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ASSESSMENT OF THE RISK OF TYPE I LATEX ALLERGY SENSITIZATION OR REACTION DURING USE OF PRODUCTS MADE FROM LATEX DERIVED FROM GUAYULE AND OTHER ALTERNATE RUBBER PRODUCING SPECIES

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Medical gloves provide an effective barrier to the transmission of human pathogens and help protect both healthcare professionals and patients against contracting infectious diseases. Medical gloves, historically, have been manufactured from natural rubber latex (NRL) tapped from the Brazilian rubber tree (*Hevea brasiliensis*) because of its excellent barrier properties. However, a processing change in the 1980's left soluble protein in the glove matrix and huge numbers of people were exposed. *Hevea* latex proteins in natural rubber elicit anti-*Hevea* latex protein IgE, an indication that the individual has become "sensitized". Some individuals who are sensitized to NRL proteins will subsequently experience life-threatening allergic symptoms following a subsequent airborne or contact exposure to latex allergen.

The public health risk of Type I latex allergy has led to gloves being made from alternative materials and receiving FDA 510(k) clearance. However, they are not preferred by healthcare providers due to their physical limitations.

Natural rubber latex can be produced from many plant species and guayule (*Parthenium argentatum*) has been commercialized on the basis of its very low protein content, and non-crossreactive latex.

This report summarizes and explains the information currently available on the safety of guayule latex and comments on other alternate latex contenders.

Keywords: Dandelion, Guayule, Latex allergy, Natural rubber latex

INTRODUCTION

Natural rubber latex remains the best and most suitable protective material for high performance medical gloves during surgery. However, the U.S. Food and Drug Administration (FDA) cleared synthetic surgeon's and examination gloves in order to address the public need for medical gloves by those sensitized or allergic to latex.

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Unfortunately, this required a lowering of the performance requirements of the gloves because the synthetic materials could not meet the high physical performance requirements set for natural rubber gloves (ASTM Standards D 3577 and D 3578). However, due to synthetic gloves' lack of tactile sensitivity and their proclivity to cause hand fatigue, they are not preferred by surgeons for use during surgery – especially for use in double-glove surgical procedures. Furthermore, several studies have demonstrated that synthetic gloves have barrier integrity and ergonomic disadvantages compared to *Hevea* natural rubber latex gloves. A recent British study compared *Hevea* natural rubber latex surgeon's gloves with the synthetic polyisoprene surgeon's glove during the intensive orthopedic surgeries of primary hip and knee arthroplasties (Aldlyami *et al.*, 2010). The overall glove failure rate was 2.67 times higher for the polyisoprene synthetic glove compared to the *Hevea* natural rubber latex glove. The operation failure rate for polyisoprene (latex-free) gloves was 80% compared with 34.4% *Hevea* latex gloves ($P<0.001$). The authors of the study suggest that the synthetic, latex-free glove tested cannot provide a reliable barrier between the surgeon and the patient. Similarly, a U.S. post-usage study of gloves following routine surgical procedures revealed higher after-use defects for non-latex surgeon's gloves compared with *Hevea* latex surgeon's gloves (Korniewicz *et al.*, 2004). Compared with natural rubber latex gloves, the odds ratio for defects was 1.39 (95% confidence interval ranging from 1.12 to 1.73) for polychloroprene and 1.90 (95% confidence interval ranging from 1.15 to 3.13) for nitrile gloves (Newsom *et al*., 1998). This study reported better barrier performance in *Hevea* natural rubber latex surgeon's gloves than in those made from the synthetic elastomer, polychloroprene. The polychloroprene glove also frequently tore at the donning stage. The post-puncture tearing resistance of the polychloroprene gloves was clearly less than natural rubber latex gloves and one surgeon withdrew from the study because of an "unacceptably large number of punctures with the synthetic elastomer gloves". The opposing and complementary glove safety arguments with respect to risk of glove failure versus risk of allergic reaction have been thoroughly discussed (Palosuo *et al.*, 2011). These authors concluded that synthetic gloves reduce tactile sensitivity, and thus hamper delicate surgical procedures.

The current option is to choose either: (1) a high-performance *Hevea* latex glove, which provides good protection against exposure to blood-borne human pathogens, but which also has the potential to cause an allergic reaction; or (2) a lower performance synthetic glove, which mitigates the risk of Type I latex allergy but increases the risk of glove breakage and exposure to blood-borne human pathogens. Both risks can be circumvented by use of guayule natural rubber gloves (Nguyen *et al.*, 2007; 2008). However, it is clearly unethical, even if possible, to deliberately make a human being sensitive to guayule latex proteins. This dilemma prevents scientists from being able to determine a sensitizing dose of guayule latex proteins, and so how can we be sure that the introduction of a guayule glove will not cause Type I latex allergy in either previously sensitized patients or in populations? This concern is a major cause for of the current inclusion of the *Hevea* protein caution label on guayule exam gloves by FDA even though they contain no proteins in common.

