# Evaluation & control of potential sensitizing & irritating chemical components in natural rubber latex extracted from the industrial crop guayule



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**ACRONYMS:** DNCB, dinitrochlorobenzene; HCA, hexylcinnamaldehyde; LLNA, localized lymph node assay; NRL, natural rubber latex; SB, staining buffer

## Abstract

*Guayule natural rubber is commercially available as an emulsion base-material for latex medical devices, including medical gloves and catheters. These products do not cause reactions in Type I latex allergic humans. In addition to natural rubber, guayule produces terpene resin comprised of hundreds of isoprenoid compounds. One group, the guayulins, is a major terpenoid component of guayule resin; the most abundant, guayulin A, comprises 1–10% of the resin fraction. An earlier study concluded that guayulins are contact sensitizers in guinea pigs, although they appeared much less sensitizing in humans. The possibility that residual guayulins may be present in the guayule natural rubber emulsions at a sensitizing level is of concern.*

*We quantified the levels of guayulin and resin in purified natural rubber emulsions, using HPLC. Guayulin concentrations fell rapidly, due to hydrolysis at high pH, reaching a steady state after 4–6 weeks. The localized lymph node assay (LLNA) in mice was used to assess sensitization potential of guayule resin and guayulin A. Guinea pig patch tests were used to assess irritation and sensitization. Mouse ear swelling and rabbit repeated patch testing were used to assess irritation.*

*No sensitization or irritation by guayulin A was observed. Guayule resin was irritating at concentrations of 10% and above, but no sensitization occurred. The LLNA and dermal test data indicate that it is extremely unlikely that guayule latex products will cause sensitization or irritation due to the presence of trace guayulins or other resin compounds co-extracted with the guayule natural rubber emulsion.*

## Introduction

Guayulins (*Figure 1*) are a major terpenoid component in the alternative rubber crop, guayule (*Parthenium argentatum*, Gray). Guayulin A (cinnamic acid ester, a sesquiterpene) is the most abundant form (1–10% of the resin fraction depending on germplasm identity).[1,2] Guayule is an industrial crop grown commercially in the southwestern United States[3] but which has also successfully undergone field trials in semi-arid regions of

every continent except Antarctica. Guayule produces rubber, resin and biomass. Its principle commercially available product is a high-performance natural rubber extracted and purified in the form of an aqueous latex emulsion. This emulsion forms the base material for alternative latex medical products[4] because it contains no proteins that cross-react with Type I latex allergy, a life-threatening allergy to proteins in natural rubber and latex from *Hevea brasiliensis*. Intrinsically, guayule natural rubber emulsion contains very low levels of protein, on the order of 1% of the level in well-leached (generally nonsensitizing) examination and surgical gloves made from *H. brasiliensis* latex (natural rubber latex, or NRL).[5–7] Poorly leached, and powdered *Hevea* NRL products, containing high levels of soluble proteins, were the primary inductive cause of Type I latex allergy symptoms in millions of people in the United States and around the world.[8,9] At the present time, guayule natural rubber emulsion is the only elastomeric material that can meet the stringent requirements for trace protein levels and zero latex antigenic protein defined in ASTM D1076-06 Category 4,[10] while still meeting product performance standards set for natural rubber devices by ASTM for products such as surgical and examination gloves. The FDA issued the first product clearance (a 510(k)) for a guayule natural rubber product (an examination glove) in April 2008.

An early study, using the guinea pig maximization test,[11] concluded that guayulins are contact sensitizers in guinea pigs,[1] although they appeared only to be very weak sensitizers in humans.[12] The possibility remained, however, that they might be able to cause Type IV contact allergic reactions in humans. This was of serious concern, because guayule latex has been developed as a safe alternative base material for various latex medical devices and products such as gloves, condoms, and catheters. Although Type IV allergies are not as dangerous as Type I reactions, any allergic reaction to the new products would likely confuse the user and render the new products unacceptable as a safe alternative to NRL. Natural latex alternatives to NRL are required because synthetic materials, now commonly seen in exam and surgical gloves, and other products, neither perform as well physically nor provide the same high level of protection against the transmission of blood-borne human pathogens such as HIV and hepatitis viruses.[3,13]

