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Permeation of limonene through disposable nitrile gloves using a dextrous robot hand

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Abstract: Objectives: The purpose of this study was to investigate the permeation of the low-volatile solvent limonene through different disposable, unlined, unsupported, nitrile exam whole gloves (blue, purple, sterling, and lavender, from Kimberly-Clark). Methods: This study utilized a moving and static dextrous robot hand as part of a novel dynamic permeation system that allowed sampling at specific times. Quantitation of limonene in samples was based on capillary gas chromatography-mass spectrometry and the internal standard method (4-bromophenol). Results: The average post-permeation thicknesses (before reconditioning) for all gloves for both the moving and static hand were more than 10% of the pre-permeation ones (P<0.05), although this was not so on reconditioning. The standardized breakthrough times and steady-state permeation periods were similar for the blue, purple, and sterling gloves. Both methods had similar sensitivity. The lavender glove showed a higher permeation rate (0.490±0.031 μg/cm²/min) for the moving robotic hand compared to the non-moving hand (P<0.05), this being ascribed to a thickness threshold. Conclusions: Permeation parameters for the static and dynamic robot hand models indicate that both methods have similar sensitivity in detecting the analyte during permeation and the blue, purple, and sterling gloves behave similarly during the permeation process whether moving or non-moving.

Key words: Limonene, Nitrile disposable gloves, Permeation, Robot hand

Introduction

Personal protection equipment such as chemical-resistant gloves is the first line of defense against potential skin exposure to toxic chemicals in the workplace¹. The National Institute for Occupational Safety and Health (NIOSH) emphasizes the importance of selecting the most appropriate glove for exposure to chemicals and defining the time limit it can be worn and whether it can be reused⁴. It is estimated that more than 10% of the working population in the U.S. risks potential exposure to chemicals via skin contact⁷. Considering that dermatitis is one of the top three occupational disorders, the direct and indirect costs of workers’ compensation are on the billion dollar scale⁶.

Many chemicals, whether absorbed through the skin upon contact or inhaled, can cause health problems⁸. Skin absorption for a large number of chemicals can occur without the workers noticing⁹. The risks of skin absorption are particularly important when it comes to exposure to low-volatile compounds. Because of low vapor pressure, such chemicals can remain in the work environment for extended periods of time⁸. One of the physical parameters used in categorizing chemical volatility is the boiling point. In general, volatile chemicals are those with boiling points below 150°C. Compounds that boil at or over 150°C are categorized as semi-/non-volatile⁹.

The American Society for Testing and Materials (ASTM) F739-99/12 standard addressed the glove permeation process for a challenge chemical in both open-loop and closed-loop systems⁶. ASTM F739-99/12 recommends the closed-loop method for low-volatile solvents. In this method, a liquid in the collection side is in direct contact with the permeated solvent, and at defined intervals samples are taken from the collection side for analysis.

Current research methods in both open-loop and
closed-loop systems fail to take into account many other factors that may contribute to the permeation of chemicals through the protective glove barrier. For example, disposable glove thickness is not the same across different areas: fingertips are thicker and the wrist is thinner than the palm area. In existing testing methods, permeation is measured on a small portion of the glove from the palm or back of palm area, which may not be representative of the overall glove thickness. Moreover, the ASTM and other international standards study glove permeation under static conditions in which the glove specimen is held still between two chambers at room temperature.

In contrast, using gloves in the workplace requires donning and doffing as well as frequent flexion and extensions throughout the work shift. Mechanical movement is a basic defined function for hands, and gloves would be exposed to the same dynamic forces as hands. This has been overlooked in the standards. Such shortcomings in existing methods can result in non-realistic standardized breakthrough times (SBRT) and steady-state permeation rates (SSPR) even if an analytical method with high sensitivity is used.Boeniger et al. discussed the limitations of permeation standards with regard to chemicals with low vapor pressure, and asserted that the ASTM permeation test method is not appropriate for over 80% of the chemicals in the threshold limit value (TLV) list with skin notation because they are categorized as low vapor pressure (defined here as <5 mmHg).

