DEPARTMENT OF HEALTH AND HUMAN SERVICES

Public Health Service

National Institute for Occupational Safety and Health
Robert A. Taft Laboratories
4676 Columbia Parkway
Cincinnati OH 45226-1998

2327 '99 OCT 28 A9:18

October 28, 1999

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

Dear Sir/Madam:

The National Institute for Occupational Safety and Health (NIOSH) has reviewed the proposed rule *Surgeon's and Patient Examination Gloves: Reclassification* published in the *Federal Register* on July 30, 1999 [64 FR 41710].

NIOSH strongly supports the Food and Drug Administration (FDA) effort to reduce the adverse health effects from allergic reactions caused by the natural latex protein allergens and glove powder found on surgeon's and patient examination gloves and to reduce the adverse health effects from defects in the barrier integrity and quality of surgeon's and patient examination gloves. In June 1997, NIOSH published the Alert *Preventing Allergic Reactions to Natural Rubber Latex in the Workplace* [DHHS(NIOSH) Publication No. 97-135]. We continue to support the recommendations in the Alert, including:

- Provide workers with nonlatex gloves to use when there is little potential for contact with infectious materials (for example, in the food service industry).
- Appropriate barrier protection is necessary when handling infectious materials. If latex gloves are chosen, provide reduced protein, powder-free gloves to protect workers from infectious materials.

Additional information and comments are enclosed. Please contact me at (513) 533-8302 if we can provide further assistance.

Sincerely yours,

Paul A. Schulte, Ph.D.
Director
Education and Information Division

99D-2335

Enclosures

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Clinical and Experimental Allergy, 1998, Volume 28, pages 537–544

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Exposure – response

REVIEW

Exposure–response relationships of occupational inhalative allergens

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Summary

Only a few threshold limit values exist at present for allergens in the workplace known to cause bronchial asthma. This contrasts with the great number of occupational asthma cases observed in industrialized countries. Recently published studies provide clear evidence for exposure intensity response relationships of occupational allergens of plant, microbiological, animal or man-made origin. If allergen exposure levels fall short of determined limit values, they are not associated with an increased risk of occupational asthma. Corresponding data are available for wheat flour (1–2.4 mg/m^3), fungal α-amylase (0.25 ng/m^3), natural rubber latex (0.6 ng/m^3), western red cedar (0.4 mg/m^3) and rat allergens (0.7 μg/m^3). It is suggested to stipulate legally binding threshold limit values (TLV/TWA) on this basis in order to induce more effective primary preventive measures. If no reliable data on the health risk of an occupational airborne noxa exist, the lowest reasonably practicable exposure level has to be achieved. Appropriate secondary preventive measures have to be initiated in all workplaces contaminated with airborne allergens. Verified exposure–response relationships provide the basis for risk assessment and for targeted interventions to reduce the incidence of occupational asthma also in consideration of cost benefit aspects.

Occupational asthma is a disease characterized by variable airflow limitation and/or airway hyperresponsiveness due to causes in a working environment. These causes can give rise to asthma through immunological or non-immunological mechanisms [1]. Up to 15% of all asthma cases are of occupational origin or have at least a significant causal occupational factor [1–10]. According to the New Zealand part of the European Respiratory Health Survey, an increased risk of asthma prevalence was found for several occupations such as laboratory technicians, food producers, chemical workers, plastic and rubber workers [11]. The Spain part of this study comprising 2646 Spanish subjects showed an asthma risk to be attributed to occupational exposures between 5 and 6.7% [12]. Main asthma-inducing agents in the workplace are flour, grain and feed dust, animal dander/urinary proteins and isocyanates. Further, several inhalative irritants such as chlorine, acid or alkaline aerosols play a pivotal role. Many low molecular weight chemicals have irritative as well as allergenic effects on the airways, e.g. isocyanates and acid anhydrides. In addition to chronic or repetitive exposures, also singular accidental exposure to high concentrations of irritative or toxic airborne substances can cause occupational asthma. This condition is frequently called reactive airways dysfunction [13].

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Exposure–response relationships

Recent studies on exposed subjects give strong evidence for exposure intensity-response relationships between indoor or common environmental allergens on the one hand and

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Table 1. Studies on exposure-response relationships of occupational/environmental inhalant allergens

Source substance allergen	Exposure evaluation (method)	Lowest effective antigen level	Exposure–response relation of	Group	Reference
Flour dust	Dust sampling in bakery atmospheres	1–2.4 mg/m^3 1.7 mg/m^3	Sensitization sensitization, respiratory symptoms, PD_{20}	230 bakery employees 279 bakery employees	[18] [16]
Fungal α-amylase	Dust sampling in bakery atmospheres	0.25 ng/m^3	Sensitization	230 bakery employees	[22]
Wood dust (red cedar)	Questionnaire and dust sampling in saw mill atmospheres	0.2–0.4 mg/m^3	Decrease in FVC	243 saw mill workers	[24]
Natural rubber latex	Dust sampling in hospital atmospheres	0.6 ng/m^3	Sensitization, respiratory symptoms	145 hospital employees	[30]
Cow dander/hair Bos d 2	Analysis of room dust samples	1–29 μg/g dust	Sensitization	40 farmers	[35]
Rat urine	Questionnaire and dust sampling in workplace atmospheres	0.1–68 μ/m^3	Respiratory symptoms	323 employees with contact to laboratory rats	[38]
Dog dander/hair Can f 1	Analysis of room dust samples	10 μg/g dust	Sensitization	Review of epidemiological studies	[63]
Cat dander/hair Fel d 1	Analysis of room dust samples	8 μg/g dust	Sensitization	Review of epidemiological studies	[63]
Chironomidae Chi t 1-9	Questionnaire	> 5 mg/month	Sensitization	184 Chi t 1-9 exposed subjects	[42]
Acid anhydrides	Dust sampling in workplace atmospheres	0.3–1.7 (various) 0.1–0.39 mg/m^3 (TCPA) 0.82 mg/m^3 (TMA)	Symptoms symptoms symptoms, sensitization	Overview on various studies 52 factory workers 17 factory workers	[52] [43] [69]
Isocyanates	MDA 7005	5–10 ppb	Symptoms, lung function impairment	84 industry workers	[70]

sensitization or diseases on the other hand [14,15]. As shown by an increasing number of investigations, this is also true for occupational allergens. In the following, results of several relevant studies on this issue are summarized (see also Table 1. However, in this context, it has to be differentiated between dust exposure and allergen exposure. Some of the studies mentioned below only estimated the exposure intensity related to the whole dust-concentration measured. Since the dust samples may contain different levels of allergenic and non-allergenic substances, it is particularly important to establish and employ specific assays for an accurate and precise estimates of the exposure intensity. Nevertheless, in general an increased exposure to dust is related to an increased exposure to allergen.

Bakery allergens

Flour is one of the main causes of occupational asthma. In bakeries, flour dust concentrations of 1–2.4 mg/m^3 were found to be associated with a significantly elevated risk of sensitization to wheat antigens [16–19]. Using logistic or linear regression analyses, Musk *et al.* [16] found symptoms, lung function, bronchial reactivity and immediate-type skin-prick test responses to bakery allergens to be related to current or past exposure to dust. More important than the total dust measurement is the specific ascertainment of flour antigens [18–20]. The antigen proportion in total dust depends on size and type of bakeries. Wheat antigen amounts ranged from 2.4 to 6 ng per mg bakery dust.

Fungal α-amylase from *Aspergillus oryzae* (Asp o 2) is widely used as a baking additive. Some 20–30% of symptomatic bakers are sensitized to this enzyme [21,22]. Dose-dependent effects are already detectable from 0.25 ng/m^3 air. A study on 178 bakers showed that in addition to the atopic state, the exposure to α-amylase in the air is an important factor of sensitization to this substance in bakeries [22].

Wood dust (western red cedar)

Total dust concentrations in the air of a red cedar sawmill were determined by Vedal *et al.* [23]. In addition, 652 employees were investigated using questionnaire and spirometry. The dust level was found to be up to 6 mg/m^3 in working environment. Workplace-related eye irritations were more common at concentrations above 3 mg/m^3. Workplace-related asthma was associated with an exposure of more than 10 years (found in 8% of workers). Lung function values (FEV_1 and FVC) were lower at higher wood dust concentrations.

Noertjojo *et al.* [24] investigated 243 sawmill workers in an 11-year follow-up study on the relationship between exposure to red cedar dust and lung function changes. A total of 140 office workers served as control group. Asthmatics were not included in the study. The intensity of exposure was calculated for each employee on the basis of concentration and duration of dust exposure. Three exposure groups were differentiated: low (0.2 mg/m^3), medium (0.2–0.4 mg/m^3) and high (> 0.4 mg/m^3). In the follow-up, a significant decrease of FEV_1 and FVC, inversely related to the wood dust load, was found.

Natural rubber latex

Allergy to natural rubber latex is becoming a serious occupational problem especially among health care workers. Although the direct skin contact with latex gloves is the most common way of exposure, recent studies demonstrate that latex allergens can become airborne in hospitals and doctors' surgeries with glove powder as allergen carrier [25–28]. To analyse the relationship between latex allergen load in the air and risk of sensitization, Baur *et al.* [29,30] collected dust samples in various hospital and surgical rooms and quantified the airborne latex allergen concentrations by a competitive immunoassay using pooled serum from latex-allergic patients. Specific IgE antibodies and hypersensitivity reactions of the 145 employees who worked in these rooms were measured as well.

Airborne latex allergens were detectable in all rooms in which powdered gloves were used and no ventilation systems were installed. The highest concentration of airborne latex allergens was 205 ng/m^3.