Thus, in order to maintain the highest level of user safety possible, we have determined the levels of guayulin and resin in guayule natural rubber emulsions, and attempted to determine the levels needed to induce sensitization, using the localized lymph node assay in mice. Irritation and sensitization of guayule natural rubber gloves made from the emulsion was determined by the Repeated Patch Dermal Sensitization test using guinea pigs. Irritation potential also was assessed using patch testing in rabbits and ear swelling in mice. These tests ensure that the level of guayulin and resin in guayule natural rubber emulsions and products is consistently maintained at a level well below a potentially sensitizing concentration.

## Materials & methods

### GUAYULE NATURAL RUBBER EMULSION

Three different lots of guayule natural rubber emulsion, purified from shrub harvested during August and September of 2006, were used. Summertime shrub has the highest percentage of leaves at harvest and so presents the highest level of possible contamination of the emulsions with guayulins and resin during the extraction and purification process.[14] The emulsions were sampled before the normal stabilization package was added to generate the commercially available lots produced by the Yulex pilot processing plant in Maricopa, Arizona, USA. The pH of the emulsions ranged from 11.5–12.5. Emulsions were stored for 2–6 weeks before analysis.

A 1-L sample of natural rubber emulsion was collected and split into 5 × 200 mL aliquots. One aliquot was held "as is," and 20% potassium hydroxide (KOH) was added in varying quantities to reach the target pH (pH 11.5, 12.0, 12.5) for each remaining aliquot. Percent alkalinity as KOH and pH were determined for each aliquot. Subsamples (50 mL) of each aliquot were analyzed for guayulins and their saponification products. Also, the guayulins and saponification products were quantified every two weeks for a total of six weeks, and pH and percent alkalinity as KOH also were determined.

### GUAYULE RESIN

Resin was a subsample of an extraction of 175 kg of guayule bagasse from the emulsion extraction process[15] using acetone pentane azeotrope.[16] Resin was extracted from the bagasse to obtain large quantities not possible by extracting trace levels from the latex. Resin was prepared under a toll agreement by Crown Iron Works, Roseville, Minnesota, USA. HPLC quantification, using methods described below, determined that this resin sample contained 1.54% guayulin A (cinnamic acid ester) and 0.27% guayulin B (*p*-anisic acid ester).

Resin levels in the emulsions were determined gravimetrically using established methods.[17]

### PURIFIED GUAYULIN A

Purified guayulin A was purchased from Professor Gary A. Sulikowski, Vanderbilt University, Nashville, Tennessee, USA.[18]

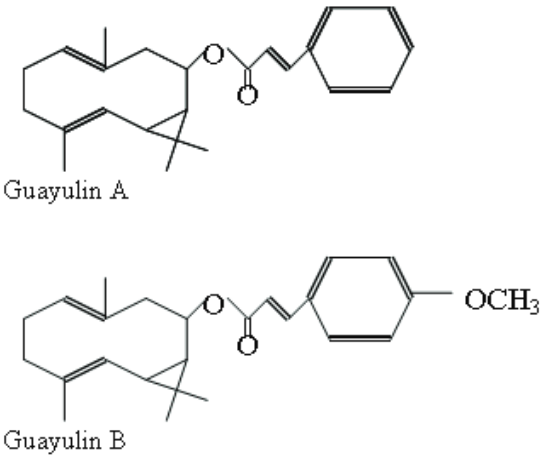


**Figure 1. Chemical structure of guayulin A (cinnamic acid ester) and guayulin B (anisic acid ester).**

## GUAYULIN QUANTIFICATION

Guayulin A and B were extracted from dried resin samples that were made up into approximately 10 mg/mL solutions with absolute EtOH (Aaper Alcohol and Chemical Co., Shelbyville, Kentucky, USA), before being analyzed by HPLC.[19] HPLC was performed using a Hitachi HPLC system (GenTech Scientific, Arcade, New York, USA) and HPLC grade degassed acetonitrile and $H_2O$ (EMD Chemicals, Gibbstown, New Jersey, USA). Chromatography was with a reverse-phase $C_{18}$ column (5 µm, 250 × 4.6 mm i.d.; Microsorb™-MV, Varian, Palo Alto, California, USA), at ambient temperature, and UV detection at 262 nm. Guayulin concentrations in the resin were determined by the following formula:

