patients reacting to the different latex extracts was plotted against the protein concentration (log) in an attempt to correlate these

TABLE I. Sensitivity of the latex-allergic population

| Reagent dilution | Bencard SPT reagent | Cumulative reactivity (%) | AL reference protein | Cumulative reactivity (%) |
|------------------|---------------------|---------------------------|----------------------|---------------------------|
| 1,000,000 | 7/39 (18%) | 18 | 2/39 (5%) | 5 |
| 100,000 | 9/39 (23%) | 41 | 4/39 (10%) | 15 |
| 10,000 | 6/39 (15%) | 56 | 9/39 (23%) | 38 |
| 1,000 | 8/39 (21%) | 77 | 8/39 (21%) | 59 |
| 100 | 2/39 (5%) | 82 | 4/39 (10%) | 69 |
| 10 | 1/39 (2.6%) | 85 | 6/39 (15%) | 84 |
| 1 | 6/39 (15%) | 100 | 3/39 (8%) | 92 |
| No reaction | 0/39 (0%) | | 3/39 (8%) | |

AL, Ammoniated latex.

TABLE II. Comparison of protein level to SPT reactivity in patients allergic to latex

| Test solution* | Percent reacting | Lowry ($\mu\text{g/ml}$) | ELISA ($\mu\text{g/ml}$) |
|----------------------|------------------|----------------------------|----------------------------|
| Saline control | 0 | bd | bd |
| Test powder-free | 5 | bd | 0.08 |
| Test polymer-coated | 8 | bd | 0.16 |
| Powder-free glove | 10 | bd | 0.08 |
| Powdered glove 3 | 15 | bd | 0.94 |
| Test powdered | 61 | 29 | 28 |
| Powdered glove 2 | 74 | 36 | 15 |
| Powdered glove 1 | 85 | 140 | 18 |
| AL reference protein | 92 | 591 | 1844 |
| Bencard reagent | 100 | 1850 | 13000 |

bd, Below detection limit of 9.3 $\mu\text{g/ml}$ for the Lowry assay and 0.06 $\mu\text{g/ml}$ for the LEAP; AL, ammoniated latex.

assays. Samples below the detection limit were not included in the analysis. As shown in Fig. 1, there was a good correlation between Lowry protein levels and SPT reactivity ($r = 0.95$). The equation for the regression line comparing Lowry protein levels with SPT reactivity was ($y = 18.8 \log[x] + 40$), where x is micrograms per milliliter of latex protein determined by the protein assay. This equation allows one to predict the percent (y) of patients allergic to latex that will react to a glove extract. Likewise, there was a good linear correlation ($r = 0.94$) between the ELISA and SPT reactivity (Fig. 1). The equation for the regression line was ($y = 19.2 \log[x] + 34$).

The major difference found between the protein assays was a reduced sensitivity of the Lowry assay. Although the Lowry assay correlated well with skin test reactivity, it has a reporting limit of 9.3 $\mu\text{g/ml}$ or 47 $\mu\text{g/gm}$ (with a 5:1 extraction ratio). If the regression line is used to predict reactivity at the reporting limits, 58% of this population would

react at the Lowry limit, whereas only 11% would react at the reporting limit of the ELISA. Thus the ELISA allows one to predict SPT reactivity over a broader protein range and at a lower protein level than does the Lowry assay.

DISCUSSION

Latex allergy is problematic for health care workers who require barrier protection and who are working in a hospital environment laden with airborne NRL allergen.^{2, 7, 25} A simple solution to reducing airborne latex is the use of powder-free gloves. In this study we compared regular powdered gloves with powder-free gloves manufactured by using chlorination and/or polymer coating processes. We attempted to determine the relationship between residual protein levels and skin test reactivity. Three brands of gloves were specially produced from a common batch of compounded latex to eliminate the variability caused by differences in the starting materials.²² Residual protein in extracts was analyzed for total protein by using the ASTM 5712-95 method, and specifically for latex antigens by using ELISA. Relative allergen levels in the extracts were determined by end-point titration skin prick testing.

It has been reported that powder-free gloves have lower protein and allergen levels than regular powdered gloves.¹⁷⁻¹⁹ The chlorination step appears to dramatically reduce latex allergen levels.²⁶ Coating latex with a polymer is another process used to eliminate the need for donning powder. We found dramatic differences between the powdered and powder-free gloves in residual protein, antigen, and SPT reactivity. We were unable to determine a significant difference in protein or SPT reactivity between the chlorinated and the polymer-coated gloves.

It is generally believed that the use of low-

protein gloves will reduce the potential for latex reactivity and sensitization. However, several important points need to be addressed including: (1) How is the residual protein or allergen level measured? and (2) What amount of protein is acceptable? Previously, total protein levels were not found to correlate with clinical reactivity.^{10, 27, 28} This was presumed to occur because not all proteins are allergens, and measuring total protein would obscure the measurement of only those proteins that are allergens. However, numerous investigations have demonstrated that multiple latex proteins are allergens^{24, 28-31}; thus, measuring total protein would be expected to correlate with allergen levels. A probable explanation for lack of correlation in previous studies is that accelerator chemicals in latex extracts strongly interfere in the protein assay, resulting in elevated protein readings.¹² To overcome this problem the ASTM 5712-95 protocol introduces a precipitation step, which serves to concentrate the protein and to remove the chemical compounds that interfere in the assay. Using this method, we now report a good correlation between the Lowry total protein levels and SPT reactivity. The ELISA, which measures antigenic protein, also demonstrated a good correlation with SPT reactivity. The rank order of SPT reactivity of the nine NRL-containing extracts matched the protein levels measured by both protein assays. The major exception observed was the test powdered glove that measured higher protein levels than expected on the basis of SPT reactivity. This glove was produced by using a calcium carbonate powder rather than the more common cornstarch powder. This processing change may have altered the allergenic potential of the residual latex proteins, or this glove may have had increased amounts of proteins that were less allergenic in this patient population. However, given the heterogeneity of reactivity to specific latex proteins,²⁸⁻³¹ our data support the conclusion that protein levels can be useful in assessing the relative allergenicity of NRL gloves.

An equally important question relates to the relevance of protein levels. The ASTM D5712-95 test has limited sensitivity, resulting in a high limit of detection. Recently, the Food and Drug Administration has allowed manufacturers to make "low protein" claims on glove packages by using the ASTM D5712-95 protocol. Because of the limitations of the assay, documenting actual protein levels below the 50 $\mu\text{g/g}$ level was not allowed. Our data demonstrate that at the detection limit of the Lowry method, 58% of patients allergic to latex

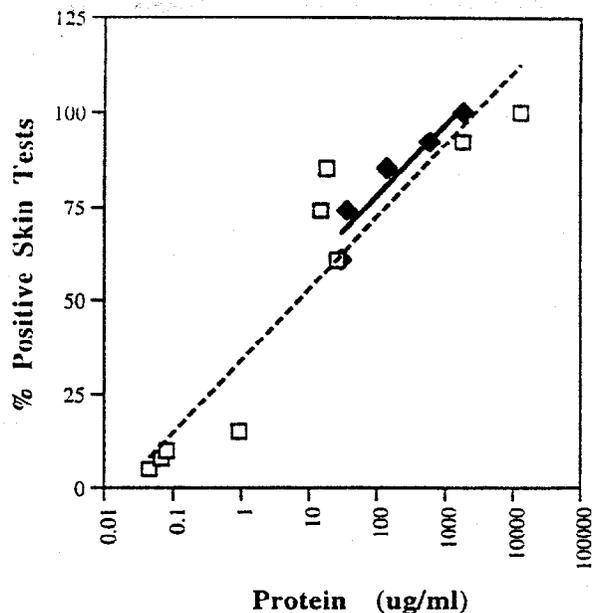


FIG. 1. Correlation of protein levels to SPT reactivity in patients allergic to latex. Patients were skin tested by end-point titration with nine NRL-containing samples. Protein levels in extracts were determined by the ASTM D5712-95 method (solid diamonds) or the LEAP method (open squares). Total percent of patients with skin test reactivity (to any dilution of extract) was plotted against protein levels, and curve fitting was performed by logarithmic regression analysis.

will still react to lower protein levels. The ASTM D5712-95 may lack the sensitivity to provide clinically relevant data.

Immunologic assays are physiologically more relevant assays in which antibodies are used to specifically measure reactive protein. The LEAP was found to be 150 times more sensitive than the ASTM 5712-95 method, and it correlates well with the RAST inhibition assay.²¹ The good correlation of the ELISA method with the SPT results and the expanded range of detection allows for a method that can more easily discriminate low protein levels. Glove extracts testing below the minimum level of detection of 0.06 $\mu\text{g/ml}$ (0.2 $\mu\text{g/g}$) would have a significantly lower potential (11%) for eliciting type I reactions in latex-sensitized individuals. However, caution must be exercised in attempting to extrapolate these results to the actual clinical situation. The results of SPT reactivity may be different than the actual in-use situation. It could be speculated that hydrophobic proteins, which might not be extracted in saline solution, may be released by the sweat on a gloved hand or by direct skin contact with latex. Latex-sensitive

individuals must still be assumed to be highly allergic to any NRL-containing glove and cautioned to only use non-latex products.

We thank Zong-Lu Shen for excellent technical assistance.

REFERENCES

- Sussman G, Tarlo S, Dolovich J. The spectrum of IgE-mediated responses to latex. *JAMA* 1991;265:2844-7.
- Swanson MC, Bubak ME, Hunt LW, Yunginger JW, Warner MA, Reed CE. Quantification of occupational latex aeroallergens in a medical center. *J Allergy Clin Immunol* 1994;94:445-51.
- Turjanmaa K. Incidence of immediate allergy to latex gloves in hospital personnel. *Contact Dermatitis* 1987;17:270-5.
- Arellano R, Bradley J, Sussman G. Prevalence of latex sensitization among hospital physicians occupationally exposed to latex gloves. *Anesthesiology* 1992;77:905-8.
- Kelly KJ, Kurup VP, Reijula KE, Fink JN. The diagnosis of natural rubber latex allergy. *J Allergy Clin Immunol* 1994;93:813-6.
- Ownby D, Ownby H, McCullough J, Shafer A. The prevalence of anti-latex IgE antibodies in 1000 volunteer blood donors [abstract]. *J Allergy and Clin Immunol* 1994;93:282.
- Tarlo SM, Sussman G, Contala A, Swanson MC. Control of airborne latex by use of powder-free latex gloves. *J Allergy Clin Immunol* 1994;93:985-9.
- Kelly KJ, Kurup V, Zacharisen M, Resnick A, Fink JN. Skin and serologic testing in the diagnosis of latex allergy. *J Allergy Clin Immunol* 1993;91:1140-5.
- Schwartz HJ. Latex: a potential hidden "food" allergen in fast food restaurants. *J Allergy Clin Immunol* 1995;95:139-40.
- Yunginger JW, Jones RT, Farnsway AF, Kelso JM, Warner MA, Hunt LW. Extractable latex allergens and proteins in disposable medical gloves and other rubber products. *J Allergy Clin Immunol* 1994;93:836-42.
- Beezhold D. Measurement of latex proteins by chemical and immunological methods. In: *Latex protein allergy: the present position. Proceedings of the International Conference*. Amsterdam: 1993:25-31.
- Beezhold D. LEAP: Latex ELISA for Antigenic Protein. A preliminary report. *Guthrie J* 1992;61:77-81.
- Melton AL. Allergenicity of latex syringe components. In: *International Latex Conference: sensitivity to latex in medical devices*. Baltimore: 1992:26.
- Beezhold D, Beck WC. Surgical glove powders bind latex antigens. *Arch Surg* 1992;127:1354-7.
- Vassallo SA, Thurston TA, Kim SH, Tradres ID. Allergic reaction to latex from stopper of a medication vial. *Anesth Analg* 1995;80:1057-8.
- Turjanmaa K, Luirila K, Mäkinen-Kiljunen S, Reunala T. Rubber contact urticaria. Allergenic properties of 19 brands of latex gloves. *Contact Dermatitis* 1988;19:362-7.
- Vandenplas O, Delwiche J-P, Depelchin S, Sibille Y, Vande Weyer R, Delaunois L. Latex gloves with a lower protein content reduce bronchial reactions in subjects with occupational asthma caused by latex. *Am J Respir Crit Care Med* 1995;151:887-91.
- Jones RT, Scheppmann DL, Heilman DK, Yunginger JW. Prospective study of extractable latex allergen contents of disposable medical gloves. *Ann Allergy* 1994;73:321-5.
- Patterson P. Latex allergies. *OR Manager* 1995;11:1-11.
- Sussman GL, Beezhold DH, Perrella F, Jones J. IgE dependent reactions to urological catheter extracts by skin testing in latex allergic patients. *Ann Allergy* 1995;75:133-7.
- Beezhold D, Swanson M, Zehr BD, Kostyal D. Measurement of natural rubber proteins in latex glove extracts: comparison of the methods. *Ann Allergy* 1996;76:520-6.
- Zehr BD, Kostyal DA, Beezhold DH. Antigenic proteins in latex glove extracts: sources of variability. *Biomed Instrum Technol* 1995;29:434-8.
- Taylor JK. *Quality assurance of chemical measurements*. Chelsea [MI]: Lewis Publishers, 1987:57.
- Beezhold DH, Chang N-S, Kostyal DA, Sussman G. Identification of a 46 kilodalton latex protein allergen in health-care workers. *Clin Exp Immunol* 1994;98:408-13.
- Swanson MC, Yunginger JW, Reed CE. Measurement of latex aeroallergens in nine dental offices. *J Allergy Clin Immunol* 1995;95:153-8.
- Subramaniam A. Reduction of extractable protein content in latex products. In: *International Latex Conference: sensitivity to latex in medical devices*. Baltimore: 1992:63.
- Hamilton RG, Charous BL, Adkinson NF, Yunginger JW. Serologic methods in the laboratory diagnosis of latex rubber allergy: study of nonammoniated, ammoniated latex and glove (end-product) extracts as allergen reagent sources. *J Lab Clin Med* 1994;123:594-604.
- Alenius H, Mäkinen-Kiljunen S, Turjanmaa K, Palosuo T. Allergen and protein content of latex gloves. *Ann Allergy* 1994;73:315-20.
- Mäkinen-Kiljunen S, Turjanmaa K, Palosuo T, Renula T. Characterization of latex antigens and allergens in surgical gloves and natural rubber by immunoelectrophoretic methods. *J Allergy Clin Immunol* 1992;90:230-5.
- Jaeger D, Kleinhans D, Czuppon AB, Baur X. Latex specific proteins causing immediate-type cutaneous, nasal, bronchial, and systemic reactions. *J Allergy Clin Immunol* 1992;89:759-68.
- Wrangsjö K, Wahlberg JE, Axelsson JGK. IgE-mediated allergy to natural rubber in 30 patients with contact urticaria. *Contact Dermatitis* 1987;19:264-71.

Title: Endotoxin Results for Absorbo® HP GJB 127/6

Author: Kimberly Gigantino

Date: September 24, 1999

Log Number/Notebook: 9909209/11181

Background:

This report gives the endotoxin levels for one starch product: **Absorbo® HP GJB 127/6**. This starch was made in Thailand and is used in Latex gloves. There was some concern that endotoxins, substances found in gram negative bacteria, could cause an adverse reaction to people wearing latex gloves.

Method:

1 gram of sample was extracted in depyrogenated test tubes with 10 mL of LAL reagent water for one hour at room temperature. The sample was vortexed for one minute every ten minutes during this hour. The sample was then allowed to settle for one additional hour at room temperature. A 1:100 dilution was made and 2 fold dilutions were made from that. Duplicate test tubes of sample at each dilution were tested. The sample was also "spiked" with endotoxin at 2 lambda as a positive control (See attached SOP for method details).

Table 1: Results on the Microbiological Testing of Samples

| Dilution | Absorbo® HP GJB 127/6 |
|------------------------------------|-----------------------|
| spiked sample | +,+ |
| 1: 100 | -, - |
| 1:2 | -, - |
| 1:8 | -, - |
| Endotoxin Activity in EU/MI | < 3 |

Key:

+ = positive gel clot

- = negative gel clot

Conclusions:

The endotoxin activity of the starch was very low. In an article by Williams and Halsey, "Endotoxin as a factor in adverse reactions to latex gloves", they found gloves to have between .9 - 28,000 EU/ML of endotoxin activity. They found the dry powder to be 3.2 EU/ML. Our starch, at < 3 EU/ML, is within the ranges the article found and is acceptable.

SOP for Detecting Endotoxin in Starch

Principle –

This SOP describes the materials and methods involved in performing the Bacterial Endotoxin Test on starch samples. Limulus Amoebocyte Lysate (LAL) is an indicator of the presence of bacterial endotoxins or Pyrogens. Endotoxins are heat stable compounds that can produce fever, shock or death if in the blood stream. The bacterial endotoxin test is a test for estimating endotoxin concentration in starch samples.

2. Safety -

Personal protective equipment such as a lab coat, safety glasses and gloves must be worn. The analyst must wash his/her hands before and after working with microorganisms. All contaminated materials must be sterilized by autoclaving and the work surfaces used must be disinfected before and after use.

3. Apparatus -

1. 15 mL Corning Polystyrene sterile, disposable centrifuge tubes for dilutions, Fisher cat. # 05-538-51
2. 10 X 75 mm soda lime glass tubes, depyrogenated, Associates of Cape Cod, cat # TSO50
3. Disposable glass pipets, from Fisher
4. Vortex Mixer
5. MLA 100 uL pipet and pipet tips
6. Incubator or water bath capable of maintaining 37⁰ C
7. Depyrogenated Erlenmeyer flasks, beakers, funnels, volumetric flasks and metal spatulas may be needed.
8. Parafilm[®]

All apparatus that comes in contact with the LAL test must be pyrogen free. This is accomplished by heating the materials at 250⁰ C for 3 hours or purchasing them pyrogen free.

4. Reagents –

1. CSE- Control Standard Endotoxin .05ug/vial- Associates of Cape Cod, 1-800-LAL-TEST
2. LAL Reagent- Pyrotell 5 mL/vial- Associates of Cape Cod
3. LAL Reagent Water- from Associates of Cape Cod or DI water sterilized in an autoclave for 15 minutes in a depyrogenated bottle.

5. Procedure -

A. Preparation

- 1) Equipment preparation- N/A
- 2) Instrumentation setup- N/A
- 3) Standards preparation- See SOP:CAG:BIO:027ROO
- 4) Sample preparation-N/A

B. Analysis -

Procedure:

1. For each sample to be tested, place 1g into 10 mL of LAL reagent water.
2. Vortex for 1 minute every 10 minutes for one hour at room temperature.
3. Let the samples stand for at least one additional hour at room temperature.
4. Take 0.10 uL of the starch extract (top liquid). This is the undiluted sample. From this undiluted sample, make a 1:100 dilution, 0.1 mL into 9.9 mL of LAL reagent water. From this 1:100 dilution, further dilutions can be made if it is necessary.
5. 1:1, 1:2, 1:4, and 1:8 dilutions can be made from the 1:100 tube. Also run a "spiked" sample which is the undiluted sample "spiked" with 2λ control endotoxin, where λ is the sensitivity of the Lysate.
6. Place 0.1 mL of each of the CSE standard series 4λ , 2λ , $\lambda/2$, $\lambda/4$ into duplicate test tubes. Also place 0.1 mL of each sample to be tested into duplicate tubes.
7. Pipet 0.1 mL of reconstituted LAL reagent into each tube. Vortex immediately and carefully place into the 37°C waterbath or incubator. The incubation time should be 60 +/- 2 minutes.
8. After 60 minutes, carefully remove each tube, invert 180 degrees once and examine for a firm clot. A positive test is indicated by a clot that maintains its integrity upon inversion.
9. Calculations:
If the spiked samples are positive and the CSE series is within a 2 fold dilution of λ , then the endotoxin concentration can be determined.
10. The last positive tube in the dilution series is used for calculations. For example: if the 1:100 tube is positive, then you would multiply 100 times the λ sensitivity or the result. If the 1:2 tube was positive, then you would multiply 2 X 100 X the λ sensitivity for the endotoxin concentration.