1. Latex allergy protection

In order to assess the allergy potential of a product and/or a situation, it is necessary to understand the nature of latex allergies, how they arose, and what can be done to ameliorate or circumvent the risk.

1.1. Protecting the latex-sensitized population

Shortly after the FDA's 510(k) clearance of Yulex® Natural Rubber Examination Gloves, FDA issued a press release stating that "anywhere from 3 to 22 per cent of all healthcare workers are sensitized to traditional (*Hevea*) latex." At particular risk of latex allergy are patients with repeated surgeries, due to the frequency of exposure to the allergens. For example, allergic reactions to latex are common in patients with spina bifida, with an incidence between 28% and 67% (Gulbahar *et al.*, 2004; Yunginger, 2003). In response to this concern, there has been a general trend in the United States towards preferential use of synthetic gloves over *Hevea* natural rubber latex gloves. A number of high profile hospitals, including Johns Hopkins Medical Center in Baltimore, Maryland (Brown *et al*., 2003; Brown *et al.*, 2009) and the Cleveland Clinic's network of nine hospitals in Ohio, have gone 'latex free'. However, studies indicate there is increasing concern that this approach is too extreme and does not account for the impact on function or even the environment. The authors of these studies conclude that the risk of a severe Type I allergic reaction from well-leached, powder-free latex gloves is much less than the risk of contagion following synthetic glove breakage (Allmers *et al.*, 2002; Kelly, 2008; Palosuo *et al.*, 2011). Published scientific data have demonstrated that guayule rubber gloves have much less potential for glove leakage than synthetic gloves, while not posing a reaction risk to those with Type I latex allergy (Cornish and Lytle, 1999; Cornish *et al.*, 2001).

1.2. Protecting the non-latex-sensitized (normal) population

Lack of a high performance, non-allergenic surgical glove affects the non-latex sensitive population too. At latex-free hospitals surgeons must use lower performance synthetic gloves (made from various petroleum-derived polymers, including polyisoprene, nitrile, and polychloroprene) even for procedures on patients who are not latex-sensitive. Use of these synthetic gloves reduces the tactile sensation felt by the surgeon's hands through the glove, increases the risk of exposure to blood-borne pathogens (due to higher failure rates) to the healthcare providers and patients, and can lead to hospital-acquired (nosocomial) infections.

Because these surgeons are forced to use synthetic gloves for treatment of all patients – including those who don't have Type I latex allergy – some surgeons have resigned from hospitals that are "latex-free". However, as discussed in the previous section, total avoidance of latex is an overreaction and is not necessary to protect the general population (Allmers *et al.*, 2002; Palosuo *et al.*, 2011). This has been proven in Germany where a combination of educating physicians and administrators, together with regulations requiring that healthcare facilities only purchase low-protein, powder-free natural rubber latex gloves, greatly reduced reactions and led to prevention of sensitization (Allmers *et al.*, 1998; Kelly *et al.*, 2011). The positive German experience of

specifically changing entirely to low-allergen gloves or to gloves with undetectable allergen contents, has been mirrored at the Mayo Clinic, Rochester, Minn., USA (Hunt *et al.*, 2003), as well as in Finland (Turjanmaa *et al.*, 2002).

As will be described below, guayule rubber gloves will provide a safer alternative to well-leached, powder-free *Hevea* latex gloves for the non-sensitized population because they exhibit outstanding physical performance characteristics and do not have the potential to induce Type I latex allergy. As discussed above, well-leached powder-free *Hevea* natural rubber latex gloves can prevent sensitization but they cannot prevent allergic reactions in all existing Type I sensitized patients. Guayule rubber gloves provide protection against sensitization allergic reaction.

2. Allergy mechanisms

We fully expect that guayule latex gloves will cause no cases of Type I allergy to guayule proteins, because of the data herein. In other words, guayule protein would not cause a guayule-specific Type I latex allergy. In order to understand why a *theoretical* case of allergy to guayule proteins would be considered, a new allergy (guayule-specific and unrelated to *Hevea* latex allergy), it is important to understand that any potential antibodies developed in humans to guayule products would be completely distinct from antibodies raised against *Hevea* latex products. This is known, definitively, through tests in rabbits and mice which demonstrate that antibodies deliberately raised against guayule proteins do not react with *Hevea* latex proteins (Siler and Cornish 1994; Siler *et al.*, 1996). In rabbits, mice, and humans, antibodies deliberately (animals) or accidentally (humans with Type I latex allergy) raised against *Hevea* proteins do not react with guayule proteins.

To assist in assessment of the sensitization risk of a guayule medical glove, the underlying technical basis for sensitization mechanisms and their relevance to guayule gloves is summarized in the next section.