$$\% \text{ guayulin A or B} = \frac{AC \times RF \times mL\ EtOH}{mg\ resin \times 100{,}000{,}000}$$

where AC = area counts and RF = response factor (RF guayulin A = 1.235 ng/1,000 AC, RF guayulin B = 0.867 ng/1,000 AC). Area counts were determined by the Hitachi HPLC System Manager Software, version 4.0 (©1994–2000, Hitachi, Ltd. Tokyo, Japan).

## DERMAL IRRITATION & SENSITIZATION IN ANIMALS

### Guinea pigs

Procedures were followed as described in ISO 10993-10.[20] The repeated patch test of Buehler was used but modified to include a longer induction exposure period for solid test articles. Tests were coordinated by Nelson Laboratories, Salt Lake City, Utah, USA, and performed by AppTec Laboratory Services, St. Paul, Minnesota, USA.

Fifteen male albino guinea pigs Hartley Strain (specific pathogen free) were randomly selected). Animal weights ranged from 300–500 g at the initiation of the test, within the required range for the test. Ten test guinea pigs and five control guinea pigs were randomly assigned. Two application sites were prepared by shaving off a 5 × 7 cm area of fur with an electric clipper. The left flank of the animals was shaved before the induction dosing on Days 0, 6, 10, 13, and 17. The right flank was shaved for the challenge on Day 31.

Guayule examination gloves were cut into 2.54 × 2.54 cm squares. Negative control squares were prepared by removing the paper backing from Hill Top Chambers® (Hill Top Research, Miamiville, Ohio, USA). Positive controls were 0.3% of dinitrochlorobenzene (DNCB) in ethanol and were applied using Hill Top Chambers. Test and control squares were applied to the left flank site of the appropriate guinea pigs and secured by wrapping with an elastic bandage (Vetrap™, 3M, St. Paul, Minnesota, USA ) secured with a hypoallergenic tape (Transpore™, 3M). The bandaging and patches were removed after at least 6 h of exposure. At 24 ± 2 h after topical application, the sites were assessed for erythema and edema using a grading scale (*Table 1*). The procedure was repeated three times per week for three weeks for a total of nine inductions.

The challenge procedure was initiated on the animals 14 days after completion of the topical induction phase. Guayule glove squares were applied to the shaved right flank of each control animal. The positive controls were tested with 0.15% DNCB in acetone. The bandaging and patches were removed after at least 6 h exposure.

**Table 1. Dermal observation scoring—Guinea pig**

| PATCH TEST REACTION | GRADING SCALE |
|---|---|
| No visible change | 0 |
| Discrete or patchy erythema | 1 |
| Moderate and confluent erythema | 2 |
| Intense erythema and swelling | 3 |

Note: Erythema is defined as redness, and edema is defined as a swelling at the challenge site. Any other adverse changes at the skin sites were recorded and reported.

The day following the challenge exposure and prior to each scoring period, each site was wiped gently with a 70% isopropyl alcohol soaked gauze sponge. The challenge sites were observed for irritation and sensitization reactions, as indicated by erythema and edema. Daily challenge scores were recorded at 24±2 h and 48±2 h after patch removal.

### Rabbits

Procedures were followed as described in ISO 10993-10.[20] Tests were coordinated by Nelson Laboratories and performed by AppTec Laboratory Services.