Sensitivity and Precision –

The minimum amount of endotoxin that can be detected by a lot of LAL reagent is termed the “LAL sensitivity” λ or label claim. The LAL reagent has a two-fold dilution range of error.

ABSORBO[®] HP Absorbable Dusting Powder, USP

TOTAL PLATE COUNT

(1996-1999)

Date: October 28, 1999

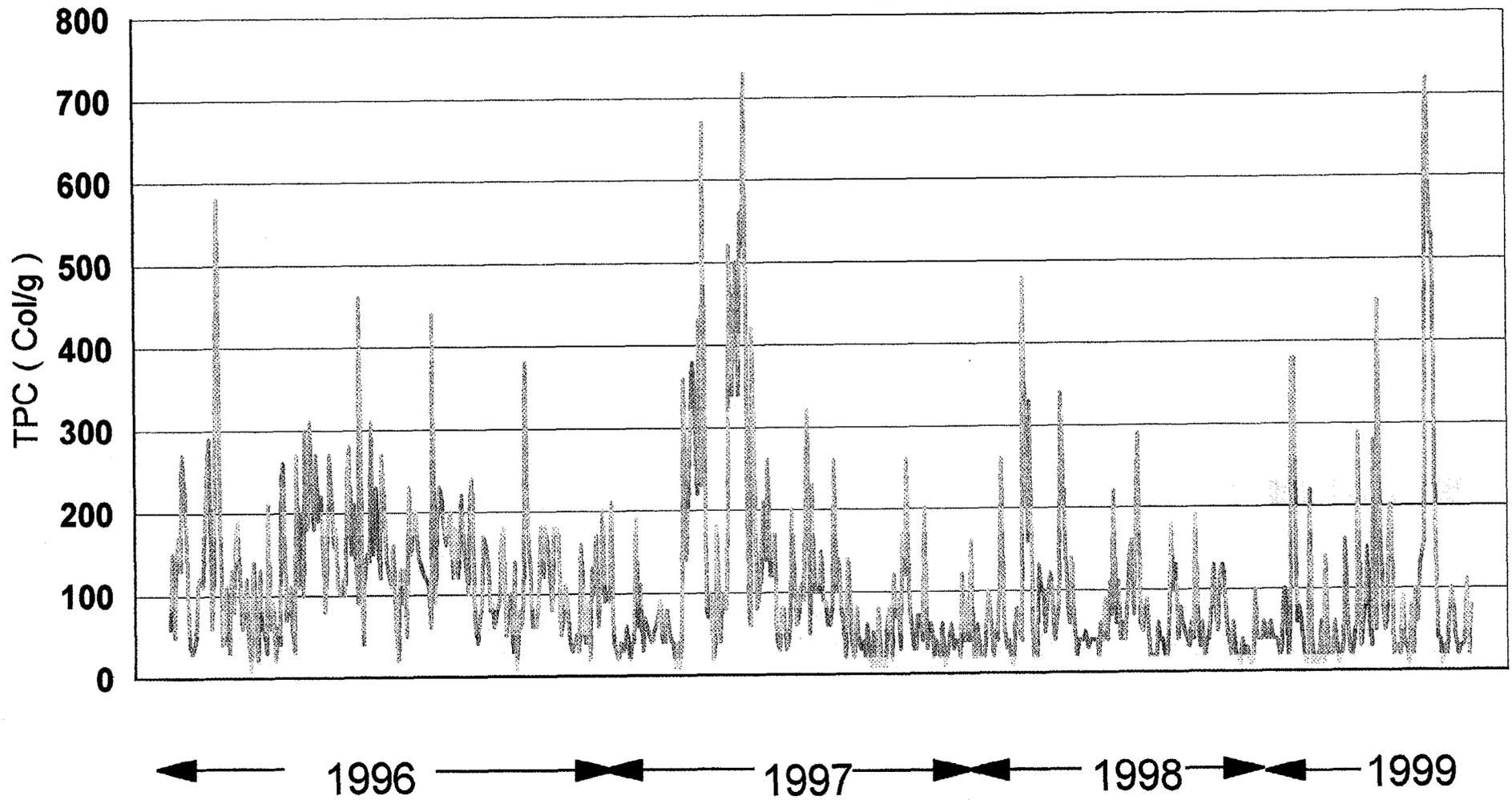
Provided by:
National Starch and Chemical Company
10 Funderne Avenue
Bridgewater, New Jersey 08807

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32872/lak

ABSORBO HP

Total Plate Count (1996-1999)



Cornstarch powder on latex products is an allergen carrier

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Allergic reactions of the upper respiratory tract during use of powdered latex rubber gloves have been recently associated with sensitivity to latex. We have studied the ability of cornstarch powder to bind latex proteins and evaluated allergenic properties of the bound protein. Allergenicity was determined by competitive inhibition of human anti-latex IgE binding to solid-phase latex antigen. Cornstarch extracted from powdered latex products and clean cornstarch exposed to latex protein extracts were evaluated in comparison with clean unexposed cornstarch. Both exposed cornstarch preparations inhibited specific binding of anti-latex IgE antibodies to latex proteins in a dose-response manner. Latex-exposed cornstarch diluted 50% vol/vol produced complete inhibition, whereas greater dilutions exhibited variable levels of inhibition, depending on the source of cornstarch-bound proteins, insolubilized latex proteins, and IgE antibody-containing human serum used. Cornstarch not exposed to latex had no inhibitory activity. The study demonstrates that cornstarch indeed binds allergenic latex proteins and supports the causative relationship between allergic reactions in individuals with latex sensitivity and the exposure to airborne particles from powdered latex products. (J ALLERGY CLIN IMMUNOL 1994;93:751-8.)

Key words: Latex, natural rubber, cornstarch, allergen, type 1 allergic reaction, IgE antibodies

Reports of immediate-type hypersensitivity reactions to latex proteins have raised a concern among manufacturers of latex medical products and the medical community.¹⁻³ A recent increase in the severity and prevalence of latex sensitivity reactions³⁻⁵ is probably associated with the increased use of natural latex rubber products as a barrier against viral infections.⁶ It has been demonstrated that a broad spectrum of allergic reactions to latex-containing products is primarily due to leachable proteins, which are common constituents of natural latex rubber.⁷⁻⁹ These proteins remain on the surface of latex products after the manufacturing process and are released during use of such products. Frequent exposure to latex proteins, combined with genetic factors predisposing individuals to atopy, may result in a gradual development of IgE-mediated latex hypersensitivity. This has led to severe anaphylactic reactions

Abbreviations used

| | |
|---------|--|
| AL: | Ammoniated latex |
| BSA: | Bovine serum albumin |
| GL-JHU: | Glove extract prepared at Johns Hopkins University |
| NAL: | Nonammoniated latex |
| PBS: | Phosphate-buffered saline |

in patients with latex sensitivity who are undergoing surgery.¹⁰⁻¹⁴

More commonly, there have been reports of contact urticaria¹⁵⁻¹⁸ and allergic reactions involving the upper airway¹⁹⁻²² during use of powdered latex gloves. These reactions were initially thought to be related to an IgE antibody specific for the cornstarch, which is used as a dry lubricant, although direct correlation between the two has never been substantiated. Only recently was an association between these reactions and latex proteins identified,²³⁻²⁵ when patients exhibiting these symptoms were found to have latex-specific IgE antibodies in their sera.²⁵ Although such cases were observed less frequently than skin reactions to latex gloves, it was speculated that the same antigen may be transferred from gloves to the cornstarch particles and thus appear as an air-

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borne antigen.^{24, 25} A report of individuals with latex allergy experiencing immediate-type reactions while being in the same room with an individual using a cornstarch-powdered latex product supports this hypothesis.²³

The goal of this study was to determine whether cornstarch adsorbs proteins from latex and therefore acts as an allergen carrier that can induce respiratory allergic reactions in individuals with latex sensitivity.

METHODS

Sources of latex proteins

Raw ammoniated latex (AL) from *Hevea brasiliensis* in Malaysia was obtained through Guthrie Latex, Inc. (Tucson, Ariz.). Nonammoniated latex (NAL) was also obtained from a Malaysian plantation by collecting *Hevea* sap directly into Tris buffer without the addition of ammonia. Latex products used for protein extraction in this study were commercially available cornstarch-powdered surgical gloves and cornstarch-powdered examination gloves.

Extraction of protein from raw latex

Unprocessed natural latex and raw AL were diluted 1:3 with phosphate-buffered saline (PBS) (Gibco, Grand Island, N.Y.) and centrifuged at 10,000 g for 40 minutes. The middle aqueous fraction containing latex proteins was collected, centrifuged at 40,000 g, and filtered through a Millipore 0.45 μ m low-protein-binding membrane (Millipore Corp., Bedford, Mass.) to eliminate any remaining latex particles. The NAL extract (NAL-3) and the AL extract (AL-6) both contained about 1% proteins as determined by the Lowry method. Both extracts were concentrated with an Amicon filtration unit (Amicon Inc., Beverly, Mass.) with a YM1 membrane (molecular weight cutoff = 1000 D).

Extraction of proteins from latex gloves

Gloves were cut open to allow contact of the extraction media with all surfaces. Proteins were extracted with sterile PBS for 1 hour at 37° C with continuous agitation. The protein extract was separated from any residual cornstarch powder by centrifugation for 10 minutes at 1000 g and then filtered through a Millipore 0.45 μ m filter. The cornstarch pellet was washed with PBS to remove unbound proteins and saved for further analysis. Proteins in the PBS extracts were also concentrated by an Amicon pressure filtration unit. All extracts were stored at 4° C for up to 1 week or at -20° C for more extended periods.

Source of cornstarch preparations

In addition to cornstarch extracted from latex gloves, we studied unexposed dry cornstarch, which is used for donning latex gloves. This preparation, kindly supplied by Baxter Healthcare Co. (Valencia, Calif.), contains

primarily carbohydrates, which are heavily cross-linked to prevent extensive swelling when in contact with moisture. The virgin cornstarch, which had never been in contact with any latex product, was exposed in our experiment to latex proteins extracted from unprocessed and ammoniated natural latex material and to those extracted from latex gloves. Treated and untreated cornstarch samples were evaluated for the level of protein, in parallel with cornstarch preparations collected directly from powdered latex gloves. For direct binding immunoassay, cornstarch was collected from 12 lots of latex gloves. PBS (10 ml/gm of latex) was carefully pipetted into gloves. Each glove was tied off, inserted in a plastic bag, and incubated for 1 hour at 37° C with agitation. The PBS-cornstarch slurry was collected by nicking a finger and milking the glove. The cornstarch was sedimented by centrifugation (10 minutes at 2000 g) and washed three times with 50-fold excess volumes of RAST buffer (PBS-0.1% bovine serum albumin [BSA] containing 0.01% sodium azide and 0.05% Tween 20) to remove any loosely bound or solution-phase proteins. Pelleted washed cornstarch was reconstituted to 50% vol/vol in RAST buffer for storage or to 1% vol/vol for immediate use. Stored cornstarch samples were rewashed with RAST buffer before dilution for use in the assay.

Protein determination methods

The protein determinations on cornstarch fractions were performed by micro-Kjeldahl's method, whereas latex protein extracts used in this study were evaluated by both the modified Lowry method and micro-Kjeldahl's method to establish correlation of the measurements.

The Lowry method used in this study was modified in our laboratory. Briefly, 2% alkaline tartrate (reagent A) and a 0.1% solution of copper sulfate (reagent B) were prepared in our laboratory, and the Folin and Ciocalteu's phenol reagent was obtained from Sigma Chemical Co. (St. Louis, Mo.). On the day of assay, reagents A and B were mixed in a ratio of 9:1, respectively (working solution). Protein samples were prepared by adding 5 ml of working solution to 0.1 ml of protein-containing extract, mixed, and left at room temperature. After 30 minutes, 0.5 ml of 1N phenol reagent was added, mixed well, and incubated for an additional 60 minutes. Protein levels were evaluated against serial dilutions of the reference protein ovalbumin, fraction V (Sigma Chemical Co.) with a DU-7 spectrophotometer (Beckman Instrument Co., Columbia, Md.) or a UV-Max microplate reader (Molecular Devices Corp., Menlo Park, Calif.) at a wavelength of 595 nm.

A modified micro-Kjeldahl procedure was used to determine protein levels in cornstarch fractions. Dried powder samples were mixed with 1 ml of 18N sulfuric acid and heated to boiling until the solution became clear or light brown. The samples were then cooled, mixed with 1 ml of 30% hydrogen peroxide, and boiled again for 1 hour. After cooling, the samples were

diluted to the desired concentration range, and total nitrogen levels were determined on a DU-7 spectrophotometer by direct comparison against an analogously processed standard protein.

Human serum specimens

The sera used in the competitive inhibition studies were collected from individuals who had a clinically documented allergy to latex products, positive skin prick test results through a rubber glove, and a positive serum IgE antibody level as measured by RAST. Sera JD and LJ (25 and 44 years old, respectively) were collected from two female health care workers who had respiratory symptoms and mild systemic reactions after exposure to latex gloves in an operating room. Serum FT was collected from a 6-year-old child, who had undergone multiple operations for correction of spina bifida. He experienced local swelling, respiratory problems, and systemic allergic reactions on exposure to latex gloves. Sera from JD and FT were used in the competitive inhibition assay to determine the presence of latex allergens attached to cornstarch. The level of latex-specific IgE antibody in the reference serum LJ was 80 ng/ml as determined by depletion analysis with latex glove extract prepared by Johns Hopkins University (GL-JHU) as the allergen source for the allergo-absorbent test.

Competitive inhibition immunoassay

The quantity of latex allergens bound to the cornstarch was determined by use of a sequential-addition, competitive antigen inhibition format of an immunoenzymometric assay for IgE antibodies.²⁶ An inhibition step was initiated 1 day before the performance of the assay. Each test and control cornstarch preparation was prewashed; diluted to 50%, 5%, and 0.5% vol/vol with PBS-1% BSA; and mixed individually with an equal volume of anti-latex IgE-containing serum. At the same time, flat-bottom Immulon IV Removawell plates (Dynatech, Chantilly, Va.) were coated overnight with either NAL-3, AL-6, or GL-JHU (10 µg/ml, 0.1 ml/well) at 4° C. Plates were washed with PBS-0.05% Tween 20 and blocked for 1 hour with PBS-1% BSA at 23° C. After blocking and washing plates, 0.1 ml of each serum, preincubated for 16 hours with cornstarch or buffer, was added to the respective wells. A standard curve was constructed with eight twofold dilutions of the LJ serum in duplicate, starting from 80 ng/ml (undiluted). All samples were pipetted within 15 minutes with no interruption. After a 2-hour incubation, plates were washed with PBS-0.05% Tween 20 and biotinylated mouse-anti-human IgE Fc (clone HP6029, Hybridoma Reagent Laboratory, Baltimore, Md.)²⁶ was added (0.1 ml, 1 µg/ml in PBS-1% BSA). After 1-hour incubation at 23° C, streptavidin-horseradish peroxidase (avidin-HRP, Sigma Chemical Co.) was added (0.1 µg/ml, 0.1 ml/well). Plates were incubated for 1 hour and washed four times; substrate (3,3', 5,5'-tetra-

methylbenzidine; KPL, Gaithersburg, Md.) was then added. The reaction was stopped with 0.1 ml of 4N H₂SO₄ at 5 minutes. Optical density was read with a Dynatech M400 spectrophotometer at a wavelength of 450 nm. The percent inhibition that resulted from binding of latex-specific IgE antibodies to insolubilized latex proteins attached to the cornstarch was calculated in comparison with antibody levels measured in control wells, when the same serum was incubated with an equal volume of buffer alone.

Direct binding immunoassay for allergen on cornstarch

A direct binding radioimmunoassay used cornstarch collected directly from the gloves as the solid-phase "allergoabsorbent."

Control (unexposed) or test cornstarch from the latex gloves was pipetted into their respective 12 × 75 mm plastic test tubes in duplicate (0.5 ml of 1% vol/vol). Human sera (LJ, LB) were then added (0.1 ml/tube; undiluted = 80 ng/ml of anti-latex IgE for LJ, 42 ng/ml anti-latex IgE for LB). A serum sample from a nonatopic volunteer (total serum IgE of 2 ng/ml) was used as a negative control. A "heterologous" reference dose-response curve was constructed with 1% vol/vol Sepharose-GL-JHU and serum LJ diluted from neat to 1:512. Antibodies were permitted to bind (16-hour rotation at 23° C), and unbound proteins were removed by four washings with RAST buffer. Iodine 125-labeled anti-human IgE Fc was added to detect IgE bound to solid-phase latex proteins. After 16 hours, unbound radiolabeled anti-IgE was removed by washing, and bound radioactivity was detected in a Capintec 16-well gamma counter (Capintec, Inc., Ramsey, N.J.). The amount of bound IgE was calculated by interpolation from the reference IgE antibody standard curve in nanograms per milliliter of anti-latex IgE. The amount of latex allergen on the cornstarch was assumed to be proportional to the amount of IgE antibody bound.

RESULTS

To identify latex proteins on the dusting cornstarch powder in gloves, we extracted proteins from commercial latex gloves and isolated the powder fraction by centrifugation and washing to remove unbound proteins.

Protein level measurements, performed by Kjeldahl's method, indicated the presence of protein in the cornstarch fraction, as well as in the PBS extract (Table I). The quantity of proteins eluted from three types of gloves varied markedly (from 6.2 to 17.3 mg per glove). Protein levels in the cornstarch samples varied over a wider range, from 0.1 to 1.3 mg per glove. On the basis of this limited sampling, it appears that the amounts of protein bound to the cornstarch depend on the total amount of leachable protein per glove and

TABLE I. Protein levels in the PBS extract and donning powder components from latex gloves

| Extract fraction | Source of latex proteins | | |
|--|--------------------------|-------|-------|
| | 1 (S) | 2 (E) | 3 (E) |
| PBS extract (mg protein per glove) | 17.3 | 6.2 | 9.0 |
| Cornstarch (mg protein per glove) | 1.3 | 0.1 | 1.1 |
| Cornstarch (% of total protein) | 7 | 1.7 | 11.3 |
| Cornstarch (mg starch per glove) | 0.22 | 0.1 | 0.17 |
| Cornstarch (mg protein per gm of starch) | 5.9 | 1.2 | 7.4 |

Protein levels were evaluated by the Kjeldahl method.
S, Surgical glove; E, examination glove.

TABLE II. Binding of proteins to cornstarch powder

| Protein source* | Protein level in cornstarch (mg/gm)† | | | |
|-----------------|--------------------------------------|----------------|---------------|-------------|
| | Before exposure | After exposure | Protein bound | Binding (%) |
| PBS (control) | 1.9 | 1.79 | -0.11 | -9.4 |
| NAL-3 | 1.9 | 2.91 | 1.0 | 34.6 |
| AL-4 | 1.9 | 2.24 | 0.34 | 15.2 |
| Glove no. 4‡ | 1.9 | 2.40 | 0.5 | 20.8 |
| Glove no. 5‡ | 1.9 | 2.5 | 0.6 | 24.0 |

One hundred milligrams of cornstarch was mixed with 1 ml of protein solution and incubated overnight at room temperature.

*Protein concentrations in extracts were between 1.2 and 1.8 mg/ml.

†Protein levels were determined by the Kjeldahl method.

‡Gloves no. 4 and no. 5 were commercially available latex examination gloves.

the quantity of cornstarch powder applied to the glove. Glove no. 2 contained the lowest quantity of protein and the smallest amount of powder of all of the gloves studied. Only 1.7% of the total protein present in the eluate was detected in the cornstarch fraction. Cornstarch from the other two gloves had greater amounts of bound protein, ranging from 7% to 11% of total protein found in the eluate. This demonstrates that cornstarch effectively adsorbs proteins from the glove surface.