2.1. Dose

Sensitization is a dose-response phenomenon. Reaching an individual's sensitization threshold is dependent on the dose-response relationship. The dose-response relationship is a function of the duration of exposure, the frequency of exposure, and the amount of the offending substance in the material to which an individual is exposed (*i.e.*, that which comes in contact with the individual). When comparing products or materials used in a particular application, the duration of exposure and the frequency of exposure are constant. In this specific case, guayule natural rubber surgeon's gloves will be used in exactly the same way as (*Hevea*) natural rubber latex surgeon's gloves – no more and no less. The content (amount and type) of the sensitizing substances is an inherent material characteristic. This content is the only variable, and it entirely governs the potential for sensitization. A glove that contains more available sensitizer will be more likely to cause sensitization, and a glove containing less available sensitizer will be less likely to cause sensitization. The individual's propensity to develop and allergy also plays into the dose response.

The total amount of exposure to a sensitizer is termed the "dose". As FDA has

recognized, the proteins in guayule are different from those in *Hevea*. In addition, the amount of protein in guayule natural rubber latex is less than 1% of the amount of protein contained in natural rubber latex from *Hevea* (Cornish *et al.,* 2006).

In leached *Hevea* latex gloves, about half of the latex protein (consisting of hydrophobic proteins) is entrained in the glove matrix. In leached guayule natural rubber gloves, even if all of the protein is entrained in the guayule latex, they still can contain no more than 2% of the level of protein entrained in leached *Hevea* gloves, *e.g.*, gloves with less than 50 μg/g (200 μg/dm^2) of total protein per ASTM D 5712. In other words, leached *Hevea* gloves contain 50 times more protein than guayule gloves. Even so, this 50 times greater amount of protein in leached *Hevea* natural rubber gloves has been shown to be an insufficient protein dose for sensitization. The lack of sensitization potential of low allergenic content, powder-free gloves has been clearly demonstrated in European countries (Crippa *et al.,* 2006). These countries permitted use only of leached, powder-free latex gloves or synthetic materials and managed to halt the spread of the epidemic, apparently eliminating the incidence of new cases – especially among health care workers (Reunala *et al.,* 2004). Personal protection also has proved effective (Hamilton and Brown, 2000).

Guayule gloves should be thoroughly leached in accordance with good manufacturing practices, and no extractable protein could be detected in prototype gloves using ASTM Standard D 5712. (None can be detected with ASTM Standard D 6499 or ASTM Standard D 4247 either, but these latex allergy-related standards are specific to proteins that cross-react with antibodies deliberately raised against *Hevea* latex antigenic and allergenic protein, respectively, and thus cannot detect the different guayule proteins.)

History has shown that Type I latex allergy development involved widespread sensitization (development of symptoms in millions of people) to high levels of soluble proteins in unleached (not washed during manufacture) *Hevea* latex products (Palosuo *et al.,* 2011; Cohen *et al.,*1998; Garabrant *et al.,* 2001). Respirable glove powder with adsorbed latex proteins was the prime route of transmission for sensitizing exposures of health care workers (Cohen *et al.,* 1998). IgE antibody responses are readily elicited to the 13 most prevalent allergenic *Hevea* proteins (Yeang *et al*, 2002; Yeang, 2004). These include the hydrophobic rubber particle bound proteins Hev b1 and b3. Hev b1 and Hev b3 are important allergens for individuals who are exposed to latex allergen through mucosal contact as a result of multiple surgeries or latex catheter use (*e.g.*, children with spina bifida). In addition, workers occupationally-exposed to latex products may be sensitized through exposure to Hev b5 and Hev b6.02 (Yeang, 2004; Yunginger, 2003). In well-leached, powder-free *Hevea* products, these two allergenic proteins, which are primarily retained in the glove matrix, were not able to induce sensitization even with repeated *Hevea* glove exposure of health-care workers (Charous *et al.,* 1994). This means that the proteins in leached *Hevea* gloves do not impose a sensitizing dose even though they are proven to include at least two potent allergens.

Thus, it is clear that the high level of soluble proteins in unwashed *Hevea* gloves,

not the hydrophobic proteins entrained in the leached latex glove matrix, caused the mass sensitization to Type I latex allergy. Research (Yip, *et al*., 1995), has suggested that an extractable protein threshold of 100 μg/g is a reasonable safe level and is a concentration which will not cause sensitization. The same authors concluded that *Hevea* natural rubber latex products containing more than 400 μg/g of extractable latex protein will cause sensitization in 60% of patients. Thus, the critical sensitization threshold falls between these two values. It is generally accepted by FDA, medical allergists, and industry, that levels of extractable protein below 50 μg/g are non-sensitizing.

Neither guayule products, nor the guayule latex material from which they are made, contain more than trace levels of soluble protein. The level of extractable protein is not detectable using standard ASTM quantification methods and must be assayed at a much higher ratio of film to extractant than recommended in the D 5712 modified Lowry assay for *Hevea* latex products. The level of entrained hydrophobic protein is <2% of that in non-sensitizing *Hevea* latex gloves (*e.g*., powder-free, leached *Hevea* gloves with protein levels below 50 μg/g). Additionally, guayule gloves will not be powdered, completely eliminating the major health care worker exposure and sensitization route of inhalation (Cohen *et al*., 1998; Heilman *et al*., 1996; Liss *et al*., 1997; Liss and Tarlo, 2001; Vanderplas *et al*., 2002). Thus, repeated use of guayule products poses a very low risk that it will, or can, expose anyone to a sensitizing protein dose. This conclusion is supported unequivocally by the studies in animals and humans summarized later in the "Sensitization Potential Studies" section of this article.