Three female albino rabbits, New Zealand White strain, were randomly selected for the study. Animal weights ranged from 2.7–3.1 kg (protocol minimum = 2.0 kg). The fur of the animals was shaved on both sides of the spinal column to expose skin for patch testing. Guayule examination gloves were cut into 2.54 × 2.54 cm squares. Negative control squares of the same size were cut from absorbent gauze.

The test and control squares were wet with tap water and applied to the shaved backs of the rabbits (one square on each side of the spinal column). The patches were held in place by wrapping the trunk of the animals with an elastic bandage (Vetrap) and securing with hypoallergenic tape (Transpore). After 4 h, the animals were unwrapped and the locations of the patches were marked on the skin to aid scoring. Dermal observations were recorded at 30 to 60 min post-unwrapping, and at 24, 48, and 72 h (±2h) and scored as described in *Table 2*.

### Mice

Ninety-five nulliparous healthy female mice, strain designation CBA/J (a general purpose strain commonly used for LLNA were procured from The Jackson Laboratory (Bar Harbor, Maine, USA) and assigned to applicable experimentation groups using a computer-based random number program. Six mice were assigned to the screen for the tests with guayulin A and guayulin resin, and 25 mice were assigned to both definitive studies with these test materials. The Day 1 body-weight range was 18–22 g, and the weight variation at study initiation did not exceed ±20% of the mean body weight.

**Table 2. Dermal observation scoring—Rabbit**

| ERYTHEMA | GRADING SCALE | EDEMA | GRADING SCALE |
|---|---|---|---|
| No erythema | 0 | No edema | 0 |
| Very slight erythema | 1 | Very slight edema | 1 |
| Well defined erythema | 2 | Slight edema (raised edges) | 2 |
| Moderate to severe erythema | 3 | Moderate edema (>1 mm) | 3 |
| Severe erythema (beet red) to slight eschar formation (injuries in depth) | 4 | Severe edema (>1 mm and extending beyond area) | 4 |

Note: Erythema is defined as redness, and edema is defined as a swelling at the challenge site. Any other adverse changes at the skin sites were recorded and reported.

**Table 3. Treatment summary for the two LLNA studies**

| | Resin study | | Guayulin A study | |
|---|---|---|---|---|
| Group number | Test substance | Number of mice | Test substance | Number of mice |
| 1 | Vehicle (acetone) | 5 | Vehicle (AOO) | 5 |
| 2 | HCA, 25% | 5 | HCA, 25% | 5 |
| 3 | Resin, 2.5% | 5 | Guayulin A, 0.1% | 5 |
| 4 | Resin, 5% | 5 | Guayulin A, 0.25% | 5 |
| 5 | Resin, 10% | 5 | Guayulin A, 0.5% | 5 |
| 6 | Resin, 5%, + Guayulin A, 0.5% | 5 | | |

Note: The resin samples used in this study intrinsically contained 1.54% guayulin A (cinnamic acid ester) and 0.27% guayulin B (*p*-anisic acid ester).

A preliminary dermal irritation toxicity screen was conducted with three groups of healthy CBA/J mice (2 per group) per test article (resin, guayulin A, resin mixed with guayulin A), to determine the concentrations of the test articles to be used in the studies. The mice were treated with the test article for three consecutive days. The ears were observed for edema and/or erythema. Ear measurements were taken on Day 1 (predose control), Day 3 (approximately 48 h), and Day 6 (end of in-life phase). On Day 6, mice were sacrificed using $CO_2$ asphyxiation. Ear thickness changes on Day 3 and Day 6 were expressed as percent of Day 1 prestudy values. An increase of 25% or more in ear thickness was considered biologically significant (based on historical laboratory data) and indicative of a primary dermal irritation response.

For purified guayulin A (cinnamic acid ester), the initial screening study showed no irritation at any of the concentrations tested including the maximum concentration of 0.5% (which was the highest achievable concentration of guayulin A in the carrier vehicle of acetone:olive oil [4:1]). Based on these results, guayulin A concentrations of 0.1%, 0.25%, and 0.5% were selected for the definitive LLNA study, in 100% acetone. Similarly, guayule resin was tested in a preliminary irritation toxicity screen, in a vehicle of 100% acetone (without olive oil). In this case, the 10% and 25% treatments resulted in dermal irritation. In consequence, resin concentrations of 2.5%, 5%, and 10% were chosen for the definitive LLNA study. In addition, a test article of 5% guayule resin + 0.5% guayulin A was used, in case the resin contained compounds that might exert an adjuvant effect on the sensitization ability of guayulin A. The carrier vehicle in this case was 100% acetone.