To address the above concerns, a few studies have been conducted on different gloves using alternative methods such as flexed permeation methods, on-site permeation testing, and a whole glove permeation model. Mikatavage and Que Hee studied permeation of four chlorinated aromatic compounds through Viton and nitrile chemical protective gloves using a closed-loop system. They used a shaking water bath system to provide gentle stresses on the glove material during permeation to simulate hand flexing, and well as to obtain adequate mixing. This is termed the modified ASTM closed-loop system. The results indicated a 4 h breakthrough time for Viton gloves compared to less than 1 h for nitrile gloves. Perkins et al. reported the effects of glove flexure on permeation using chemical protective gloves as a whole glove model. Although the method, in general, did not provide enough precision, permeation results indicated a significant difference between gloves in static and flexed positions.

Phalen and Que Hee studied impacts of hand movement on permeation of captafol through disposable nitrite gloves using a robot hand. The results showed that although hand movement influenced physical and chemical degradation, it did not contribute to permeation of analyte through nitrie gloves.

Mathews studied the permeation of cyclohexanol through different disposable nitrite gloves using a whole glove moving hand system that allowed sampling at different times. The results indicated a significant difference in NBRT and SSPR between ASTM cells and the moving hand for sterling gloves, but not for blue and purple gloves.

Limonene was chosen as a challenge solvent because it has a boiling point of 175°C, and a solubility of 13.8 mg/l in water at 25°C. Moreover, there are no previously published studies on limonene permeation through disposable gloves and very few reports are available for chemical protective gloves. The worldwide annual production of limonene in 2004 was over 150 million pounds (75,000 metric tons) and it is rising fast.

The purpose of this study was to compare the permeation of limonene through different disposable nitrite exam glove materials using whole glove dynamic and still hand models.

Materials and Methods

Chemicals, Solvents, and Glove Samples

The gloves used were Kimberly-Clark blue, purple, sterling, and lavender disposable gloves, all powder-free, unsupported, unlined, nitrite exam gloves, from Fisher Scientific, Pittsburgh, PA. The selected samples were within a wide thickness range that covered most of the disposable gloves applications in medical, industry, and services. Due to the scope of the research and factors such as time, analytical, and instrumental procedures, the glove specimens from one of the major glove manufacturer were chosen. The analyte, limonene (96%), was from Acros Organics. 4-Bromophenol (99%, from Sigma Aldrich) was the internal standard (IS) for quantification purposes in the analytical method, gas chromatography-mass spectrometry (GC-MS). Helium (99.99%) from Air Products (Long Beach, CA) was used as the carrier gas for GC-MS operation. The water utilized for all aqueous solutions was produced from two filtration systems: Millipore Milli-Q Water System followed by the Millipore Simplicity portable ultrapure water purification system (Temecula, CA).

Equipment

Glove material thickness was measured at the palm region using an Electronic Digital Micrometer Model CO-030025 (0-25 mm, 0.001 mm resolution) from Fisher Scientific. A Mettler analytical balance AE260 Scale (Mettler, Hightstown, NJ) was used to weigh the glove specimens. A bionic dextrous robot hand kit was ordered from Scientifics Direct, Inc., Tonawanda, NY. Viton tubes (2.79 mm) were used for transferring and circulating the water collection medium from the glove to the sampling vial and to the glove again. Viton tubes were connected using PTFE (Polytetrafluoroethylene) tubing (1.48 mm in-
ner diameter) as well as polypropylene T-connectors (Cole Parmer, Court Vernon Hill, IL). The power for continuous water circulation from the inner side of the glove samples to the sampling point was provided through a peristaltic Reglo 2-channel Var-Speed Analog pump (Cole Parmer, Court Vernon Hills, IL). The collection medium’s temperature was stabilized at 35.0±0.5°C using a Corning Stirring Hot Plate (Fisher Scientific). The analyte’s temperature was also stabilized at 35.0±0.5°C using a Precision Laboratory Oven-Econotherm from Fisher Scientific that also housed the assembled gloved dextrous robot hand. A Traceable Lab Controller (125 VAC, 15 A) was used to control the robotic moving hand’s flexion-extension cycle at 20-second intervals (Fisher Scientific).