2.2. Reactions in Type I latex-sensitized People

It is critical to differentiate between the dose needed to induce sensitization, as discussed in the previous section, and the dose required to elicit an allergic reaction. Minute amounts of reaction-triggering protein (the protein dose sufficient to elicit an allergy reaction in an already sensitized subject) should not be confused with the much larger amounts of protein required to induce sensitization (*i.e*., >100 μg/g for *Hevea* natural rubber latex). Much less protein than the >100 μg/g required to sensitize people (Yip *et al*., 1995) can cause a severe reaction in a sensitized person. For example, controlled studies in Germany have shown that a *Hevea* latex aeroallergen level of only 0.6 ng/m^3 is the critical reaction threshold, especially for health care workers who are sensitized to *Hevea* natural rubber latex (Baur *et al*., 1998). Another study demonstrated that the minimal level of skin test reactivity was only 70 pg/cm^3 for *Hevea* natural rubber latex allergens in patients with Type I latex allergy (Sussman *et al*., 2002).

The cellular mechanism underlying an allergic reaction is summarized as follows. When a lymphocyte encounters a particle or cell with surface marker molecules that identify it as a foreign invader, it performs a microscopic version of taking fingerprints and mug shots of the invader. Because these foreign invaders cause the production of antibodies, they are called antibody generators, or antigens. After a B-cell identifies an antigen, it will make its way back to a lymph node, change into a plasma cell and produce antibodies specifically engineered to fight that particular threat. In an allergic person's immune system, the lymphocytes cannot tell that the latex protein

is not invading the body like a pathogen. The B-cells of an allergic person cause the production of large quantities of IgE antibodies that attach themselves to mast cells and basophils throughout the body. This is known as the sensitizing exposure and frequently requires repeated exposures to the antigen(s) before enough basophils and mast cells become primed with IgE antibodies. Then, if the allergen comes along again, even in tiny amounts, it triggers a destructive domino effect within the system called the allergic cascade:

- The IgE antibodies bound to the surfaces of basophils and mast cells recognize the protein surface markers of the allergen.

- The IgE antibodies react by binding to the protein surface markers while remaining attached to the mast cells or basophils.

- This binding alerts a group of special proteins called the complement complex that circulates in the blood.

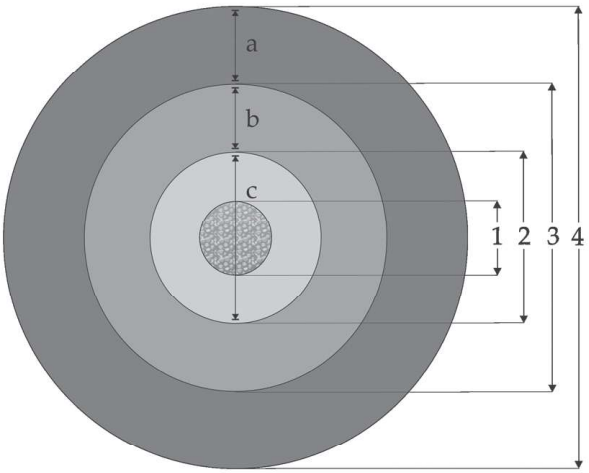
There are about 240 proteins in *Hevea* latex, of which 57 are reportedly allergenic (Slater and Chhabra, 1992) and at least 13 of these are strongly involved in the allergic-response mechanism (Yeang *et al.*, 2002; Yeang, 2004). After the IgE antibody (which is already attached to a mast cell or basophil) encounters and binds to its specific allergen, the first complement protein attaches itself to the site. This alerts the next complement protein in the sequence, which joins and alerts the next, and so on. When the string is complete, the offending cell is destroyed. This is fine in a normal immune system, as antibodies latch onto surface markers of disease cells and cause their destruction. But in an allergic episode, the cells involved are mast cells and basophils. When these cells are destroyed, their stores of histamine and other allergy mediators are released into the surrounding tissues and blood. This causes dilation of surface blood vessels and a subsequent drop in blood pressure. The spaces between surrounding cells fill with fluid and induce the various Type I allergy symptoms which can include anaphylactic shock and death.

Although minute amounts of *Hevea* latex protein can trigger an allergic reaction in a Type I latex-sensitized person, this is irrelevant with respect to guayule latex. Guayule gloves contain very little protein, and none of the guayule latex proteins are recognized by the human IgE antibodies involved in the life-threatening allergic response and thus cannot induce the allergic cascade.