## LOCAL LYMPH NODE ASSAYS IN MICE

In the definitive studies, groups of five CBA/J mice (see prior section) were treated by topical application of the test substances, the carrier vehicle alone (negative control), and a positive control of 25% alpha-hexylcinnamaldehyde (HCA), to the dorsum of each ear once daily for three consecutive days (at approximately the same time per day). The test article mixture, control or vehicle, was spread over the entire dorsal surface of the ear using a micropipette at 25 µL/ear. The dosing groups are shown in *Table 3*.

All animals in the study were observed once daily throughout the study for pharmacological effects and mortality.

Five days following the initial dose, and five hours prior to sacrifice, the mice were injected intraperitoneally with thymidine analog 5-bromo-2'-deoxy-uridine (BrdU), in Dulbecco's phosphate buffered saline (DPBS) at a dose of approximately 150 mg/kg. This thymidine analog becomes incorporated into the DNA of proliferating cells, including proliferating lymphocytes. The auricular lymph nodes were isolated post-sacrifice and single cell suspensions were generated in fetal bovine serum. Cells were fixed in 85% ethanol. Flow cytometric

analyses (FACScan Flow Cytometer equipped with an Omnichrome 25 mW argon laser emitting at 488 nm with 15 mW of power, and Cell Quest software, both from Becton Dickinson, San Jose, California), were used to determine the percentage of proliferating cells, detected by the combination of BrdU incorporation into their DNA and determination of cell number.

For BrdU incorporation, measured aliquots of fixed cells were washed and resuspended in 2 M HCl and 1% Triton X-100 for 1 h, to allow quantitative interaction between the BrdU antibody with the BrdU incorporated into the cellular DNA. The samples were neutralized by washing with sodium tetraborate (pH 8.5). The nuclei were washed with Staining Buffer (SB: 1% BSA, 0.03% sodium azide, 0.5% Tween-20 in PBS, pH 7.4) and incubated with fluorescein-conjugated BrdU-specific antibody for 40-60 minutes (Becton Dickinson Immunocytometry Systems, San Jose, California, USA). The nuclei were then washed with SB, resuspended in DPBS containing the DNA-specific dye propidium iodide at a final concentration of approximately 25 µg/mL and the percentage of nuclei staining positive for BrdU (i.e., proliferating cells in S phase) was determined by flow cytometry.

For cell-number determination, measured aliquots of fixed cells were stained in a 0.5 mg/mL propidium iodide solution in PBS. The number of cells in each aliquot (representing a 1/500 dilution of the lymph node cells suspension) was determined by flow cytometry. For both cell-number determination and BrdU incorporation, clumps of nuclei were excluded from analysis using gates set on integrated red fluorescence signals. For each animal, lymph node cell proliferation (#BrdU-positive) was determined by multiplying the total number of cells in the node (cell-number determination) by the percent BrdU-positive cells in the node. The number of proliferating lymphocytes in each treatment group was divided by the number of proliferating lymphocytes in the vehicle control group and is called the Stimulation Index. The mean stimulation index and standard deviation were calculated for each treatment group. A test article is considered to have a sensitizing potential if treatment results in ≥3 increase in the lymph node cell proliferation rate relative to control.