The unexposed virgin cornstarch alone contained about 0.2% of native proteinaceous materials (Table II). Laboratory exposure of the untreated cornstarch to the PBS-protein extracts from gloves and comparable extracts from raw NAL and AL resulted in additional binding of protein from all extracts. The level of binding observed with NAL proteins was higher than that observed with AL proteins and glove extracts (Table II). This finding was confirmed in the repeat experiments. Decreases in protein levels of 25% to 38%, which were observed with the extracts before and after incubation with previously unexposed cornstarch, supported these findings (Table III). It appears that protein binding to the

cornstarch occurs in a random manner rather than resulting from specific interaction of the powder with a particular protein molecule.

Once latex glove proteins were shown to be readily adsorbed by the cornstarch, we next explored whether these proteins were allergens, using a competitive antigen inhibition enzyme immunoassay similar to the RAST inhibition assay. A microtiter plate solid phase method was used to bind latex allergens because the cornstarch-latex protein-antibody complex formed during the first incubation could be readily separated from antibody bound to allergenic protein on the microtiter plate well. Conventional allergo-adsorbents (e.g., Sepharose, paper disc) would not have allowed us to distinguish allergo-adsorbent-bound IgE antibody from that bound to cornstarch. The data in Fig. 1 and Table IV indicate that latex allergen is present on all washed cornstarch preparations in a sufficient quantity to competitively inhibit the binding of IgE antibodies to latex solid-phase antigens. The inhibition of IgE antibody binding to the plate allergen was dose-dependent, increasing as higher (vol/vol) amounts of the cornstarch-latex preparations were preincubated

TABLE III. Protein levels in extracts after incubation with cornstarch

| Protein extract | Protein levels in extracts (mg/ml)* | | Reduction (%) |
|-----------------|-------------------------------------|--------------------------|---------------|
| | Before exposure to starch | After exposure to starch | |
| PBS | 0 | 0.02 | — |
| NAL-3 | 1.35 | 0.9 | 33.3 |
| AL-4 | 1.84 | 1.39 | 24.5 |
| Glove no. 4† | 1.15 | 0.84 | 27.0 |
| Glove no. 5 | 1.38 | 1.0 | 27.5 |

*Protein levels were determined by a modified Lowry method.

†Gloves no. 4 and no. 5 were commercially available latex examination gloves.

with serum before enzyme immunoassay analysis. In fact, the 5% vol/vol concentration of the cornstarch that had been pretreated with NAL-3, AL-6, and GL-JHU protein extracts inhibited the binding of IgE antibodies to the plate allergen by more than 95%. All preparations showed almost complete inhibition at 50% vol/vol. The measured IgE antibody levels and percentage inhibition observed at the 0.5% vol/vol cornstarch concentration discriminate more effectively among the inhibitory capacities of the five different cornstarch-latex allergen preparations.

The cross-inhibition studies demonstrated that the homologous latex allergen extract cornstarch produced the highest degree of competitive inhibition of IgE binding to each of the allergen source materials. IgE antibody from serum JD demonstrated restricted reactivity to the NAL proteins, which prevented its use in competitive inhibition with AL-6 cornstarch. Inhibition of up to 98% with cornstarch-latex protein preparations as low as 0.05% vol/vol in concentration demonstrates clearly that latex allergen is attached to the cornstarch. Untreated "clean" cornstarch showed no inhibitory activity.

Finally, the presence of latex allergen on cornstarch isolated from gloves was confirmed by direct binding immunoassay experiments in which the cornstarch was used as the solid-phase antigen and incubated with IgE antibody-containing sera. Radiolabeled anti-human IgE detected binding of IgE antibodies to glove-extracted cornstarch (1% vol/vol) in comparison with untreated cornstarch. The level of latex-specific antibody that bound to 1% vol/vol glove-extracted cornstarch was 3.3 ± 2.3 ng/ml (mean \pm SD) (range, 1.0 to 9.6 ng/ml, $n = 12$), compared with IgE antibody binding levels of less than 0.3 ng/ml for binding of the same IgE antibody to virgin cornstarch at the same concentration. Moreover, binding levels ob-

served with the buffer and nonatopic control serum to the cornstarch from 12 lots of gloves were also less than 0.3 ng/ml.

DISCUSSION

The cornstarch used by the manufacturers of latex products as a donning powder is a heavily cross-linked carbohydrate, with particle sizes ranging from 1 to 3 μ m in diameter. There is only a slight increase in the mean diameter of particles when it is exposed to moisture. Because the cornstarch particles are in a respirable size, they can transport latex allergen into an individual's respiratory tract and induce sensitization or allergic reaction. The goal of this study was to demonstrate that allergenic latex protein is attached to the cornstarch that has been recovered from latex products such as surgical and examination gloves.

Our studies demonstrate conclusively that protein molecules from various natural latex sources are effectively bound to cornstarch donning powder. We observed that the starch removed from latex gloves had higher protein levels than could be accounted for by proteinaceous material present in unused cornstarch powder. Analysis of the cornstarch extracted from gloves suggests that the amount of protein binding to cornstarch depends on both the total amount of protein in latex material and the amount of starch deposited on the particular product. Protein levels measured in the cornstarch before and after exposure to PBS-extracted latex proteins support this hypothesis. The protein level in cornstarch was highest after exposure to proteins extracted from NAL. Unexposed cornstarch binds proteins extracted from raw unprocessed latex material and those extracted from powdered latex products. This indicates that the mechanism of binding (probably

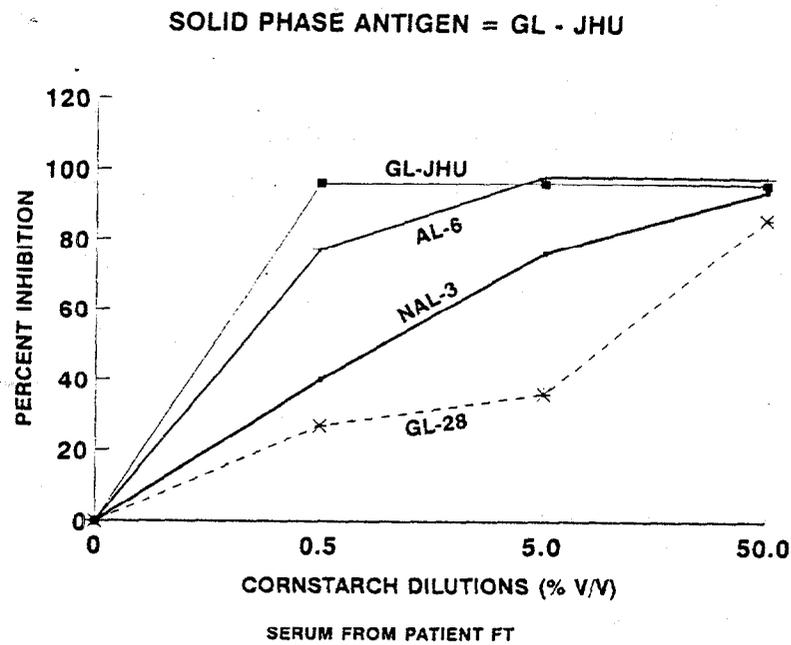


FIG. 1. Inhibition of IgE binding to latex antigens by cornstarch-bound latex proteins. Dose-response curve for 0.5%, 5.0% and 50.0% vol/vol dilutions of cornstarch preparations.

TABLE IV. Cornstarch-induced inhibition of anti-latex IgE binding to solid-phase antigens

| Latex-cornstarch inhibitor (0.5% vol/vol) | IgEa-latex serum | Microtiter plate adsorbed latex allergen | | | | | | | |
|---|------------------|--|-------|----------|-----|---------|-------|----------|-----|
| | | NAL3 | | AL6 | | GL-28 | | GL-JHU | |
| | | ng/ml | %I* | ng/ml | %I* | ng/ml | %I* | ng/ml | %I* |
| Control cornstarch | FT | 156 ± 23 | — | 246 ± 21 | — | 191 ± 7 | — | 463 ± 11 | — |
| Buffer control | FT | 142 | 9% | 240 | 2% | 182 | 5% | 462 | 0% |
| NAL3-cornstarch | FT | 39 | 75% | 87 | 65% | 53 | 72% | 279 | 40% |
| AL6-cornstarch | FT | < 10 | > 94% | 16 | 93% | < 8 | > 96% | 104 | 78% |
| GL28-cornstarch | FT | 35 | 78% | 111 | 55% | 42 | 78% | 337 | 27% |
| GL29-cornstarch | FT | < 10 | > 94% | 33 | 87% | < 8 | > 96% | 31 | 93% |
| GL-JHU cornstarch | FT | < 10 | > 94% | 6 | 98% | < 8 | > 96% | < 8 | 99% |
| Control cornstarch | JD | 287 ± 21 | — | ND | ND | ND | ND | 108 ± 11 | — |
| Buffer control | JD | 291 | -1% | ND | ND | ND | ND | 110 | -2% |
| NAL3-cornstarch | JD | 83 | 71% | ND | ND | ND | ND | 25 | 77% |
| AL6-cornstarch | JD | 142 | 50% | ND | ND | ND | ND | 38 | 77% |
| GL28-cornstarch | JD | 311 | 0% | ND | ND | ND | ND | 18 | 83% |
| GL29-cornstarch | JD | 304 | 0% | ND | ND | ND | ND | 83 | 23% |
| GL-JHU cornstarch | JD | 185 | 36% | ND | ND | ND | ND | 14 | 87% |

Control cornstarch is virgin cornstarch not previously exposed to any latex protein (mean ± 1 SD).

ND, Not done.

*%I = percent inhibition = (control cornstarch - test antigen cornstarch) ÷ control cornstarch × 100.

random ionic interaction) is not selective with regard to particular protein molecules. Results obtained with the competitive inhibition and direct binding immunoassays demonstrated that latex proteins bound to cornstarch are allergenic

proteins, which have the potential to induce allergic reactions. We were able to detect significant levels of allergens on all latex-exposed cornstarch preparations, whether they were experimentally exposed to different latex protein extracts or re-

moved directly from commercially available latex gloves.

The sera used to evaluate inhibitory activity of the cornstarch samples were obtained from children and adults who presumably had been sensitized to latex proteins by different routes of exposure. Serum FT was from a child with spina bifida, who had been repeatedly exposed to a variety of medical and consumer latex products through surgical procedures, starting very early in life. Serum JD was from an adult health care worker who was presumably sensitized later in life by repeated exposure to latex gloves. These two subjects represent populations that are presently identified to be at the highest risk for having an allergic reaction to latex.¹⁻³ The level of inhibition of IgE binding to plate-bound latex allergen by preincubation with glove cornstarch differed with these two sera, which suggested the presence of antibodies with a spectrum of latex allergen specificities. IgE antibodies in FT serum reacted four times more strongly with proteins from AL and GL-JHU extracts as compared with equivalent amounts of NAL. Cross-reactivity between NAL and AL preparations was shown when IgE antibodies in the serum from the adult health care worker (JD) reacted twice as strongly to NAL proteins as to GL-JHU-extractable proteins. The IgE antibody binding to each insolubilized protein was effectively inhibited by both homologous and heterologous latex extracts when attached to cornstarch. The interesting difference in the inhibitory activity of the glove, NAL, and AL extracts with these two sera indicate differences in the specificity of antibodies, possibly as a result of differences in the patients' mode of sensitization. Variations in the physical-chemical properties of proteins in latex-containing devices between products and among different sources may also play a role in the observed differences. Other factors, possibly specific to latex sensitization pattern in children with spina bifida, may also be contributing to the observed differences.

The important observation from both the competitive inhibition and the direct binding studies is that cross-reacting allergenic latex proteins from all three major latex sources (NAL, AL, and gloves) attach securely enough to cornstarch so that repetitive mechanical washing does not remove them. One could hypothesize that multiple allergenic proteins insolubilized on cornstarch particles may be more effective in cross-linking multiple IgE antibodies on mast cells than solution-phase latex allergens. This may explain in

part how allergic respiratory symptoms are effectively triggered in sensitized individuals by exposure to trace quantities of cornstarch that is released into the air from an individual removing powdered gloves from across a room.^{23, 25}

In summary, this study clearly demonstrates that cornstarch powder used for donning gloves has the strong propensity to bind proteins when in contact with natural latex material. Airborne particles from powdered latex devices therefore represent a serious threat to individuals with latex sensitivity.

REFERENCES

- Slater JE. Allergic response to natural rubber. *Ann Allergy* 1992;68:203-9.
- Tomazic VJ, Withrow TJ, Fisher BR, Dillard SF. Latex-associated allergies and anaphylactic reactions. *Clin Immunol Immunopathol* 1992;64:89-97.
- Sussman GL. Latex allergy: its importance in clinical practice. *Allergy Proc* 1992;13:67-9.
- Turjanmaa K, Reunala T. Contact urticaria from rubber gloves. *Dermatol Clin* 1988;6:47-51.
- Charpin D, Lagier F, Lhermet I, Vervloet D. Prevalence of latex allergy in nurses working in operating rooms [Abstract]. *J ALLERGY CLIN IMMUNOL* 1991;87:269.
- Tarlo SM, Wong L, Roos J, Booth N. Occupational asthma caused by latex in a surgical glove manufacturing plant. *J ALLERGY CLIN IMMUNOL* 1990;85:626-31.
- Spaner D, Dolovich J, Tarlo S, Sussman G, Buttoo K. Hypersensitivity to natural latex. *J ALLERGY CLIN IMMUNOL* 1989;83:1135-7.
- Slater JE, Mostello LA, Shaer C, Honsinger RW. Type I hypersensitivity to rubber. *Ann Allergy* 1990;65:411-4.
- Morales C, Bosomba A, Carreira J, Sastre A. Anaphylaxis produced by rubber glove contact. Case reports and immunological identification of the antigens involved. *Clin Exp Allergy* 1989;19:425-30.
- Meeropol E, Kelleher R, Bell S, Leger R. Allergic reactions to rubber in patients with myelodysplasia [Letter]. *N Engl J Med* 1990;323:1072.
- Slater JE, Shaer C, Mostello L. Rubber-specific IgE in children with spina bifida [Abstract]. *J ALLERGY CLIN IMMUNOL* 1990;85:293.
- Moneret-Vautrin DA, Mata E, Gueant JL, Turgeman D, Laxenaire MC. High risk of anaphylactic shock during surgery for spina bifida. *Lancet* 1990;335:865-6.
- Leynadier F, Pecquet C, Dry J. Anaphylaxis to latex during surgery. *Anaesthesia* 1989;44:547-50.
- Gold M, Braude MB, Swartz JS, Dolovich J, Shandling B. Interoperative anaphylaxis: an association with latex sensitivity [Abstract]. *J ALLERGY CLIN IMMUNOL* 1991;87:268.
- Van der Meeren HLM, Van Erp PEJ. Life-threatening contact urticaria from glove powder. *Contact Dermatitis* 1986;14:190-1.
- Fisher AA. Contact urticaria due to cornstarch surgical glove powder. *Cutis* 1986;38:307-8.
- Fisher AA. Contact urticaria and anaphylactic reaction due to corn starch surgical glove powder. *Contact Dermatitis* 1987;16:224-35.
- Assalve D, Cicioni C, Perno P, Lisi P. Contact urticaria

- and anaphylactoid reaction from cornstarch surgical glove powder. *Contact Dermatitis* 1988;19:61-78.
19. Seggev JS, Mawhinney TP, Yunginger JW, Braun SR. Anaphylaxis due to cornstarch surgical glove powder. *Ann Allergy* 1990;65:152-5.
 20. Seaton A. Latex as aeroallergen. *Lancet* 1990;336:808-9.
 21. Marcos C, Lazaro M, Fraj J, et al. Occupational asthma due to latex surgical gloves. *Ann Allergy* 1991;67:319-23.
 22. Seaton A, Cherrie B, Turnbull J. Rubber glove asthma. *Br Med J* 1988;296:531-2.
 23. Baur X, Jager D. Airborne antigens from latex gloves [Letter]. *Lancet* 1990;335:912.
 24. Turjanmaa K, Reunala T, Alenius H, Brummer-Korvenkontio H, Palosuo T. Allergens in latex surgical gloves and glove powder. *Lancet* 1990;336:1588.
 25. Jaeger D, Kleinhans D, Czuppon AB, Baur X. Latex-specific proteins causing immediate-type cutaneous, nasal, bronchial and systemic reactions. *J ALLERGY CLIN IMMUNOL* 1992;89:759-68.
 26. Hamilton RG, Adkinson NF Jr. Measurement of total serum immunoglobulin E and allergen-specific immunoglobulin E antibody. In: Rose NR, deMarcio EC, Fahey JL, Friedman H, Penn GM, eds. *Manual of clinical laboratory immunology*. Washington, DC: American Society for Microbiology, 1992:689-701.

Regulation of IgE and IgG₄ responses by allergen specific T-cell clones to bee venom phospholipase A₂ in vitro

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An in vitro antibody response to bee venom phospholipase A₂ (PLA) from peripheral blood mononuclear cells of bee sting-sensitized individuals was achieved after stimulation with PLA and pokeweed mitogen. This stimulation resulted in a secretion of T_{H1}-associated cytokines and induced PLA-specific and nonspecific IgG₄ antibody production but not IgE production. The addition of interleukin-4 (IL-4) to this system decreased the secretion of IgG antibodies, whereas secretion of polyspecific IgE was induced. The mitogen was not required if peripheral blood mononuclear cells were enriched with autologous, PLA-specific, resting T-cell clones in the presence of the antigen. In these experiments the cytokine profile of the particular clone determined the antibody class generated. Low ratios of IL-4 to interferon- γ , induced by the antigen alone or obtained by neutralizing anti-IL-4 antibodies, enhanced IgG₄ antibody formation, whereas IgE levels increased at high ratios of IL-4 to interferon- γ . These results suggest a complementary regulation of the main isotypes, IgE and IgG₄, implicated in allergic and protective hyperimmune responses. (J ALLERGY CLIN IMMUNOL 1994;93:758-67.)

Key words: T_H cells, interleukin-4, IgG₄, IgE, bee sting allergy

Physical contact of B lymphocytes with activated T helper (T_H) lymphocytes renders B cells responsive to subsequent cytokine-mediated differentiation signals.¹ These contact-dependent

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Abbreviations used

IFN- γ : Interferon- γ
IL: Interleukin
mAb: Monoclonal antibody
MHC: Major histocompatibility complex
PBMCS: Peripheral blood mononuclear cells
PLA: Bee venom phospholipase A₂
PWM: Pokeweed mitogen

signals are not restricted by major histocompatibility complex (MHC) or species.^{2,3} However, under physiologic conditions, most of the cellular interactions are antigen-specific.⁴ Cytokines have

Center for Devices and Radiological Health

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Medical Glove Powder Report**Issue**

Do current Center policies adequately address potential adverse health effects of medical glove powder?

Background

The Food and Drug Administration (FDA), as well as other state and federal agencies, has received requests to ban the use of glove powder. It has been suggested that experimental and clinical studies demonstrate that glove powder on medical gloves can enhance foreign body reactions, increase infections and act as a carrier of natural latex allergens. The National Institute of Occupational Safety and Health (NIOSH) recently issued a safety alert recommending the use of powder-free, reduced protein content latex gloves to reduce exposure to natural latex proteins (allergens).