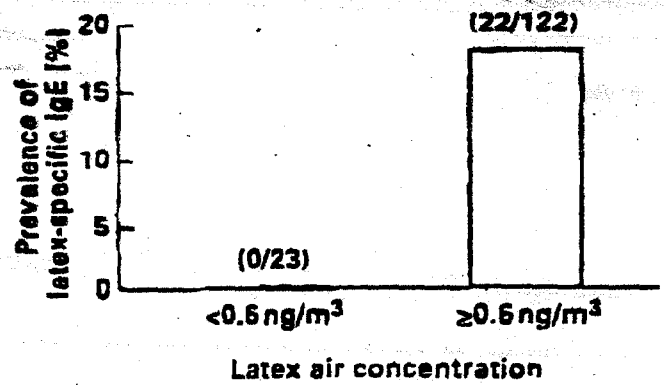

Fig. 1. Prevalence of IgE-mediated sensitization to latex of hospital employees working in rooms/units with less than 0.6 ng or ≥ 0.6 ng latex allergens per m^3 air.

As shown in Fig. 1, IgE-mediated sensitization was found to be significantly associated with the latex aeroallergen levels; at latex allergen concentrations of ≥ 0.6 ng/m^3, 18% of exposed people were found to be sensitized and 15.5% revealed hypersensitivity reactions. Lower concentrations were neither associated with IgE-mediated sensitization nor with respiratory symptoms [30].

These findings demonstrate that continuous inhalative contact with latex allergens seems to be an important risk factor for hypersensitivity reactions.

Non-occupational situations, e.g. latex allergies in spina bifida patients, also show evidence of exposure–response relations: the frequency of latex sensitization in spina bifida children was found to be strongly associated with the number of surgical procedures [31–34].

Cow dander/hair

The relationship between the levels of airborne cow dander/hair allergens and IgE-mediated sensitization in farmers was investigated by Hinze *et al.* [35]. They determined IgE antibodies to the major allergen Bos d 2 in 40 dairy farmers and analysed dust samples from living rooms. A threshold of 1–21 μg Bos d 2 per gram dust (atopics) and of 24–50 μg Bos d 2 per gram dust (non-atopics) was found to be significantly associated with an IgE level of > 0.7 kU/L.

Rat allergens

Several investigations related to laboratory animal allergy were performed by Hollander *et al.* [36]. They developed highly specific and sensitive sandwich immunoassays for rat and mouse urinary allergens. A cross-sectional study on 540 laboratory animal workers was performed to quantify the exposure–response relationship for allergy to rats. In the

group of workers with less than 4 years of working experience with laboratory animals, the prevalence rate of sensitization to rat allergens was clearly associated with exposure levels [36]. Allergies to cat and dog seemed to be an important risk factor for laboratory animal allergy [37].

A total of 323 subjects occupationally exposed to rats was studied by Cullinan *et al.* [38]. The authors measured intensity and frequency of exposure to total dust and rat urinary allergens. In addition, workplace-related symptoms and skin-prick test results (standard and rat urinary allergens) were evaluated. There was a positive correlation between the level of rat urinary allergen exposure and the frequency of positive skin-test results. The relationship between specific sensitization and work-related symptoms was more evident for atopics than for non-atopics.

Dog and cat dander/hair

Proteins from pelt and saliva of pets are mainly environmental allergens; however, sometimes they are also of occupational relevance, e.g. for veterinarians, pet shop owners and animal dealers.

Can f 1 (dog) and Fel d 1 (cat) are well-characterized allergens. Even in rooms without animals, 1–10 μg of these allergens per gram dust can be found. Obviously, the allergens are transferred by clothes, etc. Reservoirs of Can f 1 are carpets, upholstered seats in public buildings, curtains, mattresses and soft toys [39]. The investigation of different public places by Custovic *et al.* revealed values of above 10 μg Can f 1 per gram dust in 40% of upholstered seats [39]. Such concentrations are capable of provoking asthmatic symptoms in most patients allergic to dogs. A threshold value of 8 μg Fel d 1/g dust is suggested by Gelber *et al* [40] for cat allergens. The investigation of 114 sensitized asthmatic patients and 114 controls revealed that nearly all cat-allergic subjects showed symptoms at this concentration. In addition, it was the lowest value found in a house with a cat.

Chironomidae *allergens*

Red mosquito larvae of the non-biting midge (*Chironomidae*) are often used as fishfood. Their haemoglobins (Chi t 1–9) are potent allergens [41]. An association between the degree of exposure (calculated by frequency and amount of material handled) and symptoms could be found in 184 subjects exposed to this insect allergen [42]. The group consisted of fishbreeders, workers in a fish food factory and employees of zoological shops. Based on data obtained by a questionnaire, exposure levels were divided into low, medium and high. Parallel to the exposure degree, the percentage of subjects with complaints increased. In addition, a relationship between exposure and IgE-mediated sensitization could be found. Asthmatic symptoms were associated with specific IgE antibodies to Chi t 1–9. Furthermore, asthmatic patients had the highest IgE antibody concentrations [42].

Acid anhydrides

Acid anhydrides are a class of chemical agents frequently used in the production of synthetics. Lisa *et al.* [43] performed a cross-sectional study in a factory with 52 employees using tetrachlorphthalic anhydride (TCPA) as a hardener. The concentration of TCPA in the air was between 0.21 and 0.39 mg/m^3. The prevalence of work-related airway complaints was high (27–39%). After ventilation installation, the TCPA concentration decreased to 0.1 mg/m^3, and at the same time symptoms diminished considerably.

Eighteen workers exposed to trimellitic anhydride (TMA) were investigated by Bernstein *et al.* [44]. 12 of them were observed over 3 years. Five of the workers developed IgE antibodies to TMA-HSA. Three of them suffered from late respiratory systemic syndromes, one from rhinitis. After the installation of ventilation systems, TMA concentration in the air were reduced from 0.82–2.1 mg/m^3 to 0.01–0.03 mg/m^3. Subsequently, the number of workers with specific IgE antibodies and symptoms decreased. At the latter concentrations, the induction of new hypersensitivity reactions seems to be improbable.

Detergent enzymes

Proteolytic enzymes derived from *Bacillus* species have been added to synthetic detergents to enhance clearing effectiveness. They have been described as asthma-causing sensitizers [45]. Even after the introduction of encapsulated enzymes, exposures at the workplaces are still sufficient to cause IgE-mediated immunological sensitization [46]. An epidemiological study covering 11 years of enzyme-detergent production and its effects on 2344 workers revealed significant differences between minimum and maximum exposure groups with regard to FEV_1 values [47].

Isocyanates

Toluene diisocyanate (TDI), diphenylmethane diisocyanate (MDI), hexamethylene diisocyanate (HDI) as well as corresponding polyisocyanates are well known as causes of occupational airway diseases. Karol [48] investigated the relationship between exposure concentration and immunological response using an animal model (guinea-pigs). The animals were exposed for 3 h to TDI concentrations from 0.12 to 10 ppm on five consecutive days. Starting on day 22,

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specific antibodies were detected by means of passive cutaneous anaphylaxis, and allergy skin tests as well as lung function measurements were performed. No antibodies were found in the group exposed to 0.12 ppm; 55% of animals were sensitized in the group exposed to 0.36 ppm. The exposure to higher TDI concentrations resulted in an increase in antibody level and in the percentage of sensitized animals. In addition, hypersensitivity reactions were measurable by bronchial challenge test in animals exposed to 0.36 ppm or more. More than 2 ppm were found to be pneumotoxic. No immune reaction was seen after long-term exposure to a low TDI concentration (0.02 ppm for 15 weeks).

Marek *et al.* [49] found that TDI atmospheres of 10 ppb cause bronchial hyperreactivity within 4–6 h of exposure in a rabbit model of occupational lung disease. The responses were further enhanced at 30 ppb. In guinea-pigs, 10 ppb of HDI applied over 8 weeks also induced bronchial hyperreactivity [50].

Baur [51] reported an increased frequency of sensitization and symptoms in a group of isocyanate workers exposed to 5–10 ppb when compared with a group exposed to less than 5 ppb. No influence of smoking habits was found.

In most European countries, TLVs for isocyanates have been stipulated at 5 or 10 ppb.

How can threshold limit values be defined?

Avoidance of allergen exposure in the working place is the most effective approach for the prevention of occupational asthma. With regard to appropriate measures to reduce or even eliminate occupational asthma-inducing agents, the publications of Venables [52], Corn [53] and Gordon *et al.* [54] are also referred to. Depending on the allergen source and material, different preventive procedures may be effective, e.g. the substitution of hazardous substances by harmless ones, installation of exhaust systems, use of ventilated workstations, encapsulation of machines, use of granulated or liquid products instead of powdered ones.

In contrast to many toxic and/or carcinogenic substances [55,56], only few exposure limit values for allergenic substances in the workplace exist, e.g. inhalable wood dust with a TLV/TWA[1] of 2 mg/m^3 (due to carcinogenic effects) in Sweden and Germany; flour dust with a TLV/TWA of 1 mg/m^3 in the Netherlands and of 4 mg/m^3 in Germany and isocyanates with TLVs/TWAs mostly of 5 or 10 ppb.

To control the exposure to asthma-inducing agents in the workplace, threshold values or a maximum tolerated concentration of these agents should be established first. The question is how these legally binding threshold limit values (TLV) should be defined. Should they be based on the significantly increased frequency of sensitization to asthma-inducing agents or on the prevalence or incidence of occupational asthma in defined workforces? And should 'time-weighted average' (TWA) concentrations[1], 'short-term exposure limit' (STEL) concentrations[2] and/or ceiling concentrations[3] be considered? A further possibility would be to define the so-called 'no observed adverse effect level' (NOAEL) which is significantely different from the 'lowest observed adverse effect' (LOAEL) [57].