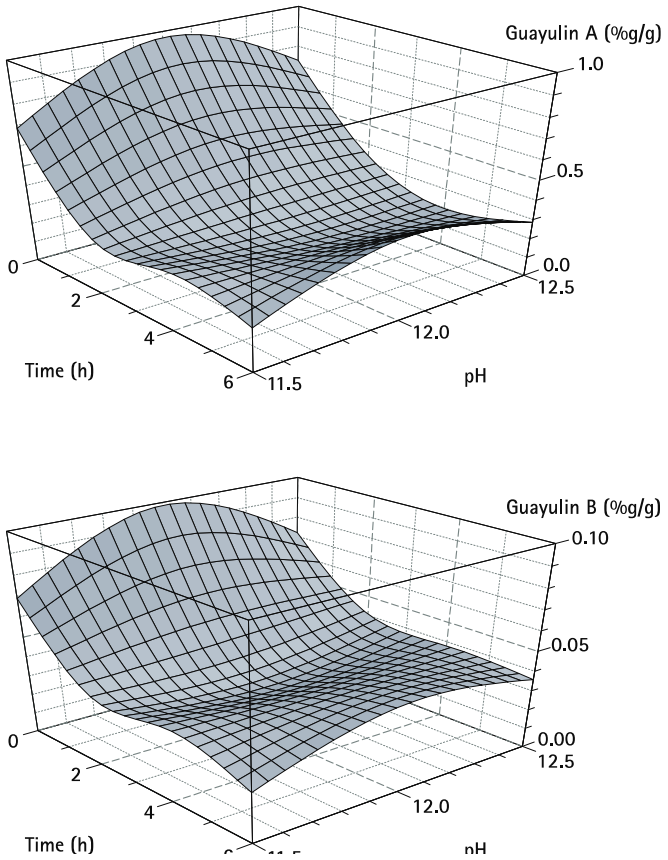


**Figure 2. Hydrolysis of guayulin A and B as a function of pH and time. HPLC proved that the respective acid increases concomitantly with the loss of guayulin (data not shown).**

## Results

### GUAYULIN LEVELS IN GUAYULE NATURAL RUBBER EMULSIONS

No significant differences were observed among the three lots of emulsion tested, and so the data (nine replicates) were combined. The resin content was 1.39% of the emulsion on a dry weight basis. The resin fraction, itself, contained 21% guayulin A and 2.6% guayulin B.[21] Thus, the emulsions contain 0.288 ± 0.024% guayulin A and 0.0359 ± 0.024% guayulin B on a dry weight basis ± standard error.

Guayule emulsions are prepared, for commercial use, at high pH of 11.5 to 12.5 in order to stabilize the emulsion and to prevent microbial attack. These pHs hydrolyze the guayulins to their respective acids, reaching a >50% lower level in two weeks (*Figure 2*). Both guayulin A and guayulin B declined at a similar rate, even though there was approximately 10 × the amount of guayulin A than guayulin B throughout.

### DERMAL IRRITATION

#### Guinea pigs

During the induction phase of the Buehler Repeated Patch Test there was no irritation observed for the ten guinea pigs tested with the guayule glove pieces or the five negative controls. The positive controls (0.3% DNCB) all reacted with strong erythema.

#### Rabbits

There were no dermal reactions at the test sites on the rabbits at the 0.5, 24, 48, and 72 h observation periods. The Primary Irritation Scores for the respective test sites of each rabbit were totaled and subtracted from the total of the control Primary Irritation Scores to generate the Primary Irritation Index for the gloves. All results were zero. The materials were classified as Irritation Response Category "negligible" and the glove as "non-irritant."

### Mice

None of the guayulin A concentrations tested in the dermal irritation toxicity screen was irritating. The positive control, 25% HCA, had a >25% increase in ear swelling by Day 6, which is consistent with normal results and occurs in about 50% of studies when using 25% HCA in acetone:olive oil, 4:1 (AOO). However, guayule resin at 10% and 25% caused a positive irritation reaction.

## SENSITIZATION

### Guinea pigs

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 Scores for the respective test sites of each guinea pig were totaled and subtracted from the total of the control Primary Irritation Scores to generate the Primary Irritation Index for the gloves. All results were zero. The materials were classified as Irritation Response Category "negligible" and the glove as "non-irritant."