For the purposes of this document, total particulate matter [glove powder] includes dusting or donning powders, mold-release compounds, and manufacturing debris. Dry lubricants such as cornstarch, silicone etc., are used to make donning gloves easier and to prevent gloves from sticking together during the manufacturing process. Cornstarch, which meets the specification for absorbable dusting powder in the United States Pharmacopoeia (USP), is the most common lubricant for patient examination gloves. Only absorbable dusting powders that have an approved Premarket Approval Application (PMA) or New Drug Application (NDA) may be used for lubricating surgeons gloves. There are no comprehensive studies of the amount of absorbable dusting powder used on powdered gloves. It is estimated that amounts of total particulates may range from 120 to 400 mg for a medium size powdered glove. [Appendix A]

Glove powder is composed of particles, thus, issues related to biologic responses to foreign bodies apply to both natural rubber latex (NRL) and synthetic gloves. Industry conversion from talcum powder, a non-absorbable lubricant, to absorbable cornstarch has greatly reduced the formation of granulomas. Adhesions of peritoneal tissue after surgery are associated with foreign bodies and remain a concern. The issue of the level of micro-organisms (bioburden) on gloves has been raised under various circumstances. However, evidence that bioburden and powder are related do not exist at this time. [Appendix B]

Experimental and clinical data demonstrate that: natural latex proteins are allergenic, natural latex proteins bind to cornstarch, aerosolized powder on NRL gloves is allergenic and can cause respiratory allergic reactions. These published studies support the conclusion that airborne glove powder represents a threat to individuals allergic to natural rubber latex and may represent an important agent for sensitizing non-allergic individuals. There are also published data (although limited) and clinical experience that cornstarch powder on NRL gloves may also be a contributing factor in the development of irritation and Type IV allergy. [Appendix B]

There are alternatives to dusting powder for lubricating natural rubber latex surfaces. The most common method is chlorination. Chlorine reacts with the natural rubber latex surface to reduce the natural tackiness, eliminating the need for adding dusting powder. The extra washing performed during the

chlorination process provides an added benefit by also greatly reducing the level of soluble natural latex proteins. However, chlorination affects some of the mechanical and physical properties. Gloves made from alternative materials, not containing natural allergens, are available, but none possess the unique mix of properties offered by natural rubber latex. [Appendix C]

Market availability must be factored into any policy decision regarding medical glove powder. The large majority of medical gloves used in the U.S. are imported. In 1996, 20.8 billion medical gloves were imported into the U.S.: 90% natural rubber latex and 10% nonlatex. Of the 90% that were natural rubber latex, 20-25% were powder-free and chlorinated. Only a small number of manufacturers are using a process other than chlorination to produce powder-free gloves. A rapid increase in the demand for non-powdered gloves could result in products with poor barrier integrity and/or unacceptable shelf life entering the U.S. market. In addition to concerns about glove quality, most alternatives to glove powder currently would entail substantially increased costs to the U.S. health care system. [Appendix D]

Conclusions

- (1) The major adverse impact of glove powder appears to be its contributing role in natural rubber latex allergies.
- (2) Glove powder acts as an airborne carrier of natural latex proteins.
- (3) Exposure to airborne natural rubber latex allergens can be most effectively reduced by considering both the level of natural latex proteins and the amount of glove powder on medical gloves.

Options

Immediately banning the use of glove powder would cause a market shortage that could result in inferior products and increased costs. Doing nothing to address the problem of airborne allergens which are carried by glove powder, would appear to be an abrogation of FDA's responsibility to protect public health. It appears that neither extreme offers a viable option. The following options are offered for consideration:

1. **Provide adequate information for the consumer to make an informed decision.** Require that the amount of water-soluble natural latex proteins and the amount of particulate present on powdered gloves be stated on the product label. In addition, establish upper limits for the amount of water-soluble natural latex proteins and glove powder allowed.

Pro:

- Should not precipitate market shortage.
- Labeling requirement is achievable using current ASTM standard protocols.
- Market forces may lower both water-soluble protein and particulate levels.

Con:

- Upper limits for water-soluble protein and particulates have to be established based on state-of-technology considerations.
- Labeling requirement would not be effective without education effort by industry and/or the FDA.
- Would require a new regulation.

2. **Ban powdered medical gloves at some predetermined time in the future.** Require manufacturers to convert to powder-free production or provide safety data, including foreign body and airborne allergen concerns, by a certain date.

Pro:

- Should not precipitate market shortage.
- Requires no education effort.
- Offers a greater degree of protection from airborne natural latex allergens than Option 1.

Con:

- Conversion date would have to be negotiated with industry to avoid market shortage.
- The effect of powder-free gloves on user preferences and needs for qualities such as tactile sensation, etc. are largely unknown.
- Would most likely result in increased costs to the U.S. health care system.
- It is not clear that the amount of particulates need to be reduced to the "powder-free" level in order to offer an acceptable level of protection from adverse health effects. Does not address natural latex protein level.
- Would require a new regulation.

Author: Mel Stratmeyer

Recommendations

These recommendations represent activities either currently ongoing or which could be initiated. Detailed action plans required to accomplish these recommendations are not addressed in this document, but will need to be developed.

Glove Powder

1. Establish a maximum allowable powder level to reduce the amount of powder on powdered medical gloves by working with ASTM. *
2. Standardize the maximum allowable amount of powder on powder-free medical gloves by working with ASTM. *
3. Adopt the use of an accepted gravimetric method (such as ASTM D 6124-97) to measure total powder to demonstrate powder-free content claims.
4. Ban medical gloves that contain talc and/or lycopodium.

Protein

5. Reduce the level of water-soluble protein on finished medical gloves by working with ASTM to establish a maximum allowable glove protein level. *

Barrier Properties

6. Define effects of processing, handling, and environment on the long-term barrier characteristics of all medical gloves (natural rubber latex and alternative materials). Establish shelf-life requirements.
7. Promote the use of Process Controls, as described in the Quality System Regulation, for controlling manufacturing processes, such as chlorination, to minimize adverse effects on glove properties.

Labeling

8. Require manufacturers to label all medical gloves with the following additional information:
 - a. the total quantity of glove powder content, unless the manufacturer has demonstrated by means of an accepted gravimetric method that the total powder is 2 mg or less;
 - b. the total quantity of remaining water-soluble protein; and
 - c. an expiration date as determined by shelf-life requirements.
9. Explore the possible need to include glove powder content labeling on all product labels.

* In addition to ASTM, work with other voluntary standards organizations when appropriate.

Appendix A

Glove Powder Background

History

Since the introduction of surgical gloves to the operating theater in 1889, various types of lubricating materials have been used to aid in glove donning. These range from various wetting techniques to the use of dusting powders such as a mixtures of Lycopodium spores and talc, talcum powder alone, calcium carbonate, and different types of starch products. The first lubricant used was a powder made of Lycopodium spores (ground pines or club moss). This lubricant was quickly accepted and was used worldwide until the 1930's, when surgeons realized that it caused granuloma and adhesion formation. Lycopodium was toxic and became unacceptable for use as a glove lubricant. As a result, talcum powder (hydrous magnesium silicate), a non-absorbable lubricant, was introduced as a replacement for Lycopodium spores. In the 1940's talcum powder was also identified as a cause of post-operative complications such as granuloma and adhesion formation. In 1947 a modified cornstarch glove powder was introduced to the medical community as an absorbable and non-irritating powder. By the early 70's, many surgical glove manufacturers replaced talc with the modified cornstarch.

Cornstarch, which is absorbable through biological degradation, that meets the specification for absorbable dusting or dusting powder in the United States Pharmacopoeia (USP) is the most common lubricant for patient examination gloves. The absorbable dusting powder used on medical gloves is a chemically cross-linked cornstarch to which no more than 2% of magnesium oxide is mixed to prevent caking or turning to paste. Talc, cotton flock, and other non-absorbable materials are not acceptable as a lubricating, dusting or donning powder. ASTM* D 3578-95 (Standard Specification for Rubber Examination Gloves), D 5250-92 (Standard Specification for Polyvinyl Chloride Gloves for Medical Application) and ASTM D 3577-91 (Standard Specification for Rubber Surgical Gloves) require the inside and outside surfaces of

medical gloves to be free of talc.

In addition to dusting powder, other lubricants may also be used in the manufacturing process. Latex and some polymers are tacky and gloves made of these materials stick to the mold or former. A mold-release lubricant such as calcium carbonate or a mixture of calcium carbonate and cornstarch is used to enable the removal of gloves from molds. The other side of the glove may be coated with a donning lubricant, such as cornstarch or silicone, to make donning gloves easier and to prevent gloves from sticking during the manufacturing process.

Over the past three years, FDA has received requests to ban the use of all glove powders. These requests have been based on repeated clinical and experimental studies reporting that cornstarch on surgical gloves can damage tissue's resistance to infection, enhance the development of infection, serve as a potential source of occupational asthma, and provide a source of natural latex protein exposure to natural latex allergic individuals. The issues regarding the use of glove powder, except for the transport of natural latex protein allergens, apply to the use of glove powder on both natural rubber latex and synthetic gloves.

As a result of continuing concern over adverse reactions to cornstarch, in 1971 FDA required manufacturers to place a warning label on the glove packages. The warning label stated, "CAUTION: After donning, remove powder by wiping gloves thoroughly with a sterile wet sponge, sterile wet towel, or other effective method." Studies have shown that efforts to remove the cornstarch from the surgical gloves using washbasins and wet cloths are unsuccessful. It has been reported that such efforts have led to added clumping, creating even less absorbable aggregates.

Because of multiple concerns about the adverse health effects of all particulate matter from the surface of medical gloves (Appendix B), there is a recognized need for "low powder" and "powder-free" glove products. Particulates found on the gloves can include dusting powder, mold- or former-release compounds, lint, dust, colloidal solids, cotton, cellulose, wood fibers, metal, paper particles from packaging, and manufacturing debris. The most common particulates on gloves are dusting powder and former-release compounds added by manufacturers. Gloves with sufficiently low amounts of residual particulates are referred to as "powder-free", or "powderless." Several brands of powder-free examination and surgical gloves have been developed, some using powder-free manufacturing processes. Gloves labeled as "powder-free" may be coated with a polymer or added powder may have been removed through washing and chlorination. Although gloves are labeled as "powder-free", they contain various amounts of powder or particulates matter. FDA has adopted 2 milligrams particulate weight (based on the ASTM test standard D 6124-97) per glove powder or less as a basis for approving powder-free gloves. Alternatively, the Office of Device Evaluation (ODE) has accepted a negative iodine test to support "powder-free" claims. However, virtually all glove manufacturers provide particulate weight. For comparison purposes, a medium size powdered glove, depending on the processing, contains about 120-400 milligrams of residual debris, former-release and dusting powder.

Problems associated with the use of powder-free examination and surgical gloves include concerns about the particulate levels remaining on the gloves, use of chlorination, and the treatment with other chemical agents that may have a deleterious effect on the physical properties and/or performance of the gloves.

Surgeon's Gloves

Surgeon's gloves, defined as "a device made of natural or synthetic rubber intended to be worn by operating room personnel to protect a surgical wound from contamination ..." are classified as Class I medical devices under 21 CFR 878.4460.

Absorbable dusting powder for lubricating a surgeon's glove is classified by the FDA General and Plastic Surgery panel under 21 CFR Part 878.4480 as a class III device which requires an approved PMA. Only absorbable dusting powders from manufacturers that have an approved PMA or NDA (before it was regulated as a device) may be used on surgeon's gloves. Powder used for lubricating examination gloves has not yet fallen under the same regulatory guidelines as those for surgical gloves.

Patient Examination Gloves

Patient examination gloves were classified as Class I medical devices in the October 21, 1980 *Federal Register* under 21 CDR 880.6250 and amended in the January 13, 1989 *Federal Register*. The amendment revoked the Premarket Notification 510(k) and Good manufacturing Practices (GMP) exemptions previously designated for examination gloves.

The description for patient examination gloves made of natural rubber, vinyl, or other materials given in regulation 880.6250 define the patient examination glove as "... a disposable device intended for medical purposes that is worn on the examiner's hand or finger to prevent contamination between patient and examiner."

Powder used for lubricating examination gloves should meet the USP monograph for absorbable dusting powder or be shown to be equivalent in terms of safety and effectiveness. The 510 (k) must state the type, specifications and source of powder or other dusting lubricant used on the gloves. ASTM is currently developing the Standard Test Method for Residual Powder on Medical Gloves (D 6124-97). The standard does not include a weight limit for the total powders on powder-free medical gloves.

Quality System Regulation

FDA published, in the Federal Register (FR) on October 7, 1996, a revised GMP or Quality Systems (QS) regulation which contains requirements on the control of naturally occurring material on medical devices such as adverse protein on gloves.

The new QS regulation has several revised definitions, such as the definition for manufacturing materials in §820.3(p) which is:

"Manufacturing material means any material or substance used in or used to facilitate the manufacturing process, a concomitant constituent, or a byproduct constituent produced during the manufacturing process, which is present in or on the finished device as a residue or impurity not by design or intent of the manufacturer."

A concomitant constituent is an ingredient that naturally exists in a component of a medical device or that exists in a manufacturing material used in, or used to facilitate, the manufacturing process. The allergenic or adverse proteins that naturally occur in the natural rubber latex component of medical devices are concomitant constituents.

Specific requirements for the use and removal of manufacturing materials are in §820.70 Process Controls where §820.70(h) states:

"Manufacturing material. Where a manufacturing material could reasonably be expected to have an adverse effect on product quality, the manufacturer shall establish and maintain procedures for the use and removal of such manufacturing material to ensure that it is

removed or limited to an amount that does not adversely affect the device's quality. The removal or reduction of such manufacturing material shall be documented."

Thus, to meet direct health care concerns and to meet GMP requirements, water-soluble proteins on medical devices have to be limited by manufacturers when such proteins can be expected to have an adverse effect on patients and users.

Authors: Terrell Cunningham, Andrew Lowery

Appendix B

Adverse Health Effects

I. Biological Reactions

Glove dusting powder is composed of particles and there are predictable biological reactions to particles. The bulk of the glove powder is cornstarch, which is a resorbable particle and reactions are expected to be minimal and of short duration. This section reviews the nature of the biological reactions and the available information on these reactions to glove powder.

General Reports

A review article appearing in the peer reviewed literature in 1990, provides background information and an excellent summary of the problems associated with the use of glove powder ⁽¹⁾. Powders have been demonstrated to cause inflammation and granulomas but a much higher dose of cornstarch is needed compared to talc. This study also cites a number of other substances such as suture material, gauze fluff, and cellulose that may cause these biological reactions more frequently than does cornstarch which is the major particulate component of glove powder. Studies on changes in starch processing were also examined and autoclaved starch is rapidly resorbed (48 hrs. in rat peritoneum) and irradiated starch was still present at 70 days. Studies on washing the powder off were also reported and washing with saline clumps the powder rather than removing it.

There are additional general reports which do not contribute much to the discussion and do not provide recent references ^(2, 3). Zaza et al ⁽⁴⁾ report a good study on natural latex sensitivity with some reference to glove powder. There was no difference in sensitivity incidences when the different kinds of gloves were compared. However, nurses with cosmetic sensitivity had higher incidence. The availability and widespread use of cosmetic powders with talc and with cornstarch is cited and is an important issue in evaluating the risks associated with glove powder.

Contamination of Surgical Wounds and Peritoneal Adhesions

Contamination of surgical wounds and peritoneal adhesions are the biological reactions most frequently cited in the literature. There were pleas for powder-free gloves ^(5, 6) and indications that glove powder does contaminate the wounds since washing of gloves is ineffective ⁽⁶⁾.

The issue of peritoneal adhesions from the use of powdered surgical gloves is the major issue in the literature and most of these studies are from Europe (11-12). The studies are well documented, and the assumption is that the glove powder is cornstarch and not talc. But this is not really proven in all cases. Peritoneal adhesions following surgery are a major complication with estimates that 60-80% of intestinal obstructions are due to adhesions. The presence of foreign bodies is a major cause of these adhesions and the reactions are likely to be to sutures. However, the overall recommendation is to keep foreign bodies out of the operative area and this includes glove powder. Powder-free gloves are recommended and some available gloves or methodologies for preparing gloves are provided.

One European study had some interesting data and is the only study to have numbers that reflect incidence of reactions to glove powder (10). In 1991-1993, 448 patients were evaluated and peritoneal granulomas were found in 26% of the patients. There were suture granulomas in 25% of the patients and the surgeons of 309 patients used powdered gloves. Of these, 14 (5%) had documented starch granulomas. The overall conclusions were: the more operations on a patient; the more likely granulomas would appear. These are related to foreign bodies with sutures being the major cause. However, they do advocate avoiding depositing glove powder into the wound.

Experimental Studies

Some very interesting animal studies, mostly done in Europe, examined glove powder. The overall conclusions can be summarized that glove powder consists of particles and there is a biological response to those particles. The presence of a foreign body increases the risk of infection and cornstarch is a foreign body. However, of all the foreign bodies studied, cornstarch promotes the least

reaction (13-16).

Other Concerns with Glove Powder

There are miscellaneous reports of glove powder being left behind on devices or instruments (17, 18).

When this literature survey began, it was anticipated that pulmonary complications and associated granulomas would be the major issue. This does not appear in the literature and pulmonary complications in patients are not described.

Powder and cancer

Chronic inflammatory responses are of concern and there is some continuing thought, but no evidence, that a site of chronic inflammatory responses may be more prone to developing a cancer. In addition, there is always the concern of foreign body carcinomas (19) demonstrated in rodents. The biggest issue with granulomas from the chronic inflammatory response is that they mimic cancers and there may be a misdiagnosis. There is no evidence of genotoxicity, mutagenicity, or carcinogenicity with cornstarch. Granulomas may mimic carcinomas and biopsies may be necessary for decision making (7).

General Issues with Cornstarch

Cornstarch is a powder of particles and as such, the reactions are as those expected to particles. However, since cornstarch is a biodegradable particle, chronic responses are rare. Any modification of cornstarch

that prolongs its degradation will increase the magnitude of the reactions. Any contamination with talc will greatly increase the biological reactions. Cornstarch is a common substance in every day life. Powders and cosmetic products with cornstarch are available over-the-counter (OTC) in all stores. In addition cornstarch is common in baking and cooking. There are numerous reports of reactions to powders in cosmetics and in the work place that are not associated with health care (20, 21).

Bioburden and Powder

The issue of the level of micro-organisms on non-sterile medical gloves has been raised under various circumstances. The only study available on bioburden is an ongoing FDA funded study. Progress reports indicate organisms of pathogenic potential were found on examination gloves in some instances. However, the issue of powder should be kept separate from the bioburden since there is no evidence that bioburden and powder are related.

Surgeons gloves are sterilized and thus, there is no remaining living bioburden on the finished product. Surgeons gloves, which are often highly powdered for ease in donning over wet hands, are routinely washed prior to use and the methods of washing and the effectiveness of the procedure are not well described and remain an area of concern for powder and bioburden from washing contamination.

Powder Free Gloves

Articles on the availability and suitability of powder-free gloves appeared with pleas to surgeons to use them (22, 23).

Review of Biological Reactions to Powdered Gloves

1. The use of cornstarch rather than talc for powdering gloves greatly reduced the formation of granulomas in surgical patients. Experimental studies in animals (mice, rats, rabbits) clearly point out that talc is a potent stimulator of granulomas. Experimental studies in the same animal models showed cornstarch did not stimulate granulomas. However, if the cornstarch was not resorbed it could stimulate granulomas and some of this was associated with irradiation rather than autoclave sterilization of the cornstarch. It is also apparent that contamination of glove powder with nonresorbable particulates will cause increased formation of granulomas.
2. Granulomas to particles from starch coated gloves were described early. There are few granulomas described in the current literature. However, adhesions of peritoneal tissue after surgery is associated with foreign bodies and remains a concern. Glove powder is implicated in these reactions. Proof is fairly substantial with some pathology sections which appear to be agglomerated cornstarch, however, sutures are a more common cause.
3. The studies on peritoneal adhesions clearly recommend the use of powder-free gloves.
4. The summary reviews on the hazards of powdered gloves, with the exception of adhesions, do not have recent (after mid 1980's) problems. They demonstrate the incidence of reactions to glove powder has diminished since elimination of talc and may still be declining.
5. Most of the literature comes from Europe.
6. Washing of gloves does not completely remove the powder and may cause clumping and delay

resorption of the glove powder.