Since most allergens have no toxic or irritative effects (exceptions are several chemicals at high concentrations) it would be appropriate to supulate legally binding TLVs/TWAs for major causes of occupational asthma based on the best available scientific work referring to the incidence (prevalence) of this disease.

If exposure to an occupational agent causes a significant increase in asthma cases in a working group when compared with the normal population, or significant increase in asthma incidence is found among the workers, the causative agent should be indicated as a hazardous substance, corresponding preventive measures should be introduced and based on the results of research works on the exposure-response relationship, a TLV/TWA for this agent should become obligatory. If this TLV/TWA is exceeded in workplaces, restrictions for work should be introduced, such as limited working duration and exposure, use of adequate personal protective devices (especially respirators) and introduction of medical surveillance programmes [30,52,58–60].

If no reliable data on asthma prevalence/incidence of a certain endangered, occupationally exposed group exist, the lowest reasonably practicable exposure level of airborne organic as well as inorganic substances has to be achieved. Furthermore, appropriate research work has to be initiated to obtain this information as soon as possible.

One major problem of defining threshold values is that methodology for quantification of different allergenic material is far from being standardized yet. This is, however, urgently needed for exact exploration of exposure-intensity relationship and it also builds the basis to establish thresholds for allergenic substances in the workplace. The recent progress on allergen analysis has been summarized by Hamilton *et al.* [61] and Esch [62]. To quantify airborne allergen concentrations in the workplace, some appropriate immunoassays, mainly based on monoclonal antibodies, already exist, e.g. for fungal α-amylase, natural rubber latex, several animal allergens and flour [22,29,38,53,54,63–68]. However, in most cases these specific assays were employed only by reporter self. Some working conditions, e.g. reference antigen or antibody are mostly not available for other investigators. In order to facilitate the comparison of exposure data obtained from different studies corroboration and standardization of these assays are necessary.

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It has to be emphasized that in the context of exposure–response relationships many other issues still need to be taken into account. One of them is the exposure assessment amongst workers studied. In addition to the quantification of asthma-eliciting agents in the workplace, occupational epidemiology studies require accurate and precise estimates of the exposure intensity to explore exposure intensity response relationships [67]. With different working conditions, the level of exposure in individuals varies; and different measurement strategies may lead to different health risk estimates. A comprehensive review of the issues involved in exposure assessment for occupational epidemiology studies has been recently given by Nieuwenhuijsen [68].

Other important aspects which also have to be investigated are the detection of early disease stages and their process, a more detailed description of the underlying exposure–response relationships of single or even mixed exposures and the interference of exposure with individual predisposing/protective factors like genetic susceptibility, atopy, smoking habits, etc. The possible booting effect of peak exposures on sensitization and symptoms/dysfunctions is especially important for the study of initiation of occupational asthma. In general, this effect can be evaluated by real-time measurements and clinical examinations. So far, the information about the relationships between a single brief high-level exposure to an irritant or sensitizing agent and the initiation of new-onset asthma is only limited. Most studies available here deal with the present average exposures, i. e. did not take into consideration variations/peaks of previous and current exposure. The data presented are mostly from cross-sectional surveys of working populations where causative factors are not always identified.

Nevertheless, there is no doubt that data are enough for assessment of exposure–response relationships for several important occupational asthma causes. More detailed studies have to be performed in order to describe precisely these relationships. We are just at the beginning of understanding such underlying complex interactions and associations.

Appendix

Definitions of well-established threshold limit values of the American Conference of Governmental Industrial Hygienists [52] are given as examples, similar definitions exist in most countries

[1]*Threshold Limit Value — Time-Weighted Average (TLV-TWA)*

- the time-weighted average concentration for a normal 8 h working day and a 40 h working week, to which nearly all workers may be repeatedly exposed, day after day, without adverse effect.

[2]*Threshold Limit Value – Short-Term Exposure Limit (TLV-STEL)*

- the concentration to which workers can be exposed continuously for a short period of time without suffering from [1] irritation [2], chronic or irreversible tissue damage, or [3] narcosis of sufficient degree to increase the likelihood of accidental injury, impair self-rescue or materially reduce work efficiency, and provided that the daily TLV-TWA is not exceeded. It is not a separate independent exposure limit; rather, it supplements the time-weighted average (TWA) limit where there are recognized acute effects from a substance whose toxic effects are primarily of a chronic nature. STELs are recommended only where toxic effects have been reported from high short-term exposures in either humans or animals.

In the USA, a STEL is defined as a 15 min TWA exposure which should not be exceeded at any time during a workday even if the 8 h TWA is within the TLV-TWA. Exposures above the TLV-TWA up to the STEL should not be longer than 15 min and should not occur more than four times per day. There should be at least 60 min between successive exposures in this range. An averaging period other than 15 min may be recommended when this is warranted by observed biological effects.

[3]*Threshold Limit Value-Ceiling (TLV-C)*

- the concentration that should not be exceeded during any part of the working exposure.

In conventional industrial hygiene practice if instantaneous monitoring is not feasible, then the TLV-C can be assessed by sampling over a 15 min period except for those substances that may cause immediate irritation when exposures are short.

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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 μg/ml). Fifty-eight percent of patients allergic to latex reacted at the 50 μg/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 μg/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-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-

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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Supported in part by The Donald Guthrie Foundation for Education and Research and by a grant from Aladan Corporation, Dothan, Alabama.

Received for publication Feb. 1, 1996; revised Apr. 4, 1996; accepted for publication May 28, 1996.

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0091-6749/96 $5.00 + 0 1/1/75630

ported from NRL inhalation, skin prick testing, and inadvertent contamination in food.[2,9]

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 variability[22] 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 µl of 0.15% (wt/vol) sodium deoxycholate and incubating the sample at room temperature for 10 minutes. Next, 50 µl of 72% (wt/vol) trichloroacetic acid and 50 µl 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 µl 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

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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 μg/ml, and the reporting limit for the method was calculated as 9.3 μg/ml (46.5 μg/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 μg/ml and a reporting limit of 0.06 μg/ml (0.3 μg/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 μg/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 ± 8.8 μg/ml (mean ± 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 μg/ml). With the LEAP, extracts of the regular powdered gloves had 28.1 ± 8.2 μg/ml (mean ± SD, $n = 4$), whereas the chlorinated gloves had 0.045 ± 0.023 μg/ml ($n = 6$) and the polymer-coated gloves had 0.067 ± 0.082 μg/ml ($n = 7$). Three of six chlorinated gloves and five of seven polymer-coated gloves were below the detection limit (0.06 μg/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

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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[a]	Percent reacting	Lowry (μg/ml)	ELISA (μg/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 μg/ml for the Lowry assay and 0.06 μg/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 μg/ml or 47 μg/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-

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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 μg/g level was not allowed. Our data demonstrate that at the detection limit of the Lowry method, 58% of patients allergic to latex

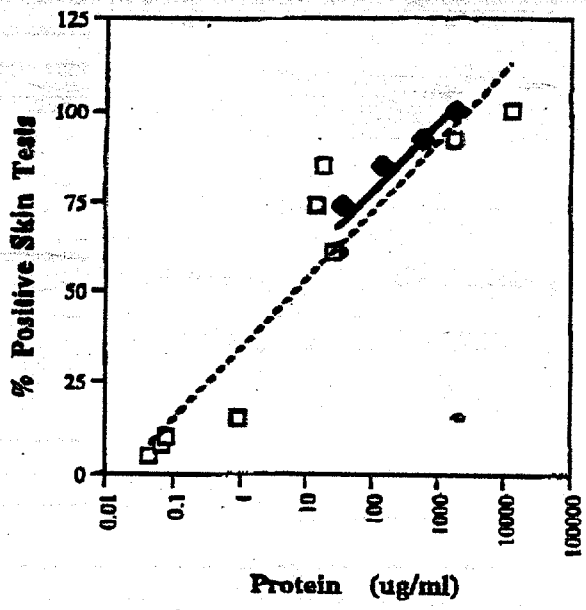

FIG. 1. Correlation of protein levels to SPT reactivity in patients allergic to latex. Patients were skin tested by end-point titration with nine NRL-containing samples. Protein levels in extracts were determined by the ASTM D5712-95 method *(solid diamonds)* or the LEAP method *(open squares)*. Total 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 μg/ml (0.2 μg/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.

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T-120 P.07/15 F-302

Oct-16-99 03:00am From-

Int Arch Occup Environ Health (1997) 70: 399–402

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ORIGINAL ARTICLE

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Allergic reactions due to glove-lubricant-powder in health-care workers

Received: 30 December 1996 / Accepted: 5 May 1997

Abstract Eight glove-wearing hospital personnel were evaluated for suspected type I-like allergic manifestations due to corn-starch powder. All subjects were clinically examined, the presence of atopy was assessed by administration of a questionnaire, the on-off test was verified (the clinical feature behavior was verified with regard to the beginning and the cessation of the work shift), levels of specific serum IgE for maize and latex were measured, and prick tests for the same allergens were performed. The on-off test was positive for everyone. The symptom associated with glove use was urticaria, which was also associated in one case with intermittent dyspnea and in another with oculorhinitis, angioedema, and asthma. Five workers were atopic. The serum IgE test found three positive responses to maize, three positive responses to both latex and maize, and two negative responses to both. However, in the two patients testing negative to IgE, the prick tests were positive: one for maize and the other for both maize and latex. All workers evaded further relapses by avoiding exposure to powdered gloves. There is general agreement that corn-starch powder may cause irritant dermatitis and that it may be a vehicle for other allergens. This study seems to suggest that corn-starch powder may act as a type I allergen itself. Further studies on a larger number of subjects and further research on the chemical properties of corn-starch powder, in particular on its protein content, are needed to confirm this hypothesis.