### Mice

The localized lymph node assay resulted in stimulation indices of <2 for resin (*Figure 3*), resin with added guayulin A (*Figure 3*), and of pure guayulin A alone (*Figure 4*) demonstrating that these substances are not dermal sensitizers at the concentrations used (a stimulation index above 3 is an indication of sensitization). The positive control (25% HCA) caused reactions in all test animals, giving stimulation indices of 16.75 ± 3.32 in the resin LLNA, and 10.10 ± 2.45 in the guayulin LLNA.

## Discussion

Acetone extracts of guayule shrub or non-stabilized emulsions (purified rubber particles) are commonly called resin. Resin contains many identified terpenoid resin compounds, such as the guayulins and pinenes, as well as a mixture of fatty acids, lipids, pigments, and other acetone-soluble materials.[14,22] When extracted at room temperature, as in this study, the resin fraction amounts to 1.39% of the dried emulsion. Other authors have reported much higher levels, such as a resin level in the emulsion of 26.2% on a dry rubber basis.[23] However, these high values appear to be an artifact of the boiling acetone extraction method used, because guayule rubber is subject to thermo- and oxidative degradation.[24] We have found (Pearson, Rath, and Cornish, unpublished results) that acetone temperatures as low as 50°C degrade the high-molecular-weight polymers to a form that is then soluble in acetone. The high "resin" levels reported[23] appear to be low molecular weight rubber polymers resulting from degradation of the high-molecular-weight rubber polymers during the 6 h Soxhlet extraction process used, during which the sample was exposed to repeated washing cycles with boiling acetone. This conclusion is supported by the finding that the amount of acetone-extractable material was reduced by 62% when the rubber polymers were first vulcanized.[22] The sulfur cross-linkages formed during the vulcanization process between the rubber polymers in the film material greatly resist the degradative process. This is because the carbon-to-carbon double bonds in the rubber polymer are converted to single bonds as the sulfur molecules form bridges from one polymer chain to the next; single bonds are much less susceptible to oxidative scission than double bonds. In contrast to purified rubber particles, commercially produced guayule natural rubber emulsions contain about 3% acetone-extractable material, the higher level resulting from the acetone-soluble surfactant stabilizers added to the emulsion.

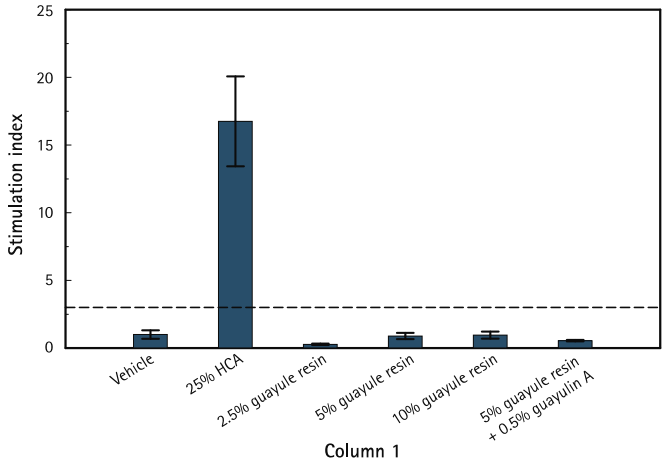


**Figure 3. Stimulation index of different concentrations of purified guayulin A in the localized lymph node assay in mice, compared to positive and negative controls. Results are 5 to 10 replicates ± se.**

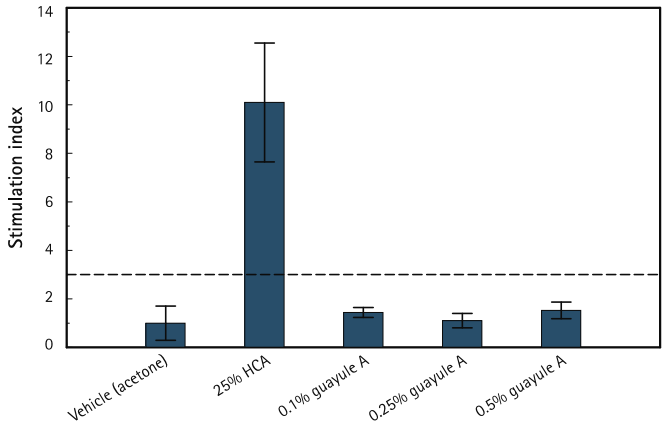


**Figure 4. Stimulation index of different concentrations of guayule resin and resin mixed with guayulin A in the localized lymph node assay in mice, compared to positive and negative controls. Results are 5 to 10 replicates ± se.**