2.3. Ability of proteins to induce immunogenic and allergenic responses

2.3.1. *Hevea* latex proteins

Proteins vary in their ability to induce immunogenic and allergenic responses in animals and in humans. This can be seen clearly in Type I latex allergy and is why three separate ASTM standards were developed. These standards allow manufacturers to select among tests for total protein (D 5712), antigenic (immunogenic) protein (D 6499), and allergenic protein (D 7427) – although FDA has yet to sanction the D 7427 method. The varying ability of proteins to induce antibody production clearly can be seen when all three tests are employed. The proteins that can be detected by D 5712, but not by the polyclonal antibodies used in D 6499, are non-immunogenic proteins (*i.e.*, they do not elicit an allergic or immunogenic response in rabbits). Similarly, immunogenic proteins



a. Immunogenically-inert proteins
b. Antigenic proteins that are non-allergenic
c. Allergenic proteins

1. Severely allergenic proteins, D7427
2. Allergenic proteins
3. Antigenic/immunogenic proteins, D 6499
4. Total protein, D 5712/ D1076

Fig. 1. Venn diagram indicating the subsets of proteins by increasing immunogenic efficacy with decreasing size. Each ring defines the type of proteins in the subset in terms of their immunogenic efficacy

that are detected by D 6499 but not by the monoclonal antibodies raised against the 13 most prominent allergens (D 7427 employs only the top four of the 13) would be weakly allergenic or non-allergenic. These concepts are presented pictorially in Fig. 1. Please note that all protein types inward from each arrow in Fig. 1 are detected by the test indicated (*e.g.*, ASTM D 5712 captures the total protein which includes immunogenically-inert protein, antigenic/immunogenic protein, allergenic protein, and severely allergenic protein).

The results of the three tests – D 5712, D 6499, and D 7427 – correlate with each other and provide a complete picture of a test material's ability to induce an immunogenic response (Kostyal *et al.*, 2009).

2.3.2. Guayule Proteins

Guayule latex has very little protein compared to *Hevea* natural rubber latex (Fig. 2, cf. Fig. 1). Furthermore, animal studies have demonstrated that the proteins which occur in trace extractable quantities in guayule products are poorly immunogenic, even when concentrated to much higher doses than could occur naturally. When scientists attempted to raise antibodies against concentrated guayule latex proteins in rabbits, initially they were unsuccessful (Cornish *et al.*, 2006). Even after a series of booster shots to increase the chance of raising antibodies, the guayule latex proteins were still 64 times less immunogenic than proteins extracted from whole plants of guayule. This difference is calculated from the relative amounts of antibody or "titer" in the different rabbit sera, which were 1:500 and 1:32,000+, for guayule latex proteins versus guayule total proteins, respectively. The poor titer of 1:500 means that these guayule latex proteins, even when concentrated and boosted, are very weakly immunogenic in rabbits.

The results are perhaps not surprising because approximately 90% of the trace protein that remains is composed of a single protein, a cytochrome P450 oxidase, allene oxide synthase (Pan *et al.*, 1995). This protein is known to be poorly immunogenic, and no members of the large P450 protein family have been associated with allergic reactions in humans (Dr. H.P. Rihs, personal communication). We have no studies of the remaining 10% of proteins to assess what percentage of this trace amount is immunologically inert in the concentrated state because the amount of these proteins is too small to permit an effective study.

When guayule latex proteins were extracted from guayule films and quantified, the D 5712 Lowry method (modified to include a larger sample and a protein concentration step, both necessary in order to allow for quantification) detected 4 µg/ck^{m} and the guayule ELISA only 0.95 µg/cm^{3}. Thus, as is the case in *Hevea* latex (see Fig. 1, region between the outer two circles), a portion of the guayule latex proteins are not immunogenic. The Lowry measures all the protein present, whereas the ELISA can only measure protein that is the same as that involved in raising the original antibodies. Therefore, numbers from the two assays would be the same if all of the protein was immunogenic. (Note: In Fig. 2, the statistic of 90% non-immunogenic protein in guayule latex was generated using the modified BCA protein quantification test (Siler and Cornish, 1995).

3. Sensitization studies

The studies relevant to assessing the risk of sensitization to guayule latex gloves are summarized in Table 1. Included are the most recent published results on animals and humans, as well as a summary of the new guayule-specific ELISA which has been tested in a series of five Round Robins. This ELISA, which should be established as a new ASTM standard in 2013, allows protein to be accurately quantified in guayule latex and when extracted from guayule products, with a sensitivity threshold of 0.02 µg/g dry rubber.