Key words Hospital personnel · Gloves · Corn-starch powder · Maize · Allergy

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Introduction

Glove use by health-care workers has increased over the last few years, principally due to universal precautions recommending barrier protection against contact with body fluids (Center for Disease Control 1987). Consequently, the number of glove-related diseases has considerably increased, particularly IgE-mediated, type I allergic reactions such as urticaria, oculorhinitis, asthma, angioedema, and anaphylactic shock that are frequently caused by latex sensitization. The main component of lubricant powder is corn-starch, a maize derivative obtained after extraction of protein fractions, in addition to magnesium oxide and calcium phosphate. In the lubricant powder of some gloves, other additives are present that may cause type IV allergic reactions, such as sorbic acid, epichloridrin, and isothiazolin-3-one derivatives (Fosserau et al. 1984; Fisher 1986, van der Meeren and van Erp 1986).

The role that lubricant powder may play in inducing glove-related diseases has not yet been fully clarified. Although there is general agreement that the crystals of lubricant powder may cause irritant dermatitis and that they may be a vehicle for other allergens such as latex and rubber additives, there is no clear evidence that corn starch may act as an allergen itself to induce type I allergic manifestations. Herein we describe eight cases of suspected type I-like allergic reactions to lubricant powder observed at our institute in the last 6 years and discuss the ethiopathogenetic role of corn-starch powder in the determination of type I allergic reactions.

Subjects and methods

A total of 154 health-care workers from our hospital and from several hospitals in the same province came to our occupational allergy clinic to be checked for suspected skin or systemic reactions to gloves from 1990 to 1995; therefore, this is a descriptive study. All subjects were clinically examined, the on-off test was verified (the clinical feature behavior with regard to the beginning and the cessation of the work shift was verified), and the presence of atopy

was assessed by administration of a detailed questionnaire. All subjects gave their informed consent and underwent allergological tests according to individual symptoms, with the sensitivity of and possible risks for each patient being taken into consideration.

Diagnosis of delayed type IV allergic dermatitis

In the presence of eczema without other manifestations the following tests were performed:

1. Patch tests (Firma Diagent) according to the European Standard Series as for rubber additives (e.g., thiurams, carbamates) and components of lubricant powder (e.g., sorbic acid, epichloridrin) were used. The application period was 48 h, with readings being taken at 48 and 96 h.
2. Patch tests with a fragment of the glove in use were carried out when the above-mentioned tests were negative.
3. Serum levels of specific IgE for both latex and maize were determined using the Pharmacia CAP FEIA method.
4. Prick tests (Lofarma Industries) for both latex and maize were performed with commercial extracts when specific IgE tests were negative.

The latter two tests were performed to determine the possiblity of a coexisting sensitization to latex or a case of protein contact dermatitis.

Diagnosis of type I allergic reactions

The main allergological tests used were specific IgE measurement and prick tests. The former in vitro tests are less risky for the patient but also less sensitive as compared with prick tests; however, the latter are not advisable for patients with severe type I symptoms.

In the presence of *urticaria* the following tests were performed:

1. Serum levels of specific IgE for both latex and maize, were determined.
2. Prick tests for both latex and maize were performed with commercial extracts.
3. Prick tests with extracts of the gloves in use (Lati and Turjanmaa 1992) were carried out when both specific IgE tests and prick tests with commercial allergens were negative.
4. A use test was conducted if the latter were negative (Lati and Turjanmaa 1992).
5. Patch tests with readings at 20 min, 48 h, and 96 h were performed using the above-mentioned allergens to determine the possiblility of a coexisting sensitization to type IV allergens.

For patients with asthma and/or oculorhinitis and/or angioedema and/or other allergic symptoms with or without skin reactions the following tests were performed: (a) allergological tests following the sequence used for the diagnosis of type I allergic urticaria, (b) monitoring of peak expiratory flow for 2 weeks, and (c) lung-function tests.

Diagnosis of irritant contact dermatitis

This diagnosis was formulated after the exclusion of allergic dermatitis and was confirmed by negative in vivo and in vitro allergological tests. Moreover, confirmation that the irritant dermatitis was due to lubricant powder was obtained after the workers had been provided with powder-free gloves and no relapse had been reported.

Results

Of the 154 subjects examined for suspected glove-related disease during the last 6 years, 27 were diagnosed with allergic type IV contact dermatitis: 31, with type I allergic reactions (urticaria with or without other symptoms); and 23, with irritant contact dermatitis due to gloves, whereas the other 73 patients showed no glove-related symptom (Table 1). Approximately 75% of the irritant contact dermatitis cases (17 patients) were probably due to the use of powdered gloves. This diagnosis was verified after the patients had used powder-free gloves, the symptoms had disappeared, and no relapse had been reported.

Table 2 reports demographical and historical data on eight cases of type I-like allergic reactions due to cornstarch powder. All eight subjects had used poor-quality gloves containing a large quantity of lubricant powder for 2–4 years. Five patients were atopic and their clinical history revealed allergic rhinitis, atopic eczema, and/or asthma. The symptom associated with glove use was urticaria, which was predominantly localized on the hands but was also associated in one case with intermittent dyspnea (case 2) and in another with oculorhinitis, angioedema, and asthma (case 3).

In all subjects, levels of specific serum IgE for maize and latex were measured; three subjects tested positive for maize only, three tested positive for both maize and latex, and two tested negative for both. Prick tests for the same allergens were performed on only six patients, since one patient (case 3) showed severe symptoms that contraindicated further testing and another (case 1) refused to undergo further tests. In the two patients testing negative for IgE the prick tests were positive: one for maize (case 6) and the other for both maize and latex (case 4). Thus, the allergological tests revealed four pa-

Table 1 Allergic and irritant reactions to gloves observed in health-care workers during the period 1990–1995 at the Institute of Occupational Health, University of Brescia, Italy (*ACD* Allergic contact dermatitis, *ICD* irritant contact dermatitis)

Job	Subjects (*n*)	ACD (*n*)	ICD (*n*)	Urticaria (*n*)	Urtic. + other sympt.[a] (*n*)	Atopy (*n*)
Operating-theater nurse	29	7	11	6	5	5
Nurse	25	7	8	10		9
Surgeon/physician	12	6	3	2	1	3
Nurse assistant	11	6	1	3	1	2
Others	4	1		2	1	2
Totals	81	27	23	23	8	21

[a] Other symptoms: rhinitis, conjunctivitis, asthma, angioedema

Table 2 Allergy to corn-starch powder observed in health-care workers during the period 1990–1995 (*U* Urticaria, *OR* oculorhinitis, *R* rhinitis, *AE* atopic eczema, *A* asthma, *An* angioedema, *D* dyspnea, *Op. th.* operating theater, *diag.* diagnosis, *spec.* specific, *lat.* latex)

Cases	1	2	3	4	5	6	7	8
Age (years)	37	37	47	23	28	37	37	41
Job	Surgeon	Op. th. nurse	Surgeon	Nurse	Op. th. nurse	Nurse	Surgeon	Op. th. nurse
Atopy	AE, R, A	R	OR	AE, R	OR	Neg.	Neg.	Neg.
Clin. picture	U	U+D	U, An, OR, A	U	U	U	U	U
Date of diag.	1992	1990	1993	1994	1991	1995	1994	1990
Patch	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Spec. IgE	Maize	Lat.+maize	Lat.+maize	Neg.	Maize.	Neg.	Lat.+maize	Maize
Prick	–	Lat.+maize	–	Lat.+maize	Maize	Maize	Lat.+maize	Maize

tients sensitized to maize and 4 sensitized to both maize and latex.

Discussion

The possibility that corn-starch powder, a maize derivative used as a glove-lubricant powder, may cause type I allergic reactions has been generally ascribed to its role as a vehicle of other allergens, especially latex proteins. However, only a few authors have hypothesized that IgE-mediated effects may be caused by the powder acting as an allergen itself (Fisher 1986; van der Meeren and van Erp 1986; Assalye et al. 1988; Seggev et al. 1990; Heese et al. 1991). Furthermore, it is relevant that maize can cause food allergies and type I allergic reactions in occupationally exposed subjects such as bakers and food handlers (Monneret-Vautrin et al. 1988; De Zotti et al. 1994). Sensitization to maize can be reliably demonstrated by specific IgE and/or prick tests.

All our subjects showed type I-like allergic reactions after occupational contact or inhalation of corn-starch powder. They were exposed to maize derivatives only in the workplace when they used powdered gloves and had no previous history of other possible occupational exposure to maize. Although cultivation of maize is common in our region, the symptoms observed in our patients appeared only at the workplace; neither seasonal manifestations related to air-dispersed allergens from maize cultivation nor food allergies due to maize were reported.

Moreover, the etiopathogenetic role of corn-starch powder was confirmed by a positive on-off test and by the efficacy of the protective measures adopted by the subjects, which prevented any relapses as confirmed by periodic follow-ups carried out several years after the diagnosis. For subjects sensitized to maize the use of powder-free gloves was sufficient, whereas for subjects sensitized to both maize and latex, various protective measures were employed, depending on the type of job performed: in subjects 3 and 7, both of whom are surgeons, the use of synthetic rubber gloves, washed to remove the powder, proved efficacious; in subject 2, an operating-room nurse, synthetic rubber gloves with polyethylene gloves were prescribed; and in subject 4, a nurse, the use of both powder-free vinyl gloves and of polyethylene gloves underneath them was effective.