Schloman et al[23] also reported that the latex sample they analyzed contained 3.9% guayulin A, on a dry rubber basis and 0.98% guayulin B. The highest values we have observed are 1% guayulin A in fresh latex. However, both guayulin A and guayulin B are rapidly hydrolyzed at high pH (guayulin A, to cinnamic acid and a remaining 10-membered ring structure; and guayulin B, to anisic acid, also yielding a 10-membered ring structure, which is probably the respective aromadendrene diol);[25] all production of commercial guayule natural rubber emulsion is exposed to high pH for more than two weeks, resulting in a minimum of a 50% reduction in guayulin concentration (*Figure 2*). The significantly lower levels of guayulin A and B we report (0.288 and 0.0359%, respectively) seem to be the worst case in the commercially available materials which, in these tests, were produced from summer-harvested material when the leaf and guayulin content is at its highest.[26] The 3.9% value was obtained from latex that had been coagulated with acid and dried before extraction, which might have enhanced solubility and contributed to the higher level extracted.[23] Nevertheless, even if occasional samples did contain 3.9% guayulin A, the acetone in olive oil vehicle, used in the LLNA study, was only able to dissolve 0.5%. Thus, 0.5% seems a maximum realistic dose that a person might be able to experience by product extraction with sweat and skin oils, although it should be noted that guayulins are insoluble in water and will likely largely remain within the product matrix and so true exposures are likely even lower.

Our observation that neither resin nor purified guayulin A are sensitizing in the LLNA assay is surprising in light of the earlier study by Rodriguez et al[1] using the guinea pig maximization test.[1,11] It is unclear why these two very different results were obtained, although it is possible that the guinea pigs are simply more sensitive to guayulin A than are the mice. The accuracy of allergy testing varies, and even one test subject may respond differently at different times. Also, a test subject may react to a substance during testing, but never react during normal exposure. False negatives, however, are rare.

Although purified guayulin A caused no positive reactions, guayule resin was irritating at concentrations of 10% and above in the mouse preliminary dermal irritation toxicity screen. Another plant-based source of resin, from the pine tree, is irritating because of the rosin (also called colophony) component of the resin, which contains abietic acid and hydroabietyl alchohol, both contact sensitizers.[27] Guayule resin has a different chemical composition than pine resin and does not contain abietic acid or hydroabietyl alchohol, so a different biochemical must be responsible for the irritation observed in the mice. Other rosin esters are likely to be present, for example. However, guayule natural rubber products cannot contain more than 1.4% resin (the resin content of the emulsion) which is a non-irritating level. The guinea pigs in our patch tests, which are similar to the maximization test used in the earlier study,[1] showed no positive irritation or sensitization reactions to the guayule glove films. The disparate results obtained in the guinea pig model do not seem to be caused by an additive or adjuvant effect of compounds co-extracted with the guayulin A in the earlier study. We found no difference in the non-sensitizing response when we mixed resin and guayulin A (*Figure 4*).

The positive control in the LLNA study was 25% HCA but it has been reported that HCA is actually sensitizing at 0.02%[28]. This is considerably lower than the levels of guayulin A and resin tested in the current study, again indicating that these materials are not strong irritants or sensitizers. We do not know if a person already sensitized to HCA would then react to trace guayulins due to structural similarities between the compounds.

In conclusion, it seems extremely unlikely that guayule natural rubber products will cause sensitization due to the presence of guayulins co-extracted with the emulsion.

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