7. All of these reports are based on surgical gloves since they are used on patients with whom follow-up is routine and problems would be noted.
8. Cornstarch is the major component of glove powder and is a common powder used in a variety of occupations. (The bottles of talc and cornstarch that are OTC as baby powder have instructions "do not inhale." Pulmonary reactions to baby powder are documented. There are some peritoneal reactions to OTC powder used in the genital areas.)

Author: Katharine Merritt

II. Prevalence and health impact of Type I allergy to natural rubber latex (NRL)

Millions of health care workers, including groups such as physicians, nurses, respiratory technicians, and phlebotomists, use NRL gloves on a daily basis. The advent of universal precautions policies dramatically altered the usage of NRL gloves by the health care workers. Prior to universal precautions, gloves were only employed in instances when the patient was known to be infected with a given infectious agent, such as the hepatitis B virus. A multi-state study by Kaczmarek et al ⁽²⁴⁾ found 100% compliance with universal precautions policies by the health care facilities in the study. Actual observed compliance by health care workers during routine procedures that could involve contact with patient body fluids was substantial, but not universal, ranging up to 92% during arterial blood gas procedures. Although many devices employed in the health care environment include natural latex, it is clear that NRL gloves are a crucial source of exposure to natural latex allergens for many health care workers.

Health care workers are recognized as comprising a high-risk group for natural latex allergy. Every study of health care workers has demonstrated an appreciable prevalence of natural latex sensitization as evidenced by natural latex-specific IgE antibodies and/or positive skin tests for natural latex allergy. For example, a study by Kibby and Akl ⁽²⁵⁾ reported that 8.2% of hospital employees were skin test positive for natural latex reagent and 6.7% of them had class II or higher ELISAs for natural latex-specific IgE antibodies. A national, multi-center study by Kaczmarek et al ⁽²⁶⁾ found that 5.5% of health care workers had natural latex-specific IgE antibodies. Nine point nine percent of the natural latex skin prick tests of 101 physicians were positive in a study by Arellano and colleagues. ⁽²⁷⁾ Operating room nurses have also been studied. A study by Lagier et al ⁽²⁸⁾ reported a prevalence of 10.7% natural latex skin prick test positivity among 197 operating room nurses. Finally, in a study that included dental personnel with hospital employees, Yassin et al ⁽²⁹⁾ observed a prevalence of natural latex skin prick test positivity of 17%.

The general population is exposed to natural latex from a variety of sources, including consumer products such as natural latex balloons, as well as medical devices such as barrier contraceptives and the NRL gloves of health care providers, e.g., dental personnel. The prevalence of natural latex allergy among the general population has been estimated to range between 1% and 6%, lower than the corresponding range for health care workers. The upper end of the range is based on a study of blood donors in southeastern Michigan ⁽³⁰⁾. This study has been questioned because blood donors may not be fully representative of the general population. There is a consensus that further study is warranted. The CDRH Epidemiology Team is currently conducting a seroprevalence study of natural latex-specific IgE antibodies among NHANES (National Health and Nutrition Examination Survey) III participants. This study, with an estimated sample size of several thousand individuals, will substantially increase the understanding of the epidemiology of

natural latex allergy among the general population.

Author: Ron Kaczmarek

III. Role of glove powder in allergic reactions to natural rubber latex (NRL)

Clinical studies

A number of publications since the mid 1980's, reported respiratory problems and asthma like attacks in hospital employees and patients. The problem was ascribed to inhalation of airborne natural latex allergen in the areas of heavy use of powdered gloves (31-39). Affected individuals were frequent users of medical gloves, mainly nurses and physicians. The reactions to airborne natural latex allergens were also reported in other occupationally exposed individuals (38, 40) and/or environmentally exposed individuals (35). It is estimated that roughly 30% of natural latex sensitive individuals develop respiratory problems (31), and that aerosolized glove powder in areas of frequent glove use may affect direct users as well as those who do not use natural latex products, but are in the same areas (41). Furthermore, a recent study from Finland demonstrated a rather low prevalence of respiratory allergy reactions in one hospital, in which powder-free gloves were used for an extended period of time (42). The conclusions regarding the role of glove powder in the above clinical reports were based on medical histories of individuals presenting symptoms, on positive skin tests and, in some cases, on positive inhalation test.

Binding of natural latex proteins to cornstarch powder

The propensity of cornstarch to bind natural latex proteins was studied in detail in two recent publications. Three preparations of cornstarch: a) clean, unused dusting powder, b) cornstarch exposed to natural latex protein extracts and c) cornstarch extracted from powdered gloves, were evaluated for total protein levels (43) and for allergenic protein levels (43, 44). Unexposed cornstarch contained no allergenic proteins, while both natural latex exposed cornstarch preparations had a significant amount of allergenic proteins bound to the particles. The results of both studies clearly demonstrate that cornstarch indeed binds allergenic proteins, which can not be detached by simply washing the powder. These findings support the causal relationship between asthmatic reactions in individuals with natural latex allergy and the exposure to airborne particles from NRL products.

Airborne glove powder as an allergen carrier

Several papers describe measurements of airborne particle levels in the environment with frequent use of NRL gloves. Airborne particles were collected through filters and analyzed for allergen content.

Airborne natural latex allergen levels were evaluated in the laboratories using either powdered gloves or powder-free gloves (45). This study showed much higher allergen levels ranging from 39-311 ng/m³ in laboratories where powdered gloves were used in comparison with the levels of less than 20 ng/m³ in laboratories where powder-free gloves were used. More detailed measurements of the airborne allergen were done in the operating rooms, comparing airborne allergen levels on days when high-allergen gloves were used with days when low-allergen gloves were used and finally with no surgery days (46). The median allergen level of 13.7 ng/m³ on high-allergen glove days was down to 1 ng/m³ and 0.6 ng/m³ on low allergen glove days or no surgery days, respectively. In the environment where powdered gloves were used, large quantities of allergen could also be collected from personnel lab coats and scrub suits (47).

These studies demonstrate that the level of airborne allergen is directly related to the frequency of powdered glove usage in particular areas and to the level of allergen/powder on the gloves used.

Respiratory problems in natural latex allergic individuals

A number of published papers provide direct evidence that natural latex protein allergens, bound to corn starch particles are a cause of respiratory allergic reactions and asthma like attacks. This has been documented by the bronchial provocation test, performed by exposing allergic individuals to inhalation from powders on NRL gloves. A change in the Forced Expiration Volume (FEV), a measure of pulmonary function, is an indication of intensity of the reaction to allergen.

Patients who developed rhinitis, conjunctivitis and dyspnea when in the operating room theater or in other hospital environments with a heavy use of NRL gloves, were evaluated for natural latex allergy (medical history, specific IgE antibodies, skin test). After positive diagnosis of existing allergy to natural latex proteins, patients underwent the bronchial provocation test with airborne powder particles from NRL gloves. Test subjects were asked to handle powdered NRL gloves and powdered non-NRL gloves while their respiratory functions were monitored. They could handle up to 20 pairs of non-NRL gloves inhaling the powder particles, without any respiratory symptoms, while the same individuals, after handling as few as one pair of NRL gloves started to develop airway resistance⁽⁴⁸⁾. Furthermore, the preparation of glove powder from NRL gloves tested by bronchial provocation test and skin test, demonstrated positive reactions in both cases⁽⁴⁹⁾. In another study, a provocation test with clean cornstarch that has not been in the contact with a natural latex product did not provoke any respiratory reaction, while in the same individuals, powder from NRL induced asthmatic reaction⁽⁵⁰⁾. The control individuals with no natural latex allergy, did not develop any symptoms during provocation with allergenic powder.

In a more recent well controlled study⁽⁵¹⁾, the bronchial provocation test was performed with the extracts from powder-free surgical gloves, from powdered surgical gloves and with a clean cornstarch powder extract. A clean cornstarch powder caused no bronchial reaction in sensitized subjects. Exposure to a nebulized powder-free NRL surgical glove extract induced immediate bronchoconstriction in two of four tested subjects. However, when nebulized powdered glove extract was tested, a 1:10 dilution of the extract induced bronchoconstriction in all four tested subjects and the intensity of the reaction was the same as with undiluted powder-free glove extract.

A recent study from Belgium⁽⁵²⁾ revealed that 4.7% of hospital personnel were allergic to natural latex, confirmed by medical history and skin testing. Allergic individuals were pretested for bronchial responsiveness and then exposed to the provocation test with powdered NRL gloves. A total of 58% of allergic participants or 2.6% of the entire surveyed population developed an asthmatic reaction, while the provocation with vinyl glove powder did not cause any change in bronchial functions.

In summary, the studies reviewed above lend support to the conclusion that airborne glove powder may represent a threat to individuals allergic to natural latex proteins. Avoidance of use of natural latex products by such individuals may provide insufficient protection from natural latex proteins if they are in the environment of powdered glove use. Since there is not current safe and effective therapy for natural latex allergy, avoidance of all sources of natural latex allergen is the only available therapeutic option.

Role of glove powder in irritation and contact dermatitis development

Another issue that has to be addressed is a possible causal relationship of glove powder with the irritation

and contact dermatitis development.

It is known that cornstarch used for donning is a strong absorbing powder and has a tendency to cause dryness of the skin leading to cracking and itching. A compromised epithelium can have serious health consequences. Not only that barrier properties for infectious agents are reduced, but also in this case, chemicals used in the production of NRL gloves and natural latex proteins can penetrate a damaged skin enhancing chances of development of both Type IV and Type I allergy. Skin reactions to glove powder have been observed and interpreted as irritant reactions ⁽⁵³⁾. The major factors influencing elicitation of irritant dermatitis are dose and exposure time, and termination of exposure is the cure. Therefore, in the case of NRL gloves, a prolonged contact with glove powder may have serious impact on the user skin condition.

There are no data that directly implicate cornstarch powder as a cause of allergic contact dermatitis up to now. However, it has been reported that nonimmune proinflammatory agents can augment the response to contact sensitizers ⁽⁵⁴⁾. This augmentation occurs with subthreshold doses of both irritants and allergens and therefore, individuals that may have not presented symptoms of either reaction, can still react in case of a combined exposure ⁽⁵⁵⁾.

These published data (although limited) and clinical experience implicate that cornstarch powder on the NRL gloves, in addition to its role in Type I allergy, may also be a contributing factor in the development of irritation and Type IV allergy.

Author: Vesna Tomazic

IV. Medical Device Reporting (MedWatch) Database

FDA's adverse event databases rarely contain event text or coded information that would allow for comprehensive, automated tallies of reported medical glove related events. Reports cannot differentiate between events associated with either Type I or Type IV hypersensitivity reactions, including reactions to powder-free vs. powdered glove products. However, based on a review of all reports, it is possible to provide the following information summary.

As of August 27, 1997, 2,501 voluntary and mandatory incident reports involving natural rubber latex containing medical gloves have been entered into FDA's adverse event database. A review of database information indicates that approximately 1,550 or 62% of these medical glove related reports allege the occurrence of adverse events that involve allergic reactions, including anaphylaxis. The text of these reports indicate the occurrence of either skin reactions (Type IV or Type I) or systemic (type I) allergic reactions of one or more health care professionals or patients to medical gloves.

Approximately 100 or 4% of medical glove related adverse event reports allege specific glove powder residue complaints. These reports raise concerns regarding granuloma formation, general concerns regarding infection risk associated with powder content, low powder content making donning difficult, contamination with unidentified debris or insect parts, mold growth, and high levels of powder on gloves labeled as "powder-free." A glove powder related death report was submitted in 1986 under the procode for surgeons' gloves. The reporter, a manufacturer, indicated that a physician had questioned the role that glove powder could have played in the death of a patient who experienced post-operative peritonitis related complications.

The remaining 851 (33%) reports are primarily related to concerns regarding product barrier integrity.

However, it should be noted that problems with degradation of the desirable physical properties of medical gloves has also been associated with powder-free glove manufacturing processes such as chlorination.

Author: Sharon Dillard

Appendix C

Alternatives to Glove Powder

As discussed in previous sections of this report, glove powder has been implicated in the post-operative formation of adhesions, and in some instances, in granuloma formation. Also as discussed previously, natural latex allergens bound to airborne glove powder are known to cause respiratory problems for natural latex allergic individuals. Although the use of glove powder as a dusting lubricant is very common, there are other alternatives available. This section discusses several alternatives to powdered NRL gloves.

Chlorinated natural latex rubber (NRL) gloves

Although lubrication of the NRL glove surface can be accomplished with various dusting powders, the powder can be rubbed off and become airborne during use. A more permanent method of reducing surface drag in natural rubber latex products is known as halogenation. When carried out using chlorine as the active element - as is commonly done with NRL gloves - the process is called chlorination.

Chlorination of the NRL gloves is performed by immersing the gloves in a dilute solution containing free chlorine ions. The chlorine reacts with the natural rubber surface to reduce the natural tackiness of the natural latex, hence eliminating the need to add a dusting powder to the glove. After immersion of the glove into the dilute chlorine solution (usually between 0.05-0.30%), the gloves are washed in water, dipped in a neutralizing solution (e.g., 1% ammonia solution), rinsed again, and then dried⁽⁵⁶⁾. This extra washing performed during and after chlorination greatly reduces the level of extractable latex proteins in the product. Some latex proteins are even converted to insoluble forms during chlorination itself⁽⁵⁷⁾.

One significant drawback to using chlorinated NRL gloves is that some of the mechanical and physical properties of the natural latex are compromised. Woods et al⁽⁵⁸⁾ states that the chlorination process adversely affects shelf life, grip and in-use durability of the glove. In addition, strong odors may be present in chlorinated gloves, as well as possible skin irritants.

An FDA study of the effects of elevated temperature on the tensile strength of NRL gloves showed very dramatic results for powder-free examination gloves that are believed to have been chlorinated. Various styles of NRL gloves were placed in paper envelopes and oven-aged in air for 7, 14, and 21 days at 70° Celsius, and then subjected to tensile testing per ASTM D 412. (Accelerated aging in the laboratory at 70° C is common for NRL gloves, and is one of two recommended temperatures for aging of gloves in ASTM D 3577 and ASTM D 3578.) Five of seven powder-free styles exhibited dramatic decreases in tensile strength after just 7-14 days at 70° C, with total decreases in tensile strength ranging from 70% to over 90% at 21 days of aging. Although the details of the manufacture of these five styles are proprietary, it is believed that all were chlorinated. In contrast, almost half of the powdered gloves subject to the same

conditions showed no statistically significant decrease in tensile strength, while the remaining powdered gloves decreased a moderate 10 to 25% by 21 days of exposure (59). A progress report from an ongoing federal-state contract study on NRL exam gloves recently indicated similar results: extreme degradation of chlorinated exam gloves observed after 14 to 21 days of aging at 70° C (60).

Slight variations in the chlorination process are known (56, 61, 62). For example, variations in solution strength, immersion time, neutralizing agents, time elapsed between chlorination and neutralization, drying temperature and drying time can all influence the effects of chlorination. Aziz (56) tested gloves chlorinated with 0.01%, 0.03%, 0.05%, 0.1% and 0.3% chlorine solutions. For unaged samples, tensile strength was maintained from 1 to 20 minutes of chlorination time for all samples except those chlorinated with the 0.3% solution, in which tensile strength decreased by approximately 25%. For samples aged 7 days at 70° C, original tensile strength decreased slightly for up to 20 minutes of chlorination, except for the 0.3% samples, where the tensile strength decreased by roughly 50% for 20 min. of chlorination. For samples aged 22 hours at 100° C, original tensile strength was maintained only for the 0.01% solution. The strengths of the remaining samples decreased 50-95% after only 2-6 minutes of chlorination.

Aziz also showed the higher concentrations of chlorine lead to microscopic cracks in the surface of the natural rubber latex. Chlorination time and solution strength also affect the color of the finished product (longer times and higher concentrations lead to a more yellow product). Thus, in order to avoid the potential negative effects of chlorination, chlorine concentrations and immersion times should be carefully chosen.

Synthetic polymer linings

Another alternative to powdered gloves is a NRL glove having a synthetic polymer lining on the internal surface of the glove. The slippery surface of such a lining facilitates donning of the glove. Synthetic polymer coatings may be made of a hydrogel, silicone, or another polymer. It appears that no shelf-life data exist to substantiate the long-term barrier properties of synthetic polymer-coated NRL gloves.

In the case of hydrogel polymer linings, the NRL glove is dipped into a solution of the hydrogel prior to the final curing stage of glove manufacture. The hydrogel lining is physically bonded to the natural rubber latex (58) and lies on the internal skin-contacting surface of the finished product. Due to its low coefficient of friction, the hydrogel lining facilitates donning with either wet or dry hands (63, 64, 65).

Other approaches

From the late 1800s to the mid-twentieth century, surgeons used water as the primary lubricating agent when donning gloves. The protective rubber gloves utilized at that time were designed for multiple use, and thus were pulled onto wet hands after being "sterilized" [sic] in boiling water (58, 63, 66). Water is not an effective glove lubricant for today's thin, close-fitting NRL gloves.

Glove liners in the form of cotton or nylon stretch gloves, or liners made of materials designed to resist puncture, are sometimes worn underneath NRL gloves, between the bare skin and the glove. Although liners are not used to facilitate donning, they will provide a layer of protection to the user, and thus reduce the risk of skin irritation. They also reduce discomfort due to hand sweating. Gloving creams are sometimes used to facilitate the donning of gloves and at other times, are used to reduce the wearer's potential for skin irritation. However, if used with powdered gloves, such glove liners and creams will do nothing to eliminate the occurrence of airborne natural latex allergens.

Gloves made from materials other than natural rubber latex (e.g., synthetic rubbers or other synthetic polymers) are available, but none possess the unique mix of properties (high elasticity and tensile strength, excellent film-forming characteristics) found in NRL gloves (57, 66). Gloves made from some of these alternative materials, such as plasticized PVC, include high levels of chemical additives which may cause skin irritation and/or allergic reactions (66, 67). Furthermore, the barrier properties of alternative glove materials must be thoroughly examined prior to their selection for use.

Summary

Chlorination of NRL gloves is a common alternative to the use of glove powder. Chlorination has an adverse affect on various mechanical and physical glove properties, which may affect shelf-life. Thus, the chlorination process should be tightly controlled. Gloves made of synthetic materials are available, but none possess the unique mix of physical properties offered by natural rubber latex. Synthetic polymer-coated gloves are another possibility, but as is the case with both NRL and non-NRL gloves, it appears that little or not shelf-life data exist in the current literature to substantiate the long-term barrier properties of this type of medical glove.

Author: Donna Walsh

Appendix D

Glove Market Availability

In 1996, the U.S. imported 20.8 billion medical gloves, 62% of which came from Malaysia. Since 1991, the number of medical gloves imported into the U.S. has increased by 247%. See the table below provided by the Division of Small Manufacturers Assistance (DSMA).

| U.S. Medical Glove imports | | | | | | |
|-----------------------------------|-------------|-------------|-------------|-------------|-------------|-------------|
| (in billions) | | | | | | |
| | 1991 | 1992 | 1993 | 1994 | 1995 | 1996 |
| Malaysia | 3.9 | 7.6 | 9.9 | 10.4 | 11.8 | 13.0 |
| Thailand | 0.9 | 1.8 | 2.0 | 1.7 | 2.2 | 3.2 |
| Indonesia | 0.2 | 0.6 | 0.8 | 0.8 | 1.1 | 1.9 |
| Sri Lanka | 0.2 | 0.5 | 0.3 | 0.5 | 0.6 | 0.7 |
| India | 0.1 | 0.3 | 0.5 | 0.5 | 0.5 | 0.6 |
| Taiwan | * | * | * | * | 0.4 | 0.3 |
| China | 0.5 | 0.4 | 0.7 | 0.6 | 0.6 | 0.8 |
| Others | 0.2 | 0.2 | 0.3 | 0.4 | 0.2 | 0.3 |
| Total Imports | 6.0 | 11.4 | 14.5 | 14.9 | 17.4 | 20.8 |
| % Increase | | 90% | 27% | 3% | 17% | 20% |

* Number of imports not enough to be included in top seven countries in this table.