To formulate this diagnosis we carefully reviewed the clinical and anamnestic data and the allergological test results. These results must be examined carefully, since methodological problems might exist. Indeed, commercial extracts are available for maize but not for corn starch, and maize and corn starch are not the same thing. Moreover, commercial maize extracts, used for both the prick tests and the specific IgE tests, contain proteins, and according to the information provided by the manufactures, glove corn-starch powder should undergo treatments that remove the proteins. However, it should be emphasized that van der Meeren et al. (1986) and Heese et al. (1991) have demonstrated that before it is put into the gloves, corn starch can contain not only amylose and amylopectin but also protein fractions with possible antigenic activity. It is probable that lubricant powder purity (the absence of any protein) can vary according to the glove brand, since there are many different types on the market, including those of poorer quality (Yunginger et al. 1994). Furthermore, proteins in trace amounts could be present in gloves even after the latter have undergone treatment for removal of proteins and could therefore cause IgE-mediated reactions in maize-sensitized subjects.

On the basis of these observations we can propose some hypotheses to explain how our subjects could have been sensitized and could thereafter have developed type I allergic reactions:

1. They could have been sensitized to maize due to their job since the poor-quality gloves they used contained corn-starch powder with high levels of antigenic protein.
2. They may have previously been sensitized to maize for extraoccupational reasons and may have shown allergic reactions to gloves because the corn-starch powder contained trace amounts of proteins. However, it should be to noted that other substances apart from proteins have been considered to be possible antigens (Seggev et al. 1990).

We plan to perform prick tests during the next follow-ups of the subjects previously examined and possible new subjects, using corn starch that has not yet been put into gloves and corn starch of food grade. The possible protein content of each powder will also be determined. We could not carry out these tests during

the present study because only glove corn starch was available.

In our opinion, periodic surveillance of sensitized subjects, especially operating-room workers, is necessary since latex and starch-powder readily disperse in the workplace environment. Indeed, in the long run, for those subjects who present with respiratory symptoms the adoption of specific-modes of individual protection may become insufficient since the dispersal of allergens from other users may provoke the symptoms. In addition, surveillance should be carried out to check the possible onset of seasonal symptoms or food allergy to maize.

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NIOSH National Institute for Occupational Safety and Health

Comments to FDA

Comments of the
National Institute for Occupational Safety and Health
on the
Food and Drug Administration
Proposed Rule and Notice on
Surgeon's and Patient Examination Gloves;
Reclassification and Medical Glove Guidance Manual Availability

21 CFR Parts 801, 878, and 880
Docket No. 98N-0313

U.S. Department of Health and Human Services
Public Health Service
Centers for Disease Control and Prevention
National Institute for Occupational Safety and Health

10/28/99

The following commentary on the Food and Drug Administration (FDA) proposed rule and notice *Surgeon's and Patient Examination Gloves; Reclassification and Medical Glove Guidance Manual Availability* [21 CFR Parts 801, 878, and 880], published in the *Federal Register* on July 30, 1999 [64 FR 41710], is intended to provide additional information for the development of the proposed rule.

COMMENTS ON SPECIFIC PROVISIONS OF THE PROPOSED RULE

801.440 User labeling for powdered and powder-free surgeon's and patient examination gloves.

(a) Natural rubber latex powdered surgeon's gloves and powdered patient examination gloves shall bear the following statement: "Caution: This product contains natural rubber latex which may cause allergic reactions. FDA recommends that this product contain not more than 120 mg powder and 1200 µg extractable protein per glove. This product contains no more than [insert level] mg powder and no more than [insert level] µg extractable protein." (page 41743)

FDA proposes to recommend a limit on glove powder of 120 mg per glove. On page 41710, the Preamble cites some of the evidence that glove powder is a key vector in the dissemination of latex antigen and is a vector for endotoxin as well. However, one of the cited studies [Tarlo et al. 1994] also indicates that the use of powder-free gloves in a work environment may control symptoms in allergic workers. FDA states on page 41712 of the Preamble ". . . the scientific data to define the quantitative relationship between respiratory allergic reactions and powder level on NL gloves are not available at this time." We note that, although the quantitative relationship between the risk of allergic reactions and higher powder levels on natural latex gloves is unknown, several studies have demonstrated that the use of powder-free gloves in health care facilities markedly reduces latex antigen exposure and can control allergy symptoms [Allmers et al.1998; Tarlo et al. 1994]. Powder-free surgical gloves also minimize the risks of wound granulomas and adhesions [Haglund and Junghanns 1997]. With adoption of the special controls proposed by FDA, all medical gloves, including powder-free gloves, will meet standards for barrier protection. In light of the documentation of improved safety of powder-free gloves, and the feasibility of assuring the safety and effectiveness of these products, NIOSH suggests that FDA consider a recommended powder level of not more than 2 mg, the proposed recommended powder-free glove total trace (residual) powder level, and require labeling of all latex gloves with this recommendation. Additionally, to improve protection of both health care workers and patients, NIOSH suggests that FDA consider the glove powder level of not more than 120 mg powder per glove be required rather than recommended. NIOSH encourages the FDA to evaluate the feasibility of requiring a limit on glove powder of 2 mg/glove for powdered surgeon's and patient examination gloves.

If powdered surgeon's latex gloves continue to be available, NIOSH suggests that the caution label be modified for gloves with greater than 2 mg powder/glove. In addition to the current caution on allergic reactions, we suggest that the label on powdered gloves also inform the user that powder present on the glove, as a carrier of extractable latex protein, increases the risk of latex sensitization to users and their co-workers, as well as increasing the risk of wound granulomas and adhesions.

FDA also proposes to recommend that manufacturers limit the extractable protein in gloves to 1200 µg protein per glove. FDA refers to studies by Palosuo et al. [1998] that indicate glove extractable protein levels correlate with the proportion and size of positive skin tests among allergic persons. NIOSH concurs that in the absence of more sensitive glove antigen measures, a total protein limit is a practical and useful approach to reducing the glove antigen content, and thus the risk of sensitization and allergy. However, although limiting the total glove protein is the only practical approach at this time, it is recognized that each individual with latex allergy may show variable antibody titers to the different allergenic proteins in latex. Additionally, the ratio of antigen to protein in different brands of gloves can vary by a factor of several thousandfold. Thus, gloves which contain total protein near or below the 1200 µg extractable protein/glove recommended limit may have a relatively high antigen content and could potentially sensitize some non-allergic individuals. To assure protection of all health care workers and patients, NIOSH suggests that until new information becomes available regarding a total protein limit, FDA consider modifying the proposed rule such that a maximum of 1200 µg extractable protein per glove be required rather than recommended. A suggested rewording of the caution label is as follows:

> Caution: This product contains natural rubber latex which may cause allergic reactions. Glove powder is associated with adverse reactions in some patients and health care workers. FDA requires that this product contain not more than 120 mg powder and 1200 µg extractable protein per glove. This product contains no more than [insert level] mg powder, and no more than [insert level] µg extractable protein. Glove powder may also increase the risk of wound granulomas and adhesions.

As a final comment regarding proposed 801.440(a), in order to assist consumers in glove product selection, NIOSH suggests that an additional labeling category of "Reduced Protein Content Latex Gloves" be considered based on a glove product demonstrating protein measurements below the quantitation threshold of the Lowry method (< 50 µg/g) [ASTM 1995]. At the threshold of detection for glove protein measurements, skin tests in 58% of latex allergic patients were found to still be positive [Beezhold et al. 1996], indicating that the FDA consider adopting a more sensitive measure of glove antigenic activity when such tests become generally available. The current FDA proposed rule would allow protein levels, depending on glove weight, to be

more than four times the modified Lowry test threshold level.

801.440(d)(3) The expiration date must be supported by stability studies demonstrating acceptable physical and mechanical integrity of the product over the shelf-life of the product from its date of manufacture;

Section IV Barrier and Other Quality Issues of the Preamble presents FDA's concern that improper control of a number of processes or conditions during powdered and powder-free latex glove manufacturing, shipping, and storage may contribute to barrier degradation. NIOSH supports this proposed requirement for stability studies in paragraph (d)(3) in order to both assure barrier integrity and availability of powder-free gloves.

ADDITIONAL COMMENTS ON THE PREAMBLE

I. Background (page 41710)

The last sentence in the first paragraph should be worded: ". . . , and *skin* irritation."

The first sentence of the last paragraph on this page should be corrected as follows: "In June 1997, the National Institute *for* Occupational Safety and Health (NIOSH) . . ."

VI. Specific Request for Comments (page 41716)

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.**

This additional labeling requirement would be useful since allergies have been demonstrated to a broad range of both organic and inorganic materials. It is impossible for individuals with allergies to avoid offending allergens in products if they do not know the allergens are present. In addition, lubricating cornstarch powder on gloves may itself be a trigger for allergies [Crippa 1997].