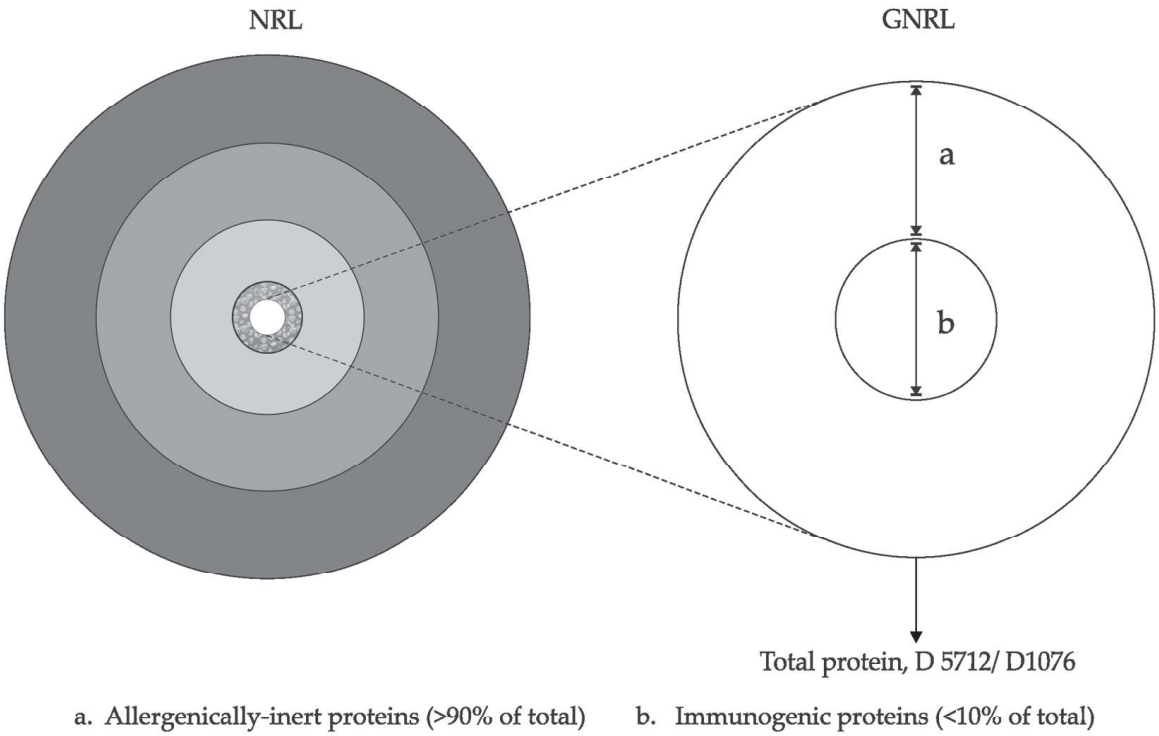


Fig. 2. Venn diagram comparing guayule (GNRL) and *Hevea* (NRL) proteins. The diagram on the left is a duplicate of Fig. 1. The white circle in the center represent the relative amount of protein in GNRL (<1% of NRL). The Venn diagram on the right expands this white dot and the areas that represent immunologically-inert and immunogenic protein are indicated. There is no evidence to suggest that any of these immunogenic proteins have the capacity to become allergenic

Table 1. **Summary of studies related to guayule rubber's sensitization potential**

Test type	Model used	Methods	Results
Guayule ELISA	Bench test method	Test method development for quantification of guayule latex proteins	Final results TBD. Initial results indicate sensitivity threshold of 0.02 mg/g dry. Test methods to be issued as ASTM standard in 2012 or 2013 rubber.
Buehler Repeated Patch Test of Guayule Natural Rubber Examination Gloves	Guinea pig	Repeat Patch test to determine sensitization potential of materials	All results were negative for incidence of sensitization response and severity at each time point.
Murine Localized Lymph Node Assays	Mice	Guayule latex non-rubber terpene compounds tested for Type IV sensitization	Negative for irritation and sensitization.
Murine Localized Lymph Node Assays	Mice	Guayule latex tested for Type IV sensitization	Negative for irritation and sensitization.
Guayule and Guayule Latex Immunogenicity Potential	Human: natural, yet high dose, occupational exposure	Guayule workers' blood sera tested for IgG and IgE responses to total plant protein and latex protein	While guayule total plant proteins and guayule latex were found to be immunogenic (IgG), the population was negative for allergic (IgE) response.

3. 1. Bench study: Guayule-specific ELISA

As indicated in Section 2.3.2., guayule natural rubber latex (GNRL) contains very low levels of protein. Current assays for total protein, such as the Lowry quantification method described in ASTM D 5712, are too insensitive to quantify extractable protein from GNRL products without using as high as possible material to extraction buffer ratio often coupled with a concentration step, and the assay can only then just detect the proteins. The more sensitive immunochemical assays, ASTM D 6499 and D 7427, are specific to *Hevea* natural rubber latex proteins, and the lack of cross-reaction between *Hevea* and guayule means that guayule proteins cannot be detected by these tests at all. Therefore, Dr. David Kostyal has been working on the development of an antigenic protein assay which can detect guayule protein as reported earlier (Kostyal *et al.*, 2011). This guayule assay is similar to the *Hevea*-specific D 6499 except that it is specific to guayule proteins instead of *Hevea* proteins. Additionally, considerable attention was paid to ensure that the polyclonal antibodies developed reacted to as complete a profile of guayule proteins as possible because the latex is mechanically purified from the plant, not tapped from a latex-containing laticifer. The approach yielded a polyclonal antibody that is sufficiently sensitive to quantify guayule proteins in purified latex and in unleached products to three significant figures. This assay provides a tool that can be used both to determine the actual level of extractable protein in finished guayule latex products

and also to assist in processing optimization and quality control during latex production.