The GC-MS system consisted of two components: an Agilent 6890N Gas Chromatograph with a non-polar, HP-5MS fused silica capillary column, 60 m length ×0.42 mm external diameter, 0.32 mm internal diameter, 1-μm internal film thickness operated in the splitless mode in tandem with an Agilent 5973 Mass Selective Detector (MSD) (Agilent Technologies).

A Thermo Nicolet Avatar 360 Fourier Transform (FT) Infrared Spectrometer was used for the acquisition of infrared reflectance spectra. The infrared spectra ranged from 4,000 to 400 cm⁻¹, with 124 scans for higher resolution and spectra uploaded with its OMNIC 6.0a software (Thermo Nicolet, Madison, WI).

Procedure

GC-MS

The MS was initially used in the total ion current mode (TIC) to obtain intensities of all mass spectral peaks over the mass-to-charge ratio (m/z) range of 50-500. Thus, the purities of the analytes, solvents, and IS were examined. The injected volume for all samples was 2.5 μl. In the next quantification phase, to define breakthrough times, the selected ion monitoring mode (SIM) was used to enhance sensitivity and selectivity for the compounds of interest (at m/z 68, 93 for limonene and m/z 172 for the 4-bromophenol IS).

1.00 ml of standard solutions of the analytes in water with concentrations of 0.0, 0.1, 0.3, 0.5, 1, 5, 10, and 100 ng/μl were made in the presence of 1.0 μg/μl of the IS (an aliquot of 2.0 μl from a concentrated IS of 1.0 μg/μl was added to each 1.0 ml standard and sample solution). After injection, the areas under the appropriate peak for the analyte and IS were measured using RTE manual integration. The area ratios of the limonene over the IS for each standard were plotted versus the corresponding standard solution concentrations or mass injected.

The linear regression parameters such as slopes, intercepts, their standard deviations, correlation coefficient r, and p-values, were defined. The Student’s t-test was used to determine whether the average of two samples was significantly different and to define the p-values of r for n-2 degrees of freedom where n is the number of data pairs. To define the relationship between dependent and independent variables, the analysis of variance technique was used.

Whole Glove Permeation

Each set of glove samples was checked to be within the expiration date. The gloves were conditioned in a desiccator at 52±1% relative humidity and 23°C for 24 h before the permeation test in an atmosphere generated by saturated aqueous potassium dichromate.

Glove physical characteristics such as weight and thickness before and after permeation experiments for still and moving robot hand experiments were also measured just after permeation (before reconditioning), and when glove specimens were blotted dry. The selected glove average thicknesses (micrometer) at three marked spots of the palm were measured in triplicate. The infrared reflectance spectra (Avatar 360) were also obtained for the challenge and collection surfaces of each glove using the clamp accessory before and after permeation.

To prepare for permeation experiments, the robot hand was donned with a chemical protective Solvex nitrile glove (size 9) to protect it. A beaker holding 100 ml of water was conditioned at 35°C on the hot plate/stirrer. Then a modified 40 ml-Pyrex vial with two holes in its screw cap (one each for supply and return water) was attached to a clamp and held on the hot plate.