10. **FDA considered allowing manufacturers to establish an initial tentative shelf life . . . FDA has been unable, however, to determine whether any validated stability study protocols exist . . . The agency invites comments or information on the availability of accelerated aging stability study protocols which are predictive of glove shelf life.**

Under NIOSH cooperative agreement *Evaluation of Health Care Worker Protection During Surgery and Effects of Storage, Chemicals, and Disinfectants on Glove Integrity,* three research studies on medical gloves are currently being conducted. One of the awardees under this cooperative agreement, The Program for Appropriate Technology in Health (PATH), will assess degradation characteristics of medical gloves. The study, *Effects of Storage, Materials, and Stress on Glove Integrity*, will test latex, tactylon, neoprene, and nitrile surgical gloves, and vinyl examination gloves. Baseline characteristics to be measured include dimensions such as thickness and length, freedom from holes, tensile strength and ultimate elongation. The artificial aging protocol includes incubation in air at four temperatures for a variety of time periods. Based on results, a mathematical model will be constructed for each glove type to predict shelf-life at specified temperatures. The gloves will also be tested to determine the effects of exposure to various chemical or biological materials, and stretch and release cycles for up to six hours. As part of this research, the FDA will be conducting viral penetration studies after flexing and fatigue. NIOSH will forward all results of this research to FDA when it is complete.

IX. Analysis of Impacts

B. Risks of NL Protein Allergic Reactions (pages 41717- 41718)

The heading terminology used in Tables 1 and 2 to distinguish the types of allergic reactions (local topical, systemic topical, and systemic respiratory) is confusing and could lead to misunderstanding. The following alternative wording is suggested:

> *local topical* to be replaced with "local skin reaction (contact dermatitis or contact urticaria [hives])"; or "dermatitis or urticaria [hives] at point of contact"; or "local dermatologic manifestation";
>
> *systemic topical* to be replaced with "distant or generalized skin reaction (dermatitis or urticaria [hives])"; or "distant dermatologic manifestation"; or "dermatitis or urticaria [hives] beyond point of contact";
>
> *systemic respiratory* to be replaced with "respiratory."

In addition, it is not clear exactly where the category of mucosal symptoms (e.g., itchy eyes, nasal congestion) fits in these tables.

G. Sensitivity Analyses

3. Linear Relationship between Environmental Protein/Powder and Allergic Reactions (page 41737)

In calculating benefits of the proposed changes, FDA assumed a relatively linear relationship between health effects and exposure, for both protein antigen and for powder. In support of the first assumption, a recent workplace investigation observed an exposure-related increase in occupational allergic sensitization with increasing exposure to a variety of antigenic proteins [Baur et al. 1998]. Data from studies of powder-free gloves (< 2 mg powder/glove) found that their use also results in diminished latex antigen exposure, and can reduce or eliminate symptoms [Allmers et al. 1998; Tarlo et al. 1994]. Thus it is likely that adverse health outcomes will be reduced if glove powder is reduced from current levels to the proposed recommended powder levels (120 mg/glove). However, data are not currently available to predict the degree of reduction at various reduced powder levels, including the recommended powder level. Because of the uncertainty in the degree of benefit from reducing the powder level to 120 mg/glove, NIOSH suggests that FDA consider recommending powder-free gloves and modifying the proposed rule such that the glove powder level of not more than 120 mg per glove be required rather than recommended.

COMMENTS ON THE DRAFT "MEDICAL GLOVE GUIDANCE MANUAL"

Adequate Directions for Use (21 CFR 801.5)

FDA recommends on page 6-3 that the following statement appear on each box of powdered examination gloves:

> "**Caution:** Users should consider the circumstances of use in deciding whether to remove powder from gloves after donning. Powder can be removed by wiping gloves thoroughly with a sterile wet sponge, sterile wet towel, or other effective method."

The message in this warning label is unclear, and could be clarified by the following suggested rewording:

> Caution: Glove powder is associated with adverse reactions. Powder can be removed after donning by wiping gloves thoroughly with a sterile wet sponge, sterile wet towel, or other effective method. Users should consider the circumstances of use in deciding whether to remove powder from gloves.

Powder and Protein Labeling (Proposed 21 CFR 801.440; page 6-3)

See NIOSH comments under 21 CFR 801.440(a) above.

Natural Rubber Latex Powder-free Gloves.

On page 6-4, the draft Medical Glove Guidance Manual states: "At present, FDA does not allow a protein labeling statement or claim below the current 50 µg/gram of glove sensitivity limit of the ASTM Lowry test method . . . For gloves to be labeled as containing 50 µg/gram or less per glove of extractable protein, the labeling should also state:

> 'Caution: Safe use of these gloves by latex sensitized individuals has not been established.'"

For clarity, we suggest that the label be modified as follows:

> Caution: Using these gloves may cause health problems for latex-sensitized individuals.

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Reduction of latex aeroallergens and latex-specific IgE antibodies in sensitized workers after removal of powdered natural rubber latex gloves in a hospital

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Background: Respiratory symptoms of natural rubber latex (NRL) allergy in health care workers (HCWs) have been reported in rooms with a detectable allergen load. Preventive measures have been proposed to reduce the risk of NRL sensitization.

Objectives: Eliminating powdered NRL gloves from the workplace and giving NRL-free material to sensitized workers has been among proposed preventive measures. To appraise the success of such procedures among HCWs, a prospective study was carried out.

Methods: Sensitization of HCWs to NRL was determined by skin prick tests and measurements of specific IgE antibodies. NRL allergen concentrations in room air were measured before and after substitution of powdered NRL gloves with powder-free or synthetic gloves in different departments of a hospital and determined by a competitive inhibition immunoassay.

Results: The prevalence of HCWs with positive skin prick test responses and NRL-specific IgE-positive HCWs was 8% ($n = 7$) among the 90 examined staff members before the intervention started. All 7 reported glove-related allergic symptoms. Six of 7 sensitized subjects had a significant decrease of latex-specific IgE antibody concentrations during follow-up examinations in April and September 1997 ($P < .003$). Within 24 hours after substitution took place, NRL aeroallergen levels (up to 49.9 ng/m^3) fell below the detection limit in areas with synthetic gloves or powder-free NRL gloves alike. Use of asthma medication and antiallergic drugs could be discontinued by 2 HCWs with NRL-related respiratory tract symptoms.

Conclusions: Our results demonstrate that elimination of powdered NRL gloves is a useful device in reducing aerogen NRL allergen loads below the detection limit and permitting sensitized or allergic personnel to remain on the job. (J Allergy Clin Immunol 1998;102:841-6.)

Key words: Immediate-type allergy, latex, latex aeroallergen, occupational asthma, natural rubber latex gloves, glove powder, IgE antibodies

Abbreviations used
HCW: Health care worker
NRL: Natural rubber latex
SPT: Skin prick test

Up to 17% of health care workers (HCWs) exposed to powdered natural rubber latex (NRL) gloves become sensitized to NRL allergens.[1-7] In a recent study we found symptoms of the upper and lower respiratory tract and NRL-specific IgE antibodies only in HCWs employed in rooms with a detectable NRL aeroallergen load.[8,9] Several authors[5,10] have issued recommendations for preventive measures (eg. elimination of powdered NRL gloves and use of nonpowdered NRL or synthetic gloves as an alternative). To determine the efficiency and success of such measures to protect HCWs and patients from the effects of NRL sensitization, allergic reactions, or both, we conducted an intervention study.

METHODS

Study setting

The study period lasted from September 1996 to September 1997.

Participants

Ninety HCWs (26 men and 64 women; age range, 22 to 53 years [median, 32 years]) representing 43% of the staff ($n = 211$) working in 4 different wards (the pediatric and adult intensive care units and the departments of anesthesiology and surgery) participated in the study. There were no exclusion criteria: anyone who wanted to participate could do so. Each subject answered a questionnaire to determine a history of atopic symptoms and NRL allergy. Follow-up examinations took place in April (history and determination of NRL-specific IgE) and September 1997 (history, skin prick tests [SPTs], and determination of NRL-specific IgE).

SPTs with controls and 8 common aeroallergens (NaCl, histamine, grass pollen, birch pollen, mugwort pollen, cat dander, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Alternaria tenuis*, and *Cladosporium herbarum*) and 7 NRL allergen extracts (latex extract Universität Münster [0.1 mg/mL protein], latex extract BGFA [0.01 mg/mL protein], Regent latex milk [Universität Münster undiluted], Regent glove extract [Universität Münster undiluted], PeHa glove extract [Universität Münster undiluted], latex Abello [commercially available], and latex Stallergen [com-

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Supported by a grant from the Berufsgenossenschaft für Gesundheitsdienst und Wohlfahrtspflege, Germany.

Received for publication May 20, 1998; revised July 21, 1998; accepted for publication July 21, 1998.