Briefly, all Round Robin participating labs were able to detect a significant difference between the assay blank and 0.008 μg/cm^3 guayule protein. However, most labs were able to detect a significant difference between the blank and 0.002 μg/cm^3, and the final detection limit will be set following the final Round Robin. The limit of quantification (LOQ) is 0.02 μg/g. This compares with 50 μg/g for the ASTM D 5712 (some individual labs can achieve 11 μg/g) and 0.2 μg/g for the ASTM D 6499. Thus, this new guayule protein quantification test is more sensitive than the previously developed tests. In actual sample tests using this guayule-specific ELISA method, guayule dental dam films averaged 0.075 μg/g, and 0.066 μg/dm^3 extractable protein when assayed using the guayule ELISA. This is well below the entire range of extractable protein found in *Hevea* natural rubber latex dental dams from eight manufacturers quantified with the ASTM D 6499 *Hevea* specific ELISA (0.64 to 198.5 μg/dm^3) (Cornish *et al.*, 2011). One more Round Robin is required to finalize the assay, which should then publish as an ASTM standard in 2013.

3.2. Animal studies

As described in this section, animal studies were carried out to assess the dermal sensitization potential of guayule in guinea pigs and mice (Cornish *et al.*, 2009). The studies performed were the Buehler repeated patch test, a murine local lymph node assay on guayule resinous compounds, and a murine local lymph node assay on guayule latex. All of these studies are internationally-recognized standard methods for assessing sensitization.

No tests showed positive reactions or sensitization.

3.2.1. Buehler repeated patch test

Procedures were followed as described in ISO 10993-10 to test guayule natural rubber examination gloves in guinea pigs. The repeated patch test of Buehler was used, and, as is standard for solid test articles, the test was modified to include a longer induction exposure period. Tests were coordinated by Nelson Laboratories, Salt Lake City, Utah, USA, and performed by AppTec Laboratory Services, St. Paul, Minnesota, USA.

In the challenge phase of the Buehler Repeated Patch Test the ten positive controls (0.15% DNCB) all reacted, indicating a 100% incidence, confirming the innate sensitivity of the guinea pigs used in the study. None of the ten guinea pigs tested with the guayule glove pieces or the five negative controls had a sensitization response at any given time point, indicating a 0% incidence. The primary irritation score for the test sites of each guinea pig were totaled and subtracted from the total of the control primary irritation score to generate the primary irritation index for the gloves. All results were zero for incidence of sensitization response and severity at each evaluated time point.

The glove was classified as acceptable in regard to dermal sensitization (Cornish *et al*., 2009).

3.2.2. Murine localized lymph node assays

The internationally-implemented murine Localized Lymph Node Assay (LLNA) offers specific advantages over guinea pig test methods, including elimination of potential pain and distress, use of fewer animals,

shorter study time, and availability of dose-response information (ICCVAM, 2011). Murine localized lymph node assays were performed by MB Laboratories primarily to test for the Type IV sensitization potential of resinous compounds in the guayule shrub which might carry over into the latex. All results were negative for sensitization.

Additionally, MB Laboratories also performed these tests in a separate study using guayule latex painted on the ears of test mice. These mice also tested negative for sensitization to guayule latex.

3.2.3. Irritation (Type IV Reactions) and Relevance to latex allergy

The lack of irritation in the animal tests (see Table 1) indicates that, in humans, use of guayule gloves is unlikely to cause any reactions that could compromise the skin barrier. Even though FDA has not accepted animal models (Aamir *et al.*, 1996) as indicators for the potential of latex proteins to induce Type I latex allergy, FDA has accepted animal models as good indicators of human Type IV allergenic potential (*i.e.*, animal models can predict the potential of a substance to cause an allergic contact reaction in humans). A 1996 study (Taylor *et al.*, 1996) indicates that skin compromised by irritation or allergic dermatitis makes the sufferer more vulnerable to a sensitizing dose of latex proteins through their skin from glove usage. In this study, the researchers found that out of 44 Type I latex allergic human patients, 36 also had allergic contact hand eczema. Thus, positive sensitization and irritation in animal models would indicate an increased susceptibility to contracting a latex allergy *via* skin exposure due to the skin barrier being compromised.