These numbers include medical gloves of all types: NRL, powder-free NRL, and non-NRL. In 1996, the distribution by type was 90% NRL and 10% non-NRL. Of the 90% natural rubber latex, 20-25% were powder-free latex and chlorinated. Only a small number of manufacturers are using a process other than chlorination to produce powder-free gloves.

Malaysia is the largest producer of natural latex worldwide. Over 90% of all patient examination gloves are made from natural latex, and it is estimated that up to 80% of NRL patient examination gloves consumed in the U.S. are manufactured in Malaysia⁽⁶⁸⁾. The Association of Malaysian Medical Industries (AMMI) represents Malaysian and multinational companies involved in the development and manufacture of medical devices, products, equipment and services in Malaysia for the health care community worldwide. The Malaysian Rubber Glove Manufacturers' Association (MRGMA) specifically represents the NRL glove manufacturers. According to the AMMI and MRGMA, any significant increase in the numbers of medical gloves available for importation is not likely. However, a shift in the types of gloves (powdered to powder-free) is already occurring.

In June 1997 as a result of the NIOSH alert, five questions regarding current and future availability of medical gloves to the U.S. were posed to the entire 20 company AMMI membership, nine of which were glove-only manufacturers, also members of MRGMA. The responses were compiled and presented to CDRH by an AMMI executive and MRGMA member at a subsequent June meeting. The questions and AMMI responses follow. Wherever appropriate, supplemental supporting documentation is included.

1. What is your current monthly and/or annual capacity for manufacturing NRL and powder-free NRL medical gloves for the U.S.?

The total capacity from Malaysia in 1996 was 13 billion (including 10% non-NRL) pieces. This capacity will not change significantly. The projected Malaysian industry trend is to shift the ratio of powdered (P) to powder-free (PF) natural rubber latex. AMMI and MRGMA project this shift to be rapid as indicated below.

| | <u>P to PF Latex</u> |
|--------------------|----------------------|
| 12 months ago | 80:20 |
| 6 months ago | 75:25 |
| today (June 1997) | 65:35 |
| 12 months from now | 50:50 |

2. How do these numbers compare to distribution outside the U.S.?

The ratio of Malaysian medical gloves for U.S. distribution to the rest of the world is 70:30. Partly due to volume and purchasing requirements, other countries are more willing to pay the higher prices of powder-free NRL gloves. As a comparison to the P to PF ratio above, the ratio in the United Kingdom is:

| | <u>P to PF Latex</u> |
|--------------------|----------------------|
| 3 months ago | 75:25 |
| today (June 1997) | 55:45 |
| 12 months from now | 40:60 |

3. If there was a request by the U.S. health care community to produce a larger quantity of powder-free medical gloves, how quickly could this increase occur and by what percent?

If the U.S. health care community could bear the "current market price" of gloves, the powder-free glove supply to other parts of the world could be significantly shifted to the U.S. Demand for powdered gloves has already dropped worldwide. One constraint to any possible shift is long-term contracts. Half or 50% of glove manufacturers have long-term contracts that stretch 6-12 months. Unless the U.S. price warranted, these contracts would not be re-negotiated.

The lines producing powder-free NRL gloves are currently working to capacity. Conversion of lines is expensive and requires 12-18 months before realizing an increased capacity. Some of the obstacles include acquiring chlorinators, which are backlogged worldwide, and water treatment enhancements. It is doubtful that the industrial process would shift to greater than 60% powder-free vs. 40% powdered NRL. Any greater erosion from powdered would be made up by a shift to non-NRL. Ten percent of the current Malaysian market is non-NRL and is growing. Although non-latex technology is not yet equal to that of natural rubber latex, glove manufacturers are attempting to perfect the nonlatex process and anticipate future increases in the nonlatex market.

However, additional FDA staff research found that non-NRL gloves, other than vinyl, are considerably more expensive than NRL gloves.

| Glove Prices to Hospitals ⁽⁶⁹⁾ | | | | |
|---|-------|----------------------------|------------------|-------------------|
| (in U.S. dollars per box of 100 pieces) | | | | |
| Exam | Vinyl | Natural Rubber Latex (NRL) | Synthetic Rubber | Synthetic Polymer |
| Powdered | 3.50 | 3.90 | 8.00 | 12.00 |
| Powder-free | 4.20 | 5.80 | 10.00 | 15.00 |
| % of Increase for Powder-free Product | 20% | 49% | 25% | 25% |

For the powdered gloves, NRL costs are 11.4% higher than vinyl but synthetic rubber is 128.5% higher than vinyl and 105% higher than NRL. For powder-free gloves, NRL costs are 39% higher than vinyl but synthetic rubber is 138% higher than vinyl and 72.4% higher than NRL. Moving to a synthetic glove is currently cost prohibitive for U.S. hospitals.

Although vinyl gloves are less expensive than NRL, research indicates they are not necessarily the best alternative. Both NRL and vinyl patient examination gloves provide protection against microorganisms; however, it has been demonstrated that NRL is preferred to vinyl for more effective and durable barrier qualities ^(70, 71). NRL is pliable allowing for natural molding for more appropriate fit and has the ability to reseal when tiny punctures occur. In general, NRL provides comfort to the wearer, adequately protects against microorganisms, and provides adequate barrier effectiveness when used for medical and nursing procedures ⁽⁷⁰⁾. Consequently, NRL is still the barrier of choice in the U.S.

4. Would an increased volume impact importation/distribution to the U.S.? If so, what obstacles may you encounter?

U.S. entry requirements can be a problem for glove manufacturers which result in delays and, in some cases, a barrier too costly to pursue. Some specific obstacles which act as a deterrent are:

- o 510(k) requirement of biocompatibility testing. There are very few laboratories available to conduct the testing causing a current 2-4 month backlog. It would be helpful if a "contingent" 510(k) approval could be granted while biocompatibility testing is being conducted. This would allow the manufacturer the opportunity to recoup some of the start-up expenses. It is cost prohibitive for a manufacturer to maintain the facility without any return, even for a relatively short period of time.
- o 510(k) processing time. The current 90 days is all the manufacturers can afford. It would be an obstacle if an increase in 510(k) applications would cause a backlog.
- o Regulatory expenses. Other countries are offering prices comparable or greater than those offered by the U.S. To avoid U.S. regulatory expenses/hassles, glove manufacturers are strongly inclined to direct their products to markets they can enter without delay or added costs.

5. What would be your special concerns and/or difficulties producing a larger quantity of powder-free NRL medical gloves, if any?

Barrier integrity is the main concern for medical gloves and glove manufacturers. Producing a product that will consistently meet water leak tests is of special concern. However, the current anxiety over natural latex allergy is resulting in a shift to materials and/or processes that may compromise barrier integrity. In a shortage situation, or even a perceived shortage situation, inconsistent quality suppliers may seize the opportunity to move into the U.S. market. This will result in poor barrier products entering the U.S., much as they did in 1988-89 when demand rapidly increased because of concern regarding universal precautions.

Producing a product that will have acceptable shelf life (one-year) is another special concern and/or difficulty. Powder-free technology is not easy and chlorination contributes to the difficulty. Most powder-free gloves are chlorinated and suppliers of auxiliary equipment are already back-ordered at least six months. However, chlorination is not the only process for producing powder-free NRL gloves. More emphasis needs to be placed on other processes which may help improve shelf life.

In summary and based on additional investigation, comprehensive labeling, including warnings and precautions, added to all medical NRL gloves would not be significant. The health care community is largely aware of natural latex allergenicity and has been making appropriate adjustments. The demand for more powder-free or lower protein gloves will most likely increase, and as refinement in other manufacturing processes improve and lower protein NRL is developed, the shift will be toward medical gloves other than chlorinated powder-free.

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References

1. Ellis H: *The hazards of surgical glove dusting powder*. Surgery, Gynecology & Obstetrics, Vol 171: 521-527, 1990.
2. Beck WC, Beezhold DH: *Starch Glove Powder should follow talc into limbo*.
3. Carlson and Fay: [Letter.] J Amer Coll Surgeons; 178: 185-186, 1994. Feb 1995. AORN Journal 61:317, 1995.
4. Zaza S, Reeder JM, Charles LE, Jarvis WR: *Latex sensitivity among perioperative nurses*. AORN Journal, 60: 806-812, 1994.
5. Edlich RF: *A plea for powder-free surgical gloves*. The J Emerg Med 12:69-71, 1994.
6. Hunt TK, Slavin JP, Goodson WH: *Starch powder contamination of surgical wounds*. Arch Surg, 129: 825-828, 1994.
7. Giercksky K-E, Qvist H, Giercksky TC, Warloe T, Nesland JM: *Multiple glove powder granulomas masquerading as peritoneal carcinomatosis*. J Amer Coll Surgeons, 179: 299-304, 1994.
8. Risberg B: *Adhesions: Preventive Strategies*. Eur J Surg, 163: (Suppl 577: 32-39, 1997.
9. Holmdahl L, Risberg B, Beck DE, Burns JW, Chegini N, diZerga GS, Ellis H: *Adhesions: Pathogenesis and Prevention-Panel Discussion and Summary*. Eur J Surg, 163 (Suppl 577) 56-62, 1997.
10. Luijendijk RW, cdLange DCD, Wauters CCAP, Hop WCJ, Duron JJ, Pailler JL, Camprodon BR, Holmdahl L, vanGeldorp HJ, Jeekel J: *Foreign material in postoperative adhesions*. Annals of Surg, 223: 242-248, 1996.
11. Becker JM, Dayton MT, Fazio VW, et al: *Prevention of postoperative abdominal adhesions by a sodium hyaluronate based bioresorbable membrane: a prospective randomized double blind*

- multicenter study. *J Am Coll Surg*, 1996 183: 297-306.
12. Hunt TK: *Can adhesions be prevented?* *J Amer Coll Surgeons*, 183: 406-407, 1996.
 13. McEntee GP, Stuart RC, Byrne PJ, Leen E, Hennessy TP: *Experimental study of starch-induced intraperitoneal adhesions.* *Br J Surg*, 77: 113-114, 1990.
 14. Ellis H: *Pathological changes produced by surgical dusting powders.* *Ann R Coll Surg Engl*, 76:5-8, 1994.
 15. Kang N, Griffin D, Ellis H: *The pathological effects of glove and condom dusting powders.* *J Appl Toxicol*, 12: 443-449, (1992).
 16. Ruhl CM, Urbanic JH, Foresman PA, Cox MJ, Rodeheaver GT, Zura RD, Edlich RF: *A new hazard of cornstarch, an absorbable dusting powder.* *The J Emerg Med*, 12: 11-14, 1994.
 17. Green MA, Lam Y, Moss RF: *Starch, gloves, and extradural catheters.* *Br J Anesth*, 75: 768-770, 1995.
 18. Oh Y, Katz LJ, Spaeth GL, Wilson RP: *Risk factors for the development of encapsulated filtering blebs.* *Ophthalmology*, 101:629-634, 1994.
 19. Schoen FJ: *Tumorigenesis and Biomaterials*, in Ratner, BD, Hoffman AS, Schoen FJ, Lemons JE, *Biomaterials Science*, Academic Press, 1996, section 4.6.
 20. Tacke J, Schmidt A, Fartasch M, Diepgen TL: *Occupational contact dermatitis in bakers, confectioners and cooks.* *Contact Dermatitis*, 33:112-117, 1995.
 21. Kanerva L, Toikkanen J, Jolanki R, Estlander T: *Statistical data on occupational contact urticaria.* *Contact Dermatitis*, 35: 229-233, 1996.
 22. Van Meter BH, Aggarwal M, Thacker JG, Edlich RF: *A new powder-free glove with a textured surface to improve handling of surgical instruments.* *The J Emerg Med*, 13: 365-368, 1995.
 23. Pavlovich LJ, Cox MJ, Thacker JG, Edlich RF: *Ease of donning surgical gloves: an important consideration in glove selection.* *The J Emerg Med*, 13: 353-355, 1995.
 24. Kaczmarek RG, Moore RM, McCrohan J, et al: *Glove use by health care workers: results of a tri-state investigation.* *Am J Inf Cont*, 19:228-232, 1991.
 25. Kibby T, Akl M: *Prevalence of latex sensitization in a hospital employee population.* *Annals of Allergy*, 78:41-44, 1997
 26. Kaczmarek RG, Silverman BG, Gross TP, et al: *Prevalence of latex-specific IgE antibodies in hospital personnel.* *Annals of Allergy*, 76:51-56, 1996.
 27. Arellano R, Bradley J, Sussman G: *Prevalence of latex sensitization among hospital employees occupationally exposed to latex gloves.* *Anesthesiology*, 77:905-908, 1992.
 28. Lagier F, Vervloet D, Lhermet I, et al: *Prevalence of latex allergy in operating room nurses.* *J Allergy Clin Immunol*, 90:319-322, 1992.
 29. Yassin MS, Lierl MB, Fischer TJ, et al: *Latex allergy in hospital employees.* *Ann Allergy*, 72:245-249, 1994.
 30. Ownby DR, Ownby HE, McCullough JA, and Shafer AW: *The prevalence of anti-latex IgE antibodies in 1000 volunteer blood donors [Abstract].* *J Allergy Clin Immunol*, 93:282, 1994.
 31. Baur X, Ammon J, Chen Z, Beckmann U, Czuppon AB: *Health risk in hospitals through airborne allergens for patients presensitized to latex.* *Lancet*, 342:1148-1149, 1993.
 32. Kujala V, Pirilä T, Niinimäki A, and Reijula K: *Latex-induced allergic rhinitis in a laboratory nurse.* *J of Laryngology and Otology*, 109:1094-1096, 1995.
 33. Field EA: *The use of powdered gloves in dental practice: a cause for concern?* *J Dent*, 25:209-214, 1997.
 34. Seggev JS, Mawhinney TP, Yunginger JW, and Braun SR: *Anaphylaxis due to cornstarch surgical glove powder.* *Ann Allergy*, 65:152-155, 1990.
 35. Ruëff F, Thomas P, and Przybilla B: *Natural rubber latex as an aeroallergen in the general environment.* *Contact Dermatitis*, 35:46-47, 1996.
 36. van der Meeren HLM and van Erp PEJ: *Life-threatening contact urticaria from glove powder.* *Contact Dermatitis*, 14:190-191, 1986.

37. Assalve D, Cicioni C, Perno P, and Lisi P: *Contact urticaria and anaphylactoid reaction from cornstarch surgical glove powder*. *Contact Dermatitis*, 19:61-78, 1988.
38. Seggev JS, Mawhinney TP, Yunginger JW, et al: *Corn starch glove powder may cause anaphylaxis*. *Ann Allergy*, 65:152-155, 1990.
39. Fisher AA: *Contact urticaria and anaphylactoid reaction due to corn starch surgical glove powder*. *Contact Dermatitis*, 16:224-235, 1987.
40. Orfan NA, Reed R, Dykewicz MS, Ganz M, and Kolski GB: *Occupational asthma in a latex doll manufacturing plant*. *J Allergy Clin Immunol*, 94:826-830, 1994.
41. Vandenplas O, Delwiche J-P, and Sibille Y: *Occupational asthma due to latex in a hospital administrative employee*. *Thorax*, 51:452-453, 1996.
42. Kujala VM and Reijula KE: *Glove-related rhinopathy among hospital personnel*. *Amer J of Industrial Medicine*, 30:164-170, 1996.
43. Tomazic VJ, Shampaine EL, Lamanna A, Withrow TJ, Adkinson, Jr. NF, and Hamilton RG: *Cornstarch powder on latex products is an allergen carrier*. *J Allergy Clin Immunol*, 93:751-758, 1994.
44. Beezhold D and Beck WC: *Surgical glove powders bind latex antigens*. *Arch Surg*, 127:1354-1357, 1992.
45. Tarlo SM, Sussman G, Contala A, and Swanson MC: *Control of airborne latex by use of powder-free latex gloves*. *J Allergy Clin Immunol*, 93:985-989, 1994.
46. Heilman DK, Jones RT, Swanson MC, and Yunginger JW: *A prospective, controlled study showing that rubber gloves are the major contributor to latex aeroallergen levels in the operating room*. *J Allergy Clin Immunol*, 98:325-330, 1996.
47. Swanson MC, Bubak ME, Hunt LW, Yunginger JW, Warner MA, and Reed CE: *Clinical aspects of allergic disease: Quantification of occupational latex aeroallergens in a medical center*. *J Allergy Clin Immunol*, 94:445-451, 1994.
48. Baur X and Jäger D: *Airborne antigens from latex gloves*. *Lancet*, 335:912, 1990.
49. Jäger D and Baur X: *Latex specific proteins as inhalative allergens causing bronchial asthma and shock during surgery*. *Clin Exp Allergy*, 20 (abstract), 1990.
50. Marcos C, Lázaro M, Fraj J, Quirce S, de la Hoz B, Fernández-Rivas M, and Losada E: *Occupational asthma due to latex surgical gloves*. *Ann Allergy*, 67:319-323, 1991.
51. Pisati G, Baruffini A, Bernabeo F, and Stanizzi R: *Bronchial provocation testing in the diagnosis of occupational asthma due to latex surgical gloves*. *Eur Respir J*, 7:332-336, 1994.
52. Vandenplas O, Delwiche J-P, Evrard G, Aimont P, van der Brempt X, Jamart J, and Delaunois L: *Prevalence of occupational asthma due to latex among hospital personnel*. *Am J Respir Crit Care Med*, 151:54-60, 1995.
53. Heese A, Hintzenstern J, Peters K-P, Koch HU, and Hornstein OP: *Allergic and irritant reactions to rubber gloves in medical health services*. *J Am Acad Dermatol*, 25:831-839, 1991.
54. Garioch J, Biagioni P, Forsyth A, Baillie A, MacPherson D: *Infrared thermography of the allergic response to nickel*. *Br J Dermatol*, 121:41, 1989.
55. McLelland J, Shuster S, and Matthews JNS: *Irritants increase the response to an allergen in allergic contact dermatitis*. *Arch Dermatol*, 127:1016-1019, 1991.
56. Aziz NAA: *Chlorination of gloves*. Paper No. 5 of the Latex Protein Workshop of the International Rubber Technology Conference, June 1993, Kuala Lumpur, Malaysia.
57. Mellstrom GA and Boman AS in *Protective Gloves for Occupational Use*, CRC Press, Boca Raton, FL, 1994, chapter 3.
58. Woods JA, Morgan RF, Watkins FH, and Edlich RF: *Surgical glove lubricants: from toxicity to opportunity*. *J Emerg Med*, 15(2), 209-220 (1997).
59. Walsh DL, Chwirut DJ, Kotz R, and Dawson J: *Environmental Degradation of Latex Gloves: The Effects of Elevated Temperature on Tensile Strength*. DMMS Report #96-05. FDA, Rockville, MD.
60. Progress report from subcontractor to FDA study. July 17, 1997.

61. *Natural Rubber Technical Information Sheet L17*. 1977 Latex Series of the Malaysian Rubber Producers' Research Association, England.
62. Gorton ADT: *Natural rubber gloves for industrial use*. NR Technology, Vol. 15, Part 1, 1984.
63. Fisher MD, Neal JG, Kheir JN, Woods JA, Thacker JG, and Edlich RF. *Ease of donning commercially available powder-free surgical gloves*. J Biomed Mater Res, 33(4), 291-295 (1996).
64. James MH, Bratby DM, Duck R, Podell HI, Goldstein A, and Blackley DC: *Dipped rubber article*. US Patent No. 4,499,154 (1985).
65. Nile JG, Gromelski SJ, Brain AA, and Hardwick ST: *Surgeon's glove having improved donning properties*. US Patent No. 5,570,475 (1996).
66. Phillips P: on <http://www.smtl.co.uk/MDRC/Gloves/jowcpaper96>; 1996.
67. Estlander T, Jolanki R, and Kanerva L in *Protective Gloves for Occupational Use*, CRC Press, Boca Raton, FL, 1994, chapter 16.
68. *Safeskin Company Report*. INVESTEXT, Thomson Financial Services, Boston, MA, January 10, 1994, p.4, from Smith Barney Shearson. Online. Dialog.Investext.545.
69. *Glove Prices to Hospitals*. Malaysian Rubber Glove Manufacturers' Association, August 1997.
70. Korniewicz D: *Barrier Protection of Latex*. Immunology and Allergy Clinics of North America, 15/1:123-137, 1995.
71. Korniewicz D, Kirwin M, Cresci K, et al: *In-use comparison of latex gloves in two high risk units: Surgical intensive care and acquired immunodeficiency syndromes*. Heart Lung, 21:81-84, 1992.