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0091-6749/98 $5.00 + 0 1/1/93401

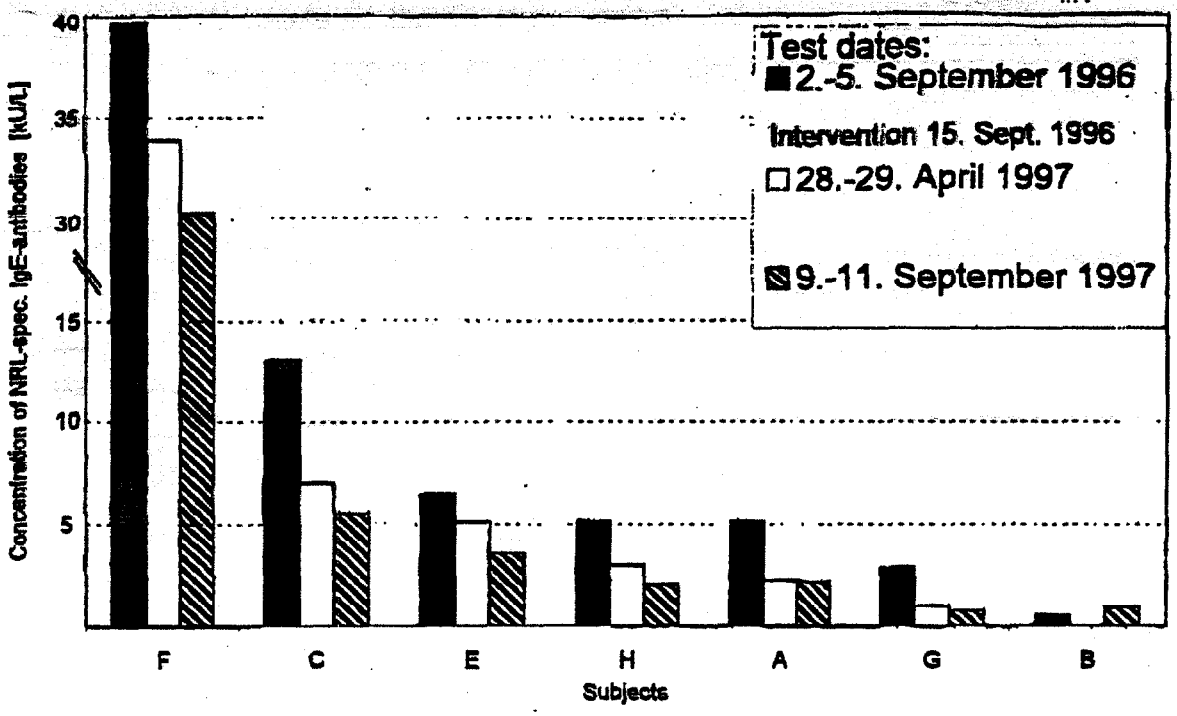

FIG 1. Concentration of NRL-specific IgE antibodies in 7 subjects exposed to aerogen NRL allergens before and 12 months after the end of exposure. IgE decrease during 12 months without exposure is highly significant as determined by using the 2-sided Page test ($P < .003$).

mercially available]) were carried out, and the results were read after 15 minutes. Total IgE and NRL-specific IgE was measured by the CAP system (Pharmacia, Uppsala, Sweden). The NRL-specific IgE antibodies were measured after each round of examination, and all samples from participants who had a detectable level of antibodies were reexamined concurrently at the end of the study.

Interventions

A floor on a surgical ward was used as the control area; powdered NRL gloves were used there throughout the study. The pediatrics intensive care unit was switched to non-NRL gloves. The pediatrics ward, general surgery, and orthopedics operating room, as well as the surgical clinic and the adult intensive care unit, were changed to powder-free NRL gloves. A changing room used by personnel working with powdered NRL gloves was also studied. Personnel with a sensitization to NRL allergens were always given a supply of non-NRL gloves after the initial examination in September 1996. To make sure that the intervention was immediate, all participating wards and areas were switched to the alternative gloves on the same day. All gloves that were not compatible with the study design were removed. One exception occurred when powdered gloves were used because of supply problems within the first 2 days after intervention took place. A physician in the surgical clinic used powdered NRL gloves. In the pediatrics examination room we assume that there was another unauthorized use of powdered NRL gloves, but the source could not be established.

Measurements

Area samplers (Probenahmepumpe MF 70, DEHA Haun & Wittmer GmbH, flow rate 5 to 70 L/min; Probenahmepumpe Tecora Bravo H 2, flow rate 5 to 70 L/min) with a flow rate of 10 L/min were used for 24-hour measurement periods in 7 different areas: the examination room in the pediatric ward (9 measurement periods), the examination and treatment room in the surgical clinic (13 measurement periods), the floor of the surgical ward (5 measurement periods), the changing room (5 measurement periods), 2 air conditioning intake ducts for the operating rooms (9 measurement periods), and the adult intensive care units (8 measurement periods). In all rooms except the changing room 3 measurement periods were carried out before the intervention took place. In March and September 1997, measurements were performed during 3 subsequent 24-hour periods at the chosen sites.

The placement of the samplers was chosen to reduce interference with routine work as much as possible while trying to measure within the area of greatest contamination (worst case scenario). Usually those areas were close to the storage areas where gloves were donned or the garbage bins in which the gloves were placed after use. Sampling took place over periods of approximately 24 hours. A flow rate of 10 L/min was used to collect air samples on a cellulose acetate filter with a pore diameter of 0.2 µm (Sartorius, Germany).

Determination of NRL aeroallergens

NRL allergen content in air samples was determined by an immune inhibition assay as described previously.[8,9] Briefly, filters with airborne particles were removed from the area air sampler and cut into pieces of approximately 2 × 1 mm. NRL allergens were extracted from the filter pieces in 20 mL of 0.1 mol/L acetate buffer, pH 8.0, containing 0.1% 3-([3-cholamidopropyl]dimethylammonio)-1-propane sulfonate (CHAPS). The extracts were then filtered

through a 0.45-μm membrane filter (Sartorius AG, Göttingen, Germany), freeze-dried, and redissolved in 400 μL distilled water. Determination of NRL allergens in extracts were performed by a competitive inhibition immunoassay with the Pharmacia CAP system in which the commercial NRL ImmunoCAP allergen from Pharmacia was used as solid-phase allergen. A standard inhibition curve was constructed by competition between a reference laboratory standard extract prepared from NRL sap from Malaysia containing 7.5 mg of protein per milliliter (determined by modified Lowry method) and the solid-phase NRL ImmunoCAP allergen for specific IgE binding by using a pooled serum of 12 NRL-sensitized subjects (IgE antibodies to latex, 15.6 kU/L). The IgE binding to the solid-phase ImmunoCAP allergen could be completely inhibited by 1 μg reference latex allergen extract, indicating that our reference extract used in the study covered all latex allergens on the solid-phase ImmunoCAP. Quantification of latex allergen in sample extracts was achieved by preincubating 20 μL extract solution with 40 μL pooled serum (overnight at 4°C) to compete with ImmunoCAP latex allergen in the inhibition assay (in duplicate). The inhibition capacity of the sample extract was then compared with that of the reference standard NRL allergen. The results were expressed as NRL allergenic protein mass per cubic meter of air.

Glove counts

To determine a correlation between the number of gloves used and the airborne allergen load, we attempted to collect all gloves being used in the following areas during 3 24-hour periods: the examination room in the pediatric ward, the examination and treatment room in the surgical clinic, the floor of the surgical ward, and the laminar flow room of the pediatric intensive care unit.

Statistics

Statistical evaluations were performed with the SPSS for Windows software package (version 6.1.3, nonparametric tests) and with StatXact 3 software package (Cytel Software, Cambridge, Mass).

RESULTS

Ninety of 211 eligible HCWs participated in the baseline examinations. Ten (11%) had NRL-specific IgE antibodies during the initial exam in September 1996. Seven of those 10 had a positive SPT response to NRL allergens and reported glove-related symptoms ranging from urticaria to asthmatic symptoms. No substantial differences were seen in SPT responses when comparing the 7 different NRL extracts (with the dilutions indicated above), and no systemic reactions occurred during SPTs in any of the participants. The other 3 subjects with NRL-specific IgE antibodies had a negative SPT response and did not report NRL-related allergic symptoms. During follow up, we reexamined 49 subjects (54%) in April 1997 and 62 subjects (69%) in September 1997. The 7 NRL- and IgE-positive subjects with positive SPT responses participated in all examinations: only 1 stopped working in the hospital because as a nurse in the neonatology ICU, she needed to be present in the delivery room where powdered gloves were still being used to take care of preterm babies. Two workers initially reported symptoms of the respiratory tract and had to use antihistamines or metered-dose inhalers during work. After switching of gloves, the symptoms disappeared, and use of medication could be terminated. Six subjects had latex-specific IgE antibody concentrations larger than 1 kU/L in September 1996. In five of those we found that concentrations were halved within 1 year. The highest concentration was found to be 40 kU/L, and it fell to 30 kU/L within the year (Fig 1). The decrease of NRL-specific IgE antibodies in all 7 subjects during 12 months without NRL exposure is highly significant (2-sided Page test, $P < .003$). No new cases of sensitization could be detected in the other participants.

TABLE I. NRL glove–related symptoms at initial examination

	Negative SPT responses (n = 83)	Positive SPT responses (n = 7)	P value*
Itching	36	7	.032
Wheals	4	6	.0004
Sneezing	6	5	.0098
Rhinitis	3	5	.005
Stuffy nose	2	3	.243

*As determined by the Kolmogorov-Smirnov test.

A topy No effect

TABLE II. History of atopy

	Negative SPT responses (n = 83)	Positive SPT responses (n = 7)	P value*
Hay fever	20	4	.481
Asthmatic symptoms	6	1	1.0
Atopic dermatitis	6	1	1.0
Atopic family history	29	5	.36

*As determined by the Kolmogorov-Smirnov test.

The 7 subjects with latex-specific IgE antibodies and positive SPT responses to latex extracts had significantly higher levels of total IgE (Mann-Whitney U test, $P = .0047$) than the other 83 participants in the study. Mean total IgE was 145.9 kU/L (SD, 98.1) in the latex-allergic group and 61.8 kU/L (SD, 80.0) in the negative group. A history of NRL glove–related symptoms was significant for wheals, sneezing, and rhinitis (Table I). Symptoms of atopy (hay fever, asthma, atopic dermatitis, and atopic family history) were not significantly associated with latex allergy (Table II).

Atmospheric allergen loads of NRL allergens between 0.86 ng/m^3 and 49.93 ng/m^3 were detectable when the sampling was performed directly inside the rooms where powdered NRL gloves were used.