3.3. Human studies

As indicated previously, the caution label has prevented guayule rubber exam gloves from being used commercially and thus has prevented the collection of extensive in-use data on sensitization potential in humans. Because it would be unethical to deliberately raise antibodies to guayule latex proteins in humans, the levels of anti-guayule protein IgG and IgE in a volunteer population of 21 guayule workers were monitored. These workers underwent a far greater exposure to guayule latex proteins than is ever likely to occur in a normal healthcare environment to users of guayule rubber gloves (Hamilton and Cornish, 2010). Exposure levels were very high due to the workers being repeatedly exposed to the guayule homogenate and the latex in the normal course of their work. Unlike the guayule workers, healthcare professionals would be exposed only to well-leached gloves containing small amounts of entrained hydrophobic proteins. Of these guayule workers, 11 of the 21 (52%) were atopic (*i.e.* they already had multiple allergies and would be predisposed to acquiring additional allergies).

This study concluded that only low IgG anti-guayule responses of <2AV/ml were observed against the residual proteins in purified latex. However, results of the study confirm that given sufficient exposure, guayule *plant* proteins (which include the subset of guayule latex proteins) can elicit a benign IgG antibody response in humans (3 of 21 samples), although no IgE antibodies could be detected. The lack of a detectable IgG immune response in human workers to guayule latex protein, who otherwise developed an IgG antibody response to the total plant proteins, reflects the absence of

an immunogenic dose of latex protein. In other words, the latex protein levels were too low to induce an immunogenic response. The results suggest that guayule latex will not induce a sensitization reaction in a naive population.

In contrast to the IgG antibody response detected in 3 of 21 of the workers to the total plant proteins, no IgE antibody or clinical evidence of allergic response was detected in any of the 21 workers. The study was unable to find evidence for guayule plant protein allergenicity, even in atopic workers who experienced extraordinarily high occupational exposures greatly exceeding the trace quantities of guayule protein in finished guayule natural rubber products.

Atopic humans have a significantly greater propensity toward developing Type I latex allergy than the general population. In a study of 44 patients with Type I latex allergy, 34 (77%) had atopy (Taylor and Praditsuwan, 1996). A study of 1351 hospital workers determined that 44.2% of atopic workers had positive skin prick tests to latex, compared with only 8.3% of non-atopic workers (Liss *et al.,* 1997). Also, researchers (Sussman and Beezhold, 1997) found that 24% of atopics and 24 to 67% of spina bifida patients had Type I latex allergy, contrasting with only 1 to 6% of the general population. Another study of Type I latex allergy in the general population in the U.K. found a 7.7% incidence of the allergy (Merritt *et al.,* 1995).

Assuming a similar sensitization rate of guayule proteins to that of *Hevea* proteins, we would expect to have seen guayule-specific IgE appearing in 1 to 2 people out of the 21 workers (general population rate) or in 2 to 3 of the 11 atopic workers (atopic rate). However, as stated above, none of the 21 workers developed IgE or other clinical allergic symptoms to guayule plant proteins or to guayule latex proteins in the three year study. Additionally, no allergic issues were reported by Yulex workers who declined to participate in the study due to fear of needle sticks.

Even though total guayule plant proteins were able to induce a benign IgG response, no evidence of IgG response was found for guayule latex-specific protein. This is probably due to the extremely low protein dose in the latex and the small percentage of these proteins in the total guayule plant protein complement.

The authors (Hamilton and Cornish, 2010) concluded that "guayule latex products, which are safe for use by *Hevea*-sensitized individuals because of their lack of cross-reactive protein, would be expected to remain non-allergenic in spite of long term use of guayule products that involves multiple exposures. The reason for this is the extremely low protein content and the general lack of allergenicity associated with any trace proteins in guayule natural rubber-containing products."

4. Other natural rubber alternatives

Many plants make rubber latex. However, it must not be assumed that, just because they are not *H. brasiliensis*, that they will be safe for use by *Hevea* allergic people. For example, many people with Type I latex allergy also are allergic to a range of tropical fruits. Similarly, latex proteins from *Taraxacum kok-sahgyz* cross-react with antibodies raised against *Hevea* latex proteins in mice, rabbits and humans (Cornish *et al.,* 2012). This species does make high quality rubber (Cornish *et al.,* 2012) and shows

promise as a supplement to *Hevea* rubber (rather than an alternative as is guayule rubber). In contrast, latex proteins from *Ficus elastica* do not cross-react (Carey *et al.*, 1995) but, unfortunately, this source of rubber is not of a sufficiently high quality for modern commercial applications (Cornish *et al.*, 1993).

CONCLUSIONS

Guayule natural rubber gloves eliminate the need to 'choose a risk' by providing the best physical properties of a *Hevea* natural rubber latex glove without exposure to allergenic proteins. Guayule natural rubber gloves exhibit exceptional physical characteristics, with respect to the fit, feel, tactility (finger sensitivity), grip, comfort, flexibility, and ease of donning. They exceed the desirable features of gloves made of natural rubber latex from the *Hevea* rubber tree. However, the strong cross-reactivity of latex proteins from Buckeye Gold (TKS) dandelion with anti-*Hevea* latex protein antibodies, indicates that it is not a latex source safe to use by Type I latex allergic patients. All new sources must be carefully evaluated to determine their immunological safety before product introduction.

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