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Endotoxin as a factor in adverse reactions to latex gloves

P Brock Williams, PhD and John F Halsey, PhD

Background: Endotoxin is an inflammatory agent made by gram negative bacteria that can irritate the skin, induce respiratory problems, fever, and shock. It is an adjuvant for both delayed hypersensitivity and IgE production and has been shown to magnify antigen specific mediator release. Since many of the clinical problems associated with natural latex products involve similar clinical sequelae, we investigated the possibility that latex gloves might be contaminated with endotoxin.

Objective: To measure the endotoxin content of a variety of natural latex gloves, investigate its distribution and origin, association with latex proteins, and determine the particle sizes associated with its release.

Methods: Endotoxin, protein, and allergen were measured using a quantitative kinetic Limulus assay, modified Lowry, and RAST inhibition, respectively. Particle size and density were determined using an Anderson multistage air sampler and CsCl₂ gradient.

Results: Endotoxin was found to be a highly significant contaminant of some latex gloves. Levels ranged from 0.09 ng to 2.8 µg/g of glove. Protein levels ranged from <25 to 1150 µg/g of glove while allergen levels ranged from <1 to 837 µg/g of glove. Endotoxin and protein eluted rapidly from the interior of the gloves tested. Greater than 70% of the endotoxin was found to be associated with particles in the <7 µm aerodynamic diameter range. The highest levels of endotoxin were found in nonsterile examination gloves with a tendency towards powdered gloves containing more endotoxin and protein. A slurry containing cross-linked dextran through which gloves were dipped revealed very high endotoxin contamination (64 µg/mL) while unused cross-linked dextran has very little associated endotoxin.

Conclusions: These data demonstrate that some natural rubber latex gloves, particularly nonsterile examination gloves, are contaminated with high amounts of endotoxin and proteins. These were found mostly on the inside of gloves and were released as very small respirable particles that were not physically associated with the powder. These findings support the hypothesis that endotoxin may be responsible for some of the tissue irritation associated with latex glove use. In addition, this material may be responsible for the enhancement of delayed and immediate hypersensitivity reactions to chemicals and proteins found in these products and offers a possible explanation for the disproportionate severity of these reactions.

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INTRODUCTION

Lipopolysaccharides unique to the outermost wall of gram negative bacteria have very potent proinflammatory effects.^{1,2} These substances referred to as "endotoxins" for historical reasons and are continually being shed from grow-

ing bacteria and are released upon their death.³⁻⁵ Specific cellular and humoral receptors exist for endotoxins that enhance their ability to induce proinflammatory cytokines and chemokines such as TNFα, IL-6, and IL-8.⁶⁻¹² Clinical reactions to endotoxins include irritation, skin rashes, fever, rhinoconjunctivitis, respiratory congestion, asthma, and anaphylactoid shock.¹³⁻¹⁸ The symptoms observed depend upon the dosage, route of entry, molecular structure, and other poorly defined suscep-

tibility factors.¹⁹⁻²⁴ Endotoxin has also been shown to act as an adjuvant for allergic contact sensitivity to haptens¹³ and to be an adjuvant for IgE responses when inhaled with antigen.²⁵⁻²⁸ In addition, endotoxin acts synergistically with allergen resulting in a magnification of specific allergen-induced mediator release.²⁹⁻³²

Since the mid-eighties the cumulative prevalence of individuals experiencing adverse reactions to products manufactured from native latex has increased dramatically.³³ Clinically these reactions range from direct irritant effects to immunologically induced contact dermatitis, urticaria, rhinoconjunctivitis, asthma, and anaphylaxis.³⁴⁻³⁶ The immunologic activity of the chemicals and the allergenicity of proteins present in natural latex products have been studied by a number of investigators and many of their molecular properties have been elucidated.³⁷⁻⁴⁷ The reasons why these substances seem to be such potent sensitizers, with often severe consequences, has not been determined. The hypotheses advanced include the large increase in the use of natural rubber latex gloves as a barrier against infectious agents and the fact that increased demand may have introduced alterations in manufacturing processes.⁴⁸

Consideration of the manner in which these latex products are manufactured, with the resulting potential for endotoxin contamination, and the nature of the clinical reactions observed, prompted us to determine the endotoxin content of natural latex gloves. In this paper, we provide data on the occurrence, distribution, and probable source of endotoxin in commercially available latex gloves. Based on these findings we propose that the presence of endotoxin in some latex

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gloves is an important factor in the etiology of the clinical reactions to these products.

METHODS

Extracts

Natural latex gloves and synthetic gloves ($n = 23$) obtained from a variety of different manufacturers were cut into 0.5 cm² pieces, weighed, and placed in depyrogenated borosilicate test tubes. Glassware was depyrogenated by heating in an oven held at 180 °C for four hours. Extracts were prepared from 0.4 g of each glove by vigorously shaking for one hour in 2 mL pyrogen-free water (BioWhittaker, Inc., Walkersville, MD). This was followed by centrifugation at 3,000 g for 20 minutes and the supernate was recovered. Fifty-microliter aliquots were removed and diluted in pyrogen-free water and assayed immediately. A fresh starch extract was prepared from unused cross-linked dextran powder (Grain Processing Corporation, Muscatine, IA) by extracting at a 1:5 ratio (wt/vol) in pyrogen-free water for one hour with shaking and centrifugation at 3,000 g for 20 minutes. A sample of cornstarch slurry from a latex glove production line was collected in Malaysia and shipped frozen to the laboratory (a gift from Russell Thompson, TNI, Hartland, WI). An extract was prepared from this material by centrifuging the thawed slurry at 3,000 g for 20 minutes.

Endotoxin

Endotoxin was measured using the kinetic chromogenic quantitative *Limulus* amoebocyte lysate assay which is nonreactive to glucans (BioWhittaker, Inc., Walkersville, MD). Aliquots of the extracts were serially diluted in 10-fold increments with pyrogen free water. A 100- μ L aliquot of each of these dilutions was mixed with 100 μ L of freshly prepared *Limulus* amoebocyte lysate containing chromogenic substrate in a pyrogen-free microtiter plate (Dynatech Corporation, Chantilly, VA). The microtiter plates were kept at 37 °C and the color development was monitored every 15 seconds

with the Dynatech MR5000 microtiter plate reading spectrophotometer. The calculation program in this instrument determines the time interval to reach 0.03 absorbance and this was compared with a standard curve covering the range of 5 ng/mL to 0.5 μ g/mL. The standards were linear over this 5-log range on a log-log plot. Unknowns were calculated by linear interpolation by the software. All dilutions were assayed in duplicate and a parallel dilution was spiked with 50 μ g endotoxin to assess any enhancement or inhibition of activity by any of the extracts. Only those dilutions that did not exhibit enhancement or inhibition and which were parallel to the standard curve were used. Values shown are averages from the same glove assayed on three different occasions. The coefficient of variation for these assays averaged 7.6%. To convert endotoxin activity to equivalent mass units of the EC6 reference standard (US Pharmacopeia), a factor of 10 EU/ng was used.

Protein

A modification of the Lowry protein determination method was used to determine protein in the glove extracts. The method (American Society for Testing Materials 5712-95) was recently adopted by the American Society for Testing Materials and is currently the method approved by the US Food and Drug Administration for determining protein content in gloves and other medical devices derived from latex. The method incorporates several protein precipitating reagents to circumvent the interference of the Lowry assay by the various chemicals used in the manufacture of these products. The method utilized ovalbumin as a standard at a range of 5 μ g/mL to 640 μ g/mL. Since the gloves were extracted at a ratio of 1:5 (wt/vol), the resulting detection limit was 25 μ g/g of glove.

Allergen Content

The quantity of latex allergen was determined by RAST inhibition using the method previously described⁴⁹ except that for this study a non-ammoniated

latex extract was used as the reference extract or standard. This extract was prepared from *H. brasiliensis* latex shipped frozen from Malaysia as described by Tomazic et al.⁵⁰ The protein content was established by the American Society for Testing Materials modified Lowry method. Dilutions of this standard were made in phosphate buffered saline containing 1 mg/mL human serum albumin. The serum pool for the RAST inhibition was prepared from 42 sera known to have high concentrations of latex-specific IgE. For comparison purposes, a reference latex extract from the FDA (E-8) was determined to contain 1.2 mg/mL of latex allergen and 2.8 mg/mL of protein by the American Society for Testing Materials modified Lowry method. The useful limit of detection of the RAST inhibition assay in these studies was estimated to be 0.03 μ g/mL and for quantitation to be 0.060 μ g/mL.

Particle Sizing

The relative size (ie. aerodynamic diameter) of the particles suspended in air when ten gloves were snapped in an enclosed room (25 m³) was determined by analyzing samples collected with an Anderson multistage air sampler (Grasby/Anderson Inc. Atlanta, GA). Prior to use this device was depyrogenated by soaking the stages overnight in 0.3 N NaOH. The glass 100-mm Petri plates were depyrogenated by heating at 180 °C for four hours. Each plate was filled with 20 mL depyrogenated water and vacuum was applied for 15 minutes at 28.3 L/min (total air volume sampled was 0.4245 m³). Aliquots of the 20 mL of water in the individual plates (ie. stages) were centrifuged and the supernates were assayed for endotoxin by the *Limulus* amoebocyte lysate method described above. These samples were assayed prior to and immediately following the application of vacuum to the Anderson sampler. A microscopic analysis of the resulting particles in the different stages of the Anderson was performed with cytopsin (Shandon Inc, Pittsburgh, PA) preparations from each stage.

Density Studies

Particles created from snapping five pairs of gloves in a small room (25 m³) were collected in a cyclone sampler (Burkard Instruments, Britain) over a period of 90 minutes. This sample was suspended in 0.5 mL depyrogenated water and layered over a 60% wt/vol solution of CsCl₂. The dextran particles were allowing to sediment for 30 minutes at which time an aliquot was removed for endotoxin testing. The results were compared with a 10- μ L sample of the suspension assayed prior to layering over the CsCl₂. Assay results were corrected for dilution and recoveries were compared.

Kinetics of Release

Endotoxin-containing gloves were either left normal or turned inside out and each was filled with 100 mL depyrogenated water. They were then attached to a 500-mL flask and placed on a rocker platform at 100 rpm. Samples (0.5 mL) were taken at times indicated and assayed for protein and endotoxin as above.

RESULTS

The results of the endotoxin, protein, and allergen assays for the 21 different gloves are listed in Table 1. The protein content of latex gloves varied over a large range, from below the assay detection limit (25 μ g/g) to 1150 μ g/g of glove. The average protein content for latex examination gloves was 460 μ g/g compared with 74.7 μ g/g of glove for latex surgical gloves (Table 2). The average protein content for powdered latex gloves was 444 μ g/g of glove while that for nonpowdered latex gloves was 47 μ g/g. One of the nonlatex control gloves (vinyl) demonstrated a high level of protein as measured by the modified Lowry test while the other was negative. The latex allergen content in commercially available latex gloves also varied over a wide range from below the detection limits of the assay (1 μ g/g) to 858 μ g/g of glove. The average allergen content for latex examination gloves was 335 μ g/g of glove while latex surgical gloves averaged much lower at 27 μ g/g of

glove. Powdered latex gloves contained on average 313 μ g/g of glove while nonpowdered latex gloves averaged 25 μ g/g of glove. Neither of the non-latex gloves demonstrated any detectable latex allergen. The endotoxin content of the latex gloves ranged from 0.09 to 2800 ng/g of glove. As with the protein and allergen measurements, there was a strong correlation of endotoxin levels with the type of glove. Examination gloves were contaminated with the highest amounts of endotoxin and averaged 404 ng/g while surgical gloves averaged a much lower endotoxin content at 3 ng/g of glove. The endotoxin content was generally higher in powdered latex gloves which averaged 338 ng/g of glove as compared with nonpowdered latex gloves which averaged 78 ng/g of glove. One powdered nonlatex control glove demonstrated 4.0 ng/g of glove while the

other was below the detection limits of the assay (0.5 pg/g of glove). On average examination gloves contained higher levels of protein, allergen, and endotoxin and this trend was apparent whether they were powdered or not. There were exceptions to this finding as one of the powdered examination latex gloves was very low in endotoxin content and two nonpowdered examination gloves were high in endotoxin content. The endotoxin content of the slurry and the unused dextran powder were found to be 64000 ng/mL and 0.3 ng/mL, respectively (Table 1).

The rate of endotoxin and allergen release into an aqueous buffer from latex gloves was very rapid (Fig 1). The majority of these materials was released in the first two minutes of incubation after which slowly increasing amounts were seen. When gloves were turned inside-out and these ex-

Table 1. Allergen, Protein, and Endotoxin Content of Surgical and Examination Gloves

| | Glove Type | | Allergen,* μ g/g | Protein,† μ g/g | Endotoxin,‡ ng/g |
|---------------|------------|---------------|-------------------------|------------------------|---------------------|
| | Powder | Surgical/Exam | | | |
| 1 | P | S | 88 | 150 | 0.09 |
| 2 | P | E | 21.9 | 145 | 0.4 |
| 3 | NP | S | 1.4 | 130 | 0.45 |
| 4 | NP | S | 1.5 | <25 | 0.92 |
| 5 | NP | S | <1 | <25 | 3.80 |
| 6 | P | S | 32 | 90 | 3.80 |
| 7 | P | S | 66 | 85 | 5.80 |
| 8 | P | S | 2.7 | 68 | 6.00 |
| 9 | P | E | 214 | 360 | 23.0 |
| 10 | NP | E | 74.5 | 55 | 37.3 |
| 11 | P | E | 837 | 735 | 82.3 |
| 12 | P | E | 635 | 685 | 87.3 |
| 13 | P | E | 26.4 | 100 | 147.1 |
| 14 | P | E | 858 | 700 | 201.0 |
| 15 | NP | E | 3.1 | 45 | 210.5 |
| 16 | NP | E | 68.6 | 50 | 216.5 |
| 17 | P | E | 686 | 840 | 337.0 |
| 18 | P | E | 44 | 655 | 662.5 |
| 19 | P | E | 553 | 1150 | 2837.5 |
| Vinyl | NP | S | <1 | 675 | <0.0005 |
| Neoprene | P | S | <1 | 25 | 4.0 |
| Dry powder | | | | 50 | 0.32 |
| Liquid slurry | | | | 405 | 64000 |

NP, nonpowdered; P, powdered; S, surgical glove; and E, examination glove.

* RAST inhibition method.

† Protein determined by the American Society for Testing Materials (ASTM) standard method D5712-95.

‡ Endotoxin by kinetic Limulus Amebocyte Lysate method (average of three determinations on same glove).

periments were repeated, only a small amount of protein was seen and no endotoxin was detected. These results indicate that both the endotoxin and protein are loosely attached to the *inside* of the gloves tested.

When powdered gloves were snapped in an enclosed room and the airborne particles were collected and analyzed, it was found that 73% of the endotoxin was associated with particles less than 7 μm aerodynamic diameter (Fig 2). Twenty-seven percent was associated with larger particles found in the first stage of the Anderson collector. The cross-linked dextran particles were only found in the first stage as their size ranged from 20 to 70 μm . Microscopic examination of air samples (Samplair) from the room during these experiments revealed mainly two types of particles. The first appeared to be typical cross-linked dextran particles and the second were much smaller and uniform in appearance and size.

The cross-linked dextran particles were found to be quite dense (>1.79 g/mL) and sedimented very quickly through 60% CsCl_2 without centrifugation. When a suspension of air sampled with the cyclone from a room in which gloves were snapped was layered over 60% CsCl_2 and the powder allowed to sediment, the endotoxin was entirely recovered from the supernatant.

DISCUSSION

This study demonstrates that natural latex gloves contain variable and in some cases very high amounts of endotoxin. Powdered gloves had an average of 338 ng of endotoxin per gram of glove. Nonpowdered gloves had roughly a quarter of that amount. Nonsterile examination gloves had an average of 135 times more endotoxin than did sterile surgical gloves. Although exceptions were observed, protein and allergen levels were higher in the powdered gloves and lower in the surgical gloves examined. No allergen was eluted from nonlatex gloves and a very small amount of endotoxin was detected in the one neoprene glove which was lightly powdered.

Table 2. Comparison of Powdered Versus Nonpowdered and Surgical Versus Examination Gloves

| | Mean Values from Gloves Tested | | |
|------------------------------|--------------------------------|---------------------------|-----------------|
| | Protein, $\mu\text{g/g}$ | Allergen, $\mu\text{g/g}$ | Endotoxin, ng/g |
| Powdered latex gloves (P) | 444 | 313 | 338 |
| Nonpowdered gloves (NP) | 47 | 25 | 78 |
| Ratio (P/NP) = | 9.5 | 12.6 | 4.3 |
| Latex examination gloves (E) | 460 | 335 | 404 |
| Latex surgical gloves (S) | 75 | 27 | 3.0 |
| Ratio (E/S) = | 6.2 | 12.3 | 135 |

Data and abbreviations from Table 1.

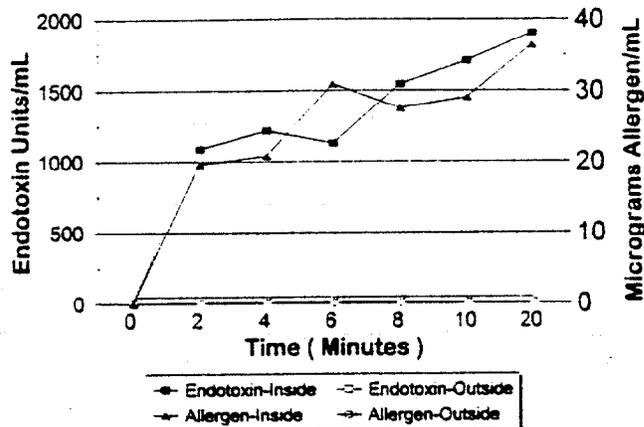


Figure 1. Kinetics of release from a latex glove: endotoxin and latex allergen. Gloves were filled with pyrogen-free water and mixed on a rocker. At the times indicated, samples were removed for analysis of endotoxin and allergen.

Both endotoxin and protein were released rapidly into an aqueous medium from the gloves tested. The similarity of the kinetics of release of both suggest that they may be associated. Levels of both endotoxin and protein increased at diminishing rates over the 20-minute observation period indicating some additional solubilization over time. Surprisingly, all the endotoxin and most of the protein were released from the interior of three different gloves tested. This would strongly suggest that the contamination with the protein and endotoxin occurred during manufacture while the gloves were on their molds in the inside-out configuration.