NRL aeroallergen was present (1.03 to 10.58 ng/m^3) on each measurement period on the surgical ward floor used as a positive control area (where powdered NRL gloves were not substituted). In a second measurement campaign in March 1997, we found NRL aeroallergens (0.61 ng/m^3) only on 1 of 3 days in the control area. In September 1997, we were not able to detect any aeroallergens there.

Within 24 hours (1 measurement period) after use of powdered gloves was terminated, allergen loads fell below the detection limit. In the surgical clinic 1 senior

TABLE III. NRL allergen concentration in the air (ng/m³)

Date of air sample	Powder-free NRL gloves; adult ICU (air duct)	Powder-free NRL gloves; operating room (air duct)	Non-NRL gloves; pediatrics, laminar flow room	Glove change: Powder-free NRL gloves; pediatrics, examination room	Powder-free NRL gloves; surgical clinic	No change of gloves; control ward	No use of gloves; changing room
Sep 11, 1996	BDL	BDL	BDL	4.13	1.97	4.25	ND
Sep 12, 1996	BDL	BDL	BDL	1.76	1.73	4.58	ND
Sep 13, 1996	BDL	BDL	BDL	BDL	49.93	10.58	ND
Sep 14, 1996	ND	ND	ND	Error	BDL	ND	ND
Sep 15, 1996 (intervention)	BDL	BDL	1.98	Error	BDL	1.03	ND
Sep 16, 1996	BDL	BDL	BDL	BDL	BDL	3.51	ND
Sep 17, 1996	BDL	BDL	BDL	0.94	0.86	ND	BDL
Sep 18, 1996	BDL	BDL	BDL	BDL	BDL	ND	BDL
Sep 19, 1996	BDL	BDL	BDL	BDL	BDL	ND	BDL
Sep 20, 1996	ND	BDL	BDL	BDL	BDL	ND	BDL
Sep 21, 1996	ND	ND	ND	ND	BDL	ND	ND
Sep 22, 1996	ND	ND	ND	ND	BDL	ND	ND
Sep 23, 1996	ND	ND	ND	ND	BDL	ND	ND
Sep 24, 1996	ND	ND	ND	ND	BDL	ND	ND
Mar 11, 1997	ND	ND	BDL	Error	BDL	0.61	ND
Mar 12, 1997	ND	ND	BDL	Error	BDL	BDL	ND
Mar 13, 1997	ND	ND	BDL	Error	BDL	BDL	ND
Sep 22, 1997	ND	ND	BDL	BDL	BDL	BDL	BDL
Sep 23, 1997	ND	ND	BDL	BDL	BDL	BDL	BDL
Sep 24, 1997	ND	ND	BDL	BDL	BDL	BDL	BDL

ICU, Intensive care unit; *BDL*, below detectable limit; *ND*, not determined.

physician did not get a supply of powder-free gloves of his size on the second day after the transition, forcing him to use powdered NRL gloves. An allergen load of 0.86 ng/m³ was measured that day. After this individual was outfitted with powder-free gloves, the detection limit was not exceeded for the remaining 7 measuring intervals.

In the examination room of the children's ward, there was a detectable load of NRL aeroallergens 2 days after the intervention took place, without the cause being known. Use of powdered gloves still in the possession of an individual was discussed but not proven.

No allergen load could be detected in the changing room where gloves were not used.

It was not possible to detect NRL aeroallergens on samples taken within the air stream of the air conditioning system for the intensive care unit and operating rooms. The results are summarized in Table III.

When measurements of aerogen latex allergens were repeated in March and September 1997, no detectable allergen load could be detected in the rooms where only powder-free latex gloves were used. The results of the glove counts in the different rooms/areas were so heterogeneous that it was not possible to determine a correlation between the number of gloves used and the aeroallergen concentration.

DISCUSSION

Several investigations demonstrate that powdered NRL gloves are the source of atmospheric NRL aeroallergen.[4,5,11,12] Heilman et al[13] showed that switching to low-allergen gloves resulted in a significant reduction of NRL aeroallergen levels. In an investigation of the staff in our health care facilities, we found that respiratory symptoms and NRL-specific IgE antibodies were only present in employees who had worked in rooms with a detectable NRL allergen load.[8,9] These findings are in accordance with our hypothesis that direct allergen contact to the mucosa of the upper and lower respiratory tract is an important cause for the development of sensitization against NRL allergens. Currently there are no epidemiologic studies linking sole skin contact and sensitization to NRL allergens. Brehler et al[14] found a significantly lower prevalence in operating room staff in the UK, where only powder-free gloves have been used in the last decades, in comparison with German HCWs working in operating rooms with powdered NRL gloves. Therefore we pursued the hypothesis that once the major source of NRL aeroallergens is removed by using powder-free NRL or synthetic gloves, NRL aeroallergen levels will drop below the detection limit, and the risk of NRL sensitization will decrease. Another advantage in this approach is the reduction or elimination of airborne exposure to NRL allergens for HCWs and patients in whom respiratory symptoms have already developed.

Except for 1 sample in the surgical clinic and 1 sample in the children's examination room, no NRL aeroallergen could be detected in any sampling period (24 hours) after the replacement of powdered gloves. These results indi-

cate that in areas such as operating rooms and intensive care units that need to be prepared for NRL-sensitized patients, a removal of powdered NRL gloves will result in an elimination of NRL aeroallergens within 24 hours and probably sooner in rooms with air conditioning.

Because of the delicate working conditions in operating rooms and intensive care units, we tried to determine whether NRL aeroallergen levels could be detected within the airstream of the air conditioning units of those areas. That way we did not interfere with any procedures that might be impaired by measuring inside of these facilities. However, it was not possible to detect any NRL allergens in our samples, probably because of the much higher air flow rate within the air ducts (10 m^3/s) as opposed to that inside the rooms (<0.5 m^3/s to 2 m^3/s).

Another very interesting aspect of the study was the fact that NRL aeroallergen was present (1.03 to 10.58 ng/m^3) on each measurement period on the surgical ward floor used as a positive control area (where powdered NRL gloves were not substituted) in September 1996. In April 1997 we only found NRL aeroallergens (0.61 ng/m^3) on 1 of 3 days, and in September 1997 we were not able to detect any aeroallergens. Inquiries at the glove-manufacturing company revealed that they had decreased the amount of corn starch powder used during manufacturing after the summer/fall period of 1996 to remain competitive in the market because awareness of the NRL allergy problem had become an issue in many hospitals at the management level (personal communication).

Swanson et al[4] showed that surgical scrub suits in operating rooms with detectable NRL aeroallergen levels were contaminated with NRL allergens. To determine whether the NRL allergens adherent to clothing of personnel working in areas contaminated with NRL aeroallergens results in detectable release of NRL allergens into the atmosphere, we performed measurements in a changing room where HCWs changed their clothing before and after work shifts. No NRL aeroallergens could be detected, suggesting that even though work clothing might be contaminated with NRL allergens, changing of these clothes will not result in significant NRL aeroallergen levels.

In our study group sensitization to NRL allergens as determined by NRL-specific IgE antibodies and positive SPT responses was 8%. No new cases of sensitization occurred in areas where only powder-free gloves were used during the 1-year follow-up period. Because of the high fluctuation of personnel, we could not determine whether any new sensitization occurred in the control ward where powdered NRL gloves were still being used. No allergic symptoms caused by NRL were reported during the follow-up period. Use of asthma medication and antihistamines could be discontinued by 2 staff members with allergic asthma and rhinitis before the intervention took place. In a recently published study Sussman et al[15] found very similar results for the measurement of aerogen latex allergen concentrations and a decline of allergen content in powdered gloves during the length of the investigation. Another very interesting finding that seems to support our hypothesis that inhalation of NRL is a main cause for sensitization was the phenomenon that conversion from negative SPT responses to NRL to positive SPT responses to NRL occurred both in the group using powdered gloves and in the group using powder-free gloves but that clinical symptoms were only reported by the participants in the powdered group.

Our earlier investigation of 26 HCWs who had given up work showed a trend of decreasing latex-specific IgE antibody concentrations over time; however, those subjects were examined only once after NRL exposure was terminated, and no follow-up data was available.[16] The results of this study clearly show a decrease of latex-specific IgE antibody concentrations within 1 year after continuous exposure to NRL allergens was terminated. Occasional contact by short exposure to NRL aeroallergens (eg, when walking through areas that were not included in the intervention and where powdered NRL gloves are still being used) does not seem to cause antibodies to be maintained at levels that continuous exposure would induce. However, it should be noted that even if NRL-specific IgE levels fall below the detection limit, severe allergic reactions might still occur on reexposure.

We conclude that substitution of powdered NRL gloves with powder-free NRL gloves or gloves not containing NRL is a useful device in reducing NRL aeroallergen loads below the assay detection limit within 24 hours. NRL allergen load adhering to clothing does not seem to cause a significant atmospheric contamination. To prevent detectable atmospheric contamination with NRL aeroallergens, the use of nonpowdered NRL gloves is a sufficient measure. HCWs sensitized and even allergic to NRL can remain on the job if exposure to NRL can be avoided. Banning of powdered NRL gloves in the workplace and a supply of NRL-free material for sensitized individuals seems to be a sufficient prevention strategy.

We thank the staff of the St Franziskus Hospital in Münster for their patience and support while participating in this investigation. Dr Renate Kolling (St. Franziskus Hospital), Jörg Schmengler, and Paul Gurock (Berufsgenossenschaft für Gesundheitsdienst und Wohlfahrtspflege) made this study possible by giving administrative and logistic support at every stage of planning and completion of this study. We are extremely grateful for their help.

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