The Viton tubing was cut in duplicate to three different dimensions, 29,” 21,” and 12” (73, 53, and 30 cm) to allow water circulation through the system. Two 21”-Viton tubes were inserted in the peristaltic pump with one Viton 3-stop tubing in each tube (channel). Each 3-stop tubing had supply and return ends. One of the 21”-pieces was connected to the supply end of the first 3-stop tubing at the first channel and the other one connected to the return section of 3-stop tubing at the second channel. The other end of both 21”-Viton tubings connected to the 40-ml vial through the separate holes made in the cap. An 18-gauge needle was used to make holes over every 0.5” at the first 9” of one of the 29”-Viton tubings, and the punctured end of the tube was heated with a burner and sealed. The holes acted as water suppliers to expose all areas of the inner surface of the test glove (medium size). The 29”-Viton tubings were used as water supply and return connections from the peristaltic pump to the whole glove in the oven with an optimized flow rate of 16 ml/min. The sealed punctured side of the 29”-Viton tubing was wrapped around the glove (Robotic hand) within 1” from the cuff and the other side fitted to the supply end of the second 3-stop tubing. The 12”-Viton tubings were placed at pronation and supination of the hand to assess leaks and collected water from the inner side of the glove. The front side of the 12”-Viton tubing was joined with a T-connector that directly attached to the second 29”-Viton tube and led to the return end of the first 3-stop tubing.
The supply end of the first 3-stop tubing led to the 40-ml vial. Aliquots of 80 ml of the purified water were added to the space between the disposable nitrile glove inner side and the chemical protective glove, and 20 ml water was added to the sampling point; a 40-ml vial. To avoid water leaks, parafilm was wrapped over the cuff of the glove. Finally, the robotic hand was held by the wrist pointing down into a desiccator containing limonene with 7.5” of the glove from the tip of the middle finger immersed in limonene. A jacket shield was made and covered the toggle switch to protect the connections from overheating and prevent physical damage during the permeation. The robotic hand test tube fingers were covered with clear duct tape to cover and remove sharp edges, protect the chemical protective glove from physical damage, and facilitate donning and doffing procedures. An overview of the schematic model of the whole glove permeation is shown in Fig. 1.

Aliquots of 1.0 ml were taken at permeation time intervals of 0, 10, 20, 40, 50, 60, and 80 min, 2, 4, 6, and 8 h, and deposited into chilled 1.5-ml vials plus 2.0 μl of IS (4-bromophenol) of 1.0 μg/μl concentration was added. The sum of all samples taken did not exceed 10% of the initial collection solvent volume for each permeation run. After the permeation test, glove specimens were blotted dry and reconditioned in the original desiccator for reflectance IR analysis as well for thickness and mass measurements.

The total permeated mass of the analyte was calculated for each time interval, and the mass taken during each prior sampling time was included in the total mass. The cumulated permeation curve was plotted by permeated mass/area of the analyte on the y-axis versus time on the x-axis as indicated in ASTM 739-127. The steady-state permeation rate was defined as the area of the permeation curve with the steepest linear slope. Statistical analysis was performed using SPSS software version 20 (IBM Corp., Armonk, NY, USA). Results were considered significant if the p-value was equal or less than 0.05.

**Results**

**GC-MS Analytical Features**

The MS was initially used in the total ion current mode (TIC) to record intensities of all mass spectral peaks over the mass-to-charge ratio (m/z) range of 50-500. The purities of the analyte and IS were verified as 96% and 99%, respectively.

The GC-MS working ranges were 0.1 ng to 1.0 ng and 1.0 ng to 10.0 ng with 0.1 ng as the lower quantifiable limit. The retention times for limonene and the IS were 5.4 min and 9.5 min, respectively. The total run time for each injection was 11.6 min.

**Thickness and Weight**

The average thickness and weight measurements for the moving and still robotic hand experiments before and after permeation are reported in Table 1. Lavender gloves in both moving and still hand permeation experiments showed the highest changes in weight and thickness before and after permeation compared to the other gloves. The average lavender glove weights before and just after exposure to limonene in moving hand module were 3.17±0.02 g (mean ± standard deviation) and 4.11±0.20 g, respectively. This reflected a significant weight gain (P<0.05). The respective numbers in static robotic hand experiment on lavender gloves were 3.17±0.02 g and 4.20±0.40 g.

The average post-permeation thicknesses (before reconditioning) for all glove materials in dynamic and static experiments were more than 10% of the pre-permeation ones but less than 25%. After reconditioning, the thickness for each glove was not statistically different from the original thickness.

The average thickness of the lavender glove specimens at the palm area before and after challenging (before reconditioning) in moving hand module was 59±3 μm and 73±3 μm, respectively (P<0.05). The respective numbers in static robotic hand experiment on lavender gloves were 58±2 μm and 72±4 μm.

Initial observations indicated swelling and discoloration after the permeation test on limonene. The discoloration was