Upon gently snapping inverted gloves, we found that the majority (ie, 73%) of the endotoxin could be found in fully respirable airborne particles less than 7 μm in aerodynamic diam-

eter. This again suggests that the endotoxin is not associated with the cross-linked dextran particles which are much larger (20 to 70 μm in diameter). In addition, the endotoxin found in the upper stage of the Anderson sampler (27%) may be an overestimate because of the trapping effects of fluid medium in the first stage. Protein was not measured in these Anderson sampling experiments. The fact that both endotoxin and protein elute with similar kinetics in aqueous medium suggests that they may be associated with each other as an aerosol. This conclusion is somewhat contradictory to the findings of Tomazic et al who provided data that the cross-linked dextran particles were carriers of allergenic proteins.⁵¹ Upon close inspection, their report suggests that only a small fraction of the protein (1% to 11%) and specific IgE binding (6%) is associated with the

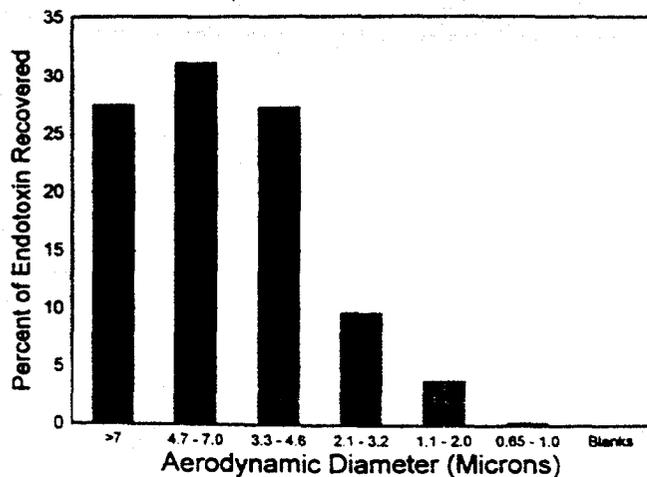


Figure 2. Size distribution of endotoxin in air. Gloves were snapped in a 25-m³ room and air samples were collected with the Anderson multistage sampler. An aliquot from each stage was assayed for endotoxin content.

cross-linked dextran particles. Since practically all the cross-linked dextran particles are too large to be fully respired, it is difficult to understand how these could reach tissues and effect allergen-specific mediator release and/or sensitization. The density of the cross-linked dextran particles we studied was shown to be very high (>1.79 g/mL). Powder of this density and size would not be expected to remain airborne for a long period of time and as such would not be likely to contribute to sensitization. This procedure also separated the endotoxin from the cross-linked dextran indicating again that the endotoxin is not closely associated with the cross-linked dextran particles.

What is the possible origin of this endotoxin? Since practically all of the endotoxin and most of the protein were found on the inner surface of the gloves, it is probable that they were passively absorbed from the dipping slurries through which the gloves were put prior to their removal from their molds. These slurries are kept at elevated temperatures, can be highly malodorous, and contain both protein and cross-linked dextran which should provide an excellent growth medium for microbes. In support of this, the slurry sample that we tested contained a high amount of endotoxin which is probably

an underestimate since the slurry had been frozen and thawed, a treatment that greatly reduces measurable endotoxin activity.⁵² Furthermore, there was only a small amount of endotoxin detected in the unused cross-linked dextran particles. It is reasonable to anticipate that the amount of endotoxin and protein in the slurry baths would increase over time during manufacture and thus with the size of a particular lot. This could result not only in lot-to-lot variations, as documented by Yunginger et al.⁵³ but also in a time-dependent increase in both endotoxin and protein during the manufacture of a single lot. As demand for gloves increased, it is likely that the number of gloves/lot also increased. In addition, with the rapid increase in demand for latex gloves, more of the production was shifted to overseas sites, particularly Malaysia, where much of the latex is harvested. It is possible that the tropical environmental conditions at these sites favors higher levels of bacterial growth and thus more endotoxin in the slurries. The conversion from talc to cross-linked dextran powder, which occurred in the mid-80s (close to when problems with latex products began to accelerate) would also be expected to favor bacterial growth. Finally, the shortened time between harvest and use in production would be

expected to allow for less breakdown of proteins and endotoxin by the action of various proteases and ammonia.

Medical problems from endotoxin are diverse and have been well documented. They include irritation, rhinoconjunctivitis, pulmonary congestion and asthma, fever, malaise, and anaphylactoid shock.¹³⁻¹⁸ These nonspecific effects have been shown to involve the induction of proinflammatory cytokines such as TNF- α , IL-6, and IL-8 via specific humoral factors (LBP-lipid binding protein-a co-factor in the binding of LPS to CD-14) and cell receptors (CD-14).⁶⁻¹² These cytokines can in turn induce chemotactic factors, cellular adhesion molecules, and effector systems such as nitric oxide synthetase and platelet activating factor.^{54,55} The clinical effects of exposure to endotoxin depends upon the dosage, the route of exposure, the structure of the endotoxin encountered, and individual susceptibility factors.^{19-24,57} Specific effects of endotoxin include its activity as an immunologic adjuvant both for IgE antibodies to bystander antigens when administered through the respiratory route and also as an adjuvant for delayed hypersensitivity to chemical haptens on the skin.^{13,25-28} Both the nonspecific and specific activities of endotoxin could be playing a role in clinical problems with natural latex products and these are not necessarily mutually exclusive. This may explain some of the difficulties in diagnosing these problems as these symptoms can arise through specific or nonspecific mechanisms. This possibility is strengthened by Sullivan's finding that a number of ill defined symptoms blamed upon latex sensitization do not seem to be associated with the presence of IgE antibodies.⁵⁶ In addition to being an immunologic adjuvant, endotoxin has been shown to enhance antigen-specific mediator release from basophils.²⁸⁻³¹ The possibility exists that endotoxin can magnify these immunologically induced reactions. This in turn could explain why so many of these reactions are so severe.

Our experiments demonstrated that ten lightly snapped gloves (with high endotoxin content) in a room of 25 m³ resulted in a concentration of endotoxin in the air of 520 ng/m³. This is a highly significant level of endotoxin as illustrated by studies indicating that levels of 10 ng endotoxin/m³ and 100 ng endotoxin/m³ can significantly compromise asthmatic and normal individuals' pulmonary problems.^{57,58} The total endotoxin exposure to a single pair of highly contaminated gloves (#19) can be calculated to be around 84 µg assuming the average glove weighs 7.5 g and the endotoxin is confined to the inside of the glove. Since many pairs of these gloves are worn during a working period, the actual exposure would be much higher. The time the gloves are worn might also be a factor in estimating exposure as moisture accumulates over time and would be expected to enhance elution of both endotoxin and proteins.

An additional complication of endotoxin-induced inflammation includes the fact that other bacterial products such as peptidoglycans and teichoic acid can not only induce similar inflammation but can act to prime animals in a way that their reactions to endotoxins are more severe.¹⁹⁻²⁴ The possibility exists that these priming effects could also affect the susceptibility of different individuals at different times to exhibit symptoms upon exposure to endotoxins and allergens.

These findings strengthen the arguments that increased demand and changes in manufacturing procedures, such as the use of cross-linked dextran, the production of larger lots of gloves, and the increase use of foreign production sites are likely factors responsible for the current high incidence of sensitization to natural latex products. They also indicate that the powder is most likely relevant to this phenomenon, not as a carrier of allergens, but as a growth source for bacteria. Collectively, these processes would be expected to increase the chances of bacterial and protein contamination.

In conclusion, given their nonspecific and specific inflammatory poten-

tial, we propose that the finding of endotoxin in some latex gloves could have serious health implications. Endotoxin would be expected to be an irritant to the skin and to increase the sensitization rate to chemicals inherent in these products. Second, since endotoxin was found in small fully respirable particles, areas of heavy glove usage may represent an environmental threat from endotoxins alone. The adjuvancy properties of endotoxin through the respiratory route would be expected to increase sensitization rates. Finally, the fact that endotoxin and other bacterial products can enhance antigen-specific mediator release and other inflammatory processes may explain why many of the reactions to these products are so severe. This latter property of endotoxin may also provide an explanation for why a high number of latex skin tests are positive in individuals with negative clinical histories.^{35,59}

Our results do demonstrate that these contaminants readily elute from natural latex gloves in aqueous medium and thus can be removed by washing. The observation that several different gloves were very low in protein, allergen, and endotoxin provides evidence that this can be accomplished on a routine basis.

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REFERENCES

1. Raetz CRH, Ulevitch RJ, Wright SD, et al. Gram-negative endotoxin: an extraordinary lipid with profound effects on eukaryotic signal transduction. *FASEB J* 1991;5:2652-60.
2. Rietschel ET, Schade U, Jensen M, et al. Bacterial endotoxins: chemical structure, biological activity and role in septicemia. *Scand J Infect Dis Suppl* 1982;31:8-21.
3. Hoekstra D, Van Der Laan JW, DeLeij L, Witholt B. Release of outer mem-

brane fragments from normally growing *Escherichia coli*. *Biochim Biophys Acta* 1976;455:889-99.

4. Cadieux JE, Kuzio J, Milazzo FD, Kropinski AM. Spontaneous release of lipopolysaccharide by *Pseudomonas aeruginosa*. *J Bacteriol* 1983;155:817-25.
5. Shenep JJ. Antibiotic-induced bacterial cell lysis: a therapeutic dilemma. *Eur J Clin Microbiol* 1986;5:11-20.
6. Wright SD, Ramos RA, Tobias PS, et al. CD14: a receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein. *Science* 1990;249:1431-3.
7. Tobias PS, Mathison JC, Ulevitch RJ. A family of lipopolysaccharide binding proteins involved in responses to gram negative sepsis. *J Biol Chem* 1988;263:13479-81.
8. Tobias PS, Soldau K, Ulevitch RJ. Identification of a lipid A binding site in the acute phase reactant lipopolysaccharide binding protein. *J Biol Chem* 1989;264:10867-71.
9. Beutler B, Cerami A. The biology of cachectin/TNF-α primary mediator of the host response. *Annu Rev Immunol* 1989;7:625-55.
10. Nordan R, Potter M. A macrophage-derived factor required for plasmacytomas for survival and proliferation in vitro. *Science* 1986;233:566-9.
11. Pober JS, Cotran RS. Cytokines and endothelial cell biology. *Physiol Rev* 1990;70:427-51.
12. Pohlman TH, Harlan JM. Endotoxin-endothelial cell interactions. In: Morrison DC and Ryan J, ed. *Bacterial endotoxic lipopolysaccharides*. Boca Raton: CRC Press, 1992:459-72.
13. Piguert PF, Grau GE, Hauser C, Vassalli P. Tumor necrosis factor is a critical mediator in hapten-induced irritant and contact hypersensitivity. *J Exp Med* 1991;173:673-9.
14. Milton DK, Amsel J, Reed CE, et al. Cross-sectional follow-up of a flu-like respiratory illness among fiberglass manufacturing employees: endotoxin exposure associated with two distinct sequelae. *Am J Ind Med* 1995;28:469-88.
15. Casale TB, Ballas ZK, Kaliner MA, Keahey TM. The effects of intravenous endotoxin on various host-effector molecules. *J Allergy Clin Immunol* 1990;85:45-51.
16. Sandstrom T, Bjermer L, Rylander R. Lipopolysaccharide (LPS) inhalation

- in healthy subjects increases neutrophils, lymphocytes and fibronectin levels in bronchoalveolar lavage fluid. *Eur Respir J* 1992;5:992-6.
17. Sandstrom T, Bjermer L, Rylander R. Lipopolysaccharide (LPS) inhalation in healthy subjects causes bronchoalveolar neutrophilia, lymphocytosis, and fibronectin increase. *Am J Ind Med* 1994;25:103-4.
 18. Michel O, Duchateau J, Sergysels R. Effects of inhaled endotoxin on bronchial reactivity in asthmatic and normal subjects. *J Appl Physiol* 1989;66:1059-64.
 19. Galanos CMA, Freudenberg, Matsuura M. Mechanisms of the lethal action of endotoxin and endotoxin hypersensitivity. In: Friedman H, Klein TW, Nakona M, Nowotny A, ed. *Endotoxin*. New York: Plenum Press, 1990: 603-19.
 20. Takada H, Kawabata Y, Kawata S, Kusumoto S. Structural characteristics of peptidoglycan fragments required to prime mice for induction of anaphylactoid reactions by lipopolysaccharides. *Infect Immun* 1996;64:657-9.
 21. Baker PJ, Hrada T, Taylor CF, et al. Structural features that influence the ability of lipid A and its analogs to abolish expression of suppressor T cell activity. *Infect Immun* 1992;60:2694-701.
 22. Millar A, Singer M, Meager A, et al. Tumour necrosis factor in bronchopulmonary secretions of patients with adult respiratory distress syndrome. *Lancet* 1989;2:712-5.
 23. Jacobs CO, Fronek Z, Lewis GD, et al. Heritable major histocompatibility complex class II-associated differences in production of tumor necrosis factor α : Relevance to genetic predisposition to systemic lupus erythematosus. *Proc Natl Acad Sci USA* 1990;87:1233-7.
 24. Li ZG, Danis VA, Brooks PM. Effect of gonadal steroids on the production of Il-1 and Il-6 by blood mononuclear cells *in vitro*. *Clin Exp Rheumatol* 1993;11:157-62.
 25. Snapper C, Peca-nha L, Levine A, Mond J. IgE class switching is critically dependent upon the nature of the B-cell activator, in addition to the presence of Il-4. *J Immunol* 1991;147:1163-70.
 26. Coffman RL, Ohara J, Bond MW, et al. B cell stimulatory factor-1 enhances the IgE response of LPS activated B cells. *J Immunol* 1986;136:4538-45.
 27. Mizoguchi K, Nakashima Y, Hasegawa K. Augmentation of antibody responses of mice to inhaled protein antigens by simultaneously inhaled bacterial polysaccharide. *Immunobiology* 1986;34:355-65.
 28. Bottaro A, Lansford R, Xu L, et al. S region transcription per se promotes basal IgE class switch recombination but additional factors regulate the efficiency of the process. *EMBO J* 1994;13:665-74.
 29. Smith TF, Alvelot M, Morrison DC. The effect of bacterial lipopolysaccharide on histamine release from human basophils. I. Enhancement of immunologic release by *S. minnesota* R595 LPS. *Clin Immunopath* 1985;34:355-65.
 30. Norm S. Microorganism-induced or enhanced mediator release: a possible mechanism in organic dust related diseases. *Am J Ind Med* 1994;25:91-5.
 31. Iida M, Shinohara S, Yamaguchi M, et al. Lipopolysaccharide primes human basophils for enhanced mediator release: requirement for plasma cofactor and CD-14. *Biochem Biophys Res Comm* 1994;203:1295-301.
 32. Norm S, Jarlov J, Clementsen P, et al. Bacteria and their products peptidoglycan and teichoic acid potentiate antigen-induced histamine release in allergic patients. *Agents Action* 1987;20:175-7.
 33. Dillard SF, MacCollum MA. Reports to the FDA: allergic reactions to latex containing medical devices. International Latex Conference: Sensitivity to latex in medical devices [Abstract]. 1992:23.
 34. Sussman GL, Tarlo S, Dolovitch J. The spectrum of IgE-mediated responses to latex. *JAMA* 1991;265:2844-7.
 35. Turjanmaa K, Alenius H, Mäkinen-Kiljunen S, et al. Natural rubber latex allergy. *Allergy* 1996;51:593-602.
 36. Ownby D, Tomlanovitch M, Sammons N, McCullough J. Fatal anaphylaxis during a barium enema examination associated with latex allergy. *Am J Roentgenol* 1991;156:903-8.
 37. Jaeger D, Kleinhans D, Czuppon AB, Baur X. Latex specific proteins causing immediate-type cutaneous, nasal, bronchial, and systemic reactions. *J Allergy Clin Immunol* 1992;89:759-68.
 38. Kaniwa M, Isama K, Nakamura A, et al. Identification of causative chemicals of allergic contact dermatitis using a combination of patch testing in patients and chemical analysis. Application to cases from rubber gloves. *Contact Dermatitis* 1994;31:65-71.
 39. Slater JE. Latex allergy. *J Allergy Clin Immunol* 1994;94:139-49.
 40. Yagami T, Sato M, Nakamura A, Shono M. One of the rubber latex allergens is a lysozyme. *J Allergy Clin Immunol* 1995;96:677-86.
 41. Alenius H, Kurup V, Kelly K, et al. Latex allergy: frequent occurrence of IgE antibodies to a cluster of 11 latex proteins with spina bifida and histories of anaphylaxis. *J Lab Clin Med* 1994;123:712-20.
 42. Alenius H, Kalkkinen N, Turjanmaa K, et al. Purification and partial amino acid sequencing of a 27 kD natural rubber allergen recognized by latex-allergic children with spina bifida. *Int Arch Allergy Immunol* 1995;106:258-62.
 43. Alenius H, Kalkkinen N, Reunala T, et al. The main IgE-binding epitope of a major latex allergen, prohevein, is present in its N-terminal 43-amino acid fragment, Hevein. *J Immunol* 1996;156:1618-25.
 44. Lu LJ, Kurup VP, Hoffman DR, et al. Characterization of a major latex allergen associated with hypersensitivity in spina bifida patients. *J Immunol* 1995;155:2721-8.
 45. Kurup VP, Kelly T, Elms N, et al. Cross-reactivity of food allergens in latex allergy. *Allergy Proc* 1994;15:211-6.
 46. Czuppon AB, Chen Z, Rennert S, et al. The rubber elongation factor of rubber tree (*Hevea brasiliensis*) is the major allergen in latex. *J Allergy Clin Immunol* 1993;92:690-7.
 47. Lavaud F, Prevost A, Cossart C, et al. Allergy to latex, avocado, pear, and banana: evidence for a 30 kD antigen in immunoblotting. *J Allergy Clin Immunol* 1995;95:557-64.
 48. Sussman GL, Beezhold DH. Allergy to latex rubber. *Ann Intern Med* 1995;122:43-6.
 49. Halsey JF. Reports to the FDA: measurement of the latex allergen content in medical devices. International latex conference: sensitivity to latex in medical devices [Abstract]. 1992:24.
 50. Tomazic VJ, Withrow TJ, Hamilton RG. Allergens, IgE mediators, inflammatory mechanisms. Characterization

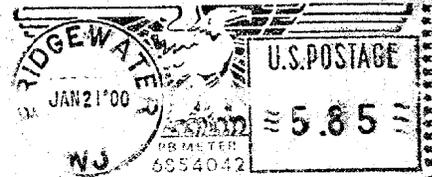
- of the allergen(s) in latex protein extracts. *J Allergy Clin Immunol* 1995; 96:635-42.
51. Tomazic VJ, Shampaine EL, Lamanna A, et al. Cornstarch powder on latex products is an allergen carrier. *J Allergy Clin Immunol* 1994;93:751-8.
52. Douwes J, Versloot P, Hollander A, et al. Influence of various dust sampling and extraction methods on the measurement of airborne endotoxin. *Appl Environ Microbiol* 1995;61:1763-9.
53. Yunginger JW, Jones RT, Fransway AF, et al. Extractable latex allergens and proteins in disposable medical gloves and other rubber products. *J Allergy Clin Immunol* 1994;93: 836-42.
54. Warren JB, Coughlan ML, Williams TJ. Endotoxin-induced vasodilatation in anaesthetized rat skin involves nitric oxide and prostaglandin synthesis. *Br J Pharmacol* 1992;106:953-7.
55. Rylander R, Beijer L. Inhalation of endotoxin stimulates alveolar macrophage production of platelet-activating factor. *Am Rev Respir Dis* 1987;135: 83-6.
56. Beebe RG, Sullivan TJ. Analysis of clinical indicators of IgE-mediated occupational latex allergy [Abstract]. *Ann Allergy Asthma Immunol* 1997: 78:86.
57. Castellan RM, Olenchock KB, Kinsley KB, Hankinson JL. Inhaled endotoxin and decreased spirometric values, an exposure-response relation for cotton dust. *N Engl J Med* 1987;317:605-10.
58. Rylander R, Haglund P, Lundholm M. Endotoxin in cotton dust and respiratory function decrement among cotton workers in an experimental cardroom. *Am J Respir Dis* 1985;131:209-13.
59. Arellano R, Bradley J, Sussman G. Prevalence of latex sensitization among hospital physicians occupationally exposed to latex gloves. *Anesthesiology* 1992;77:905-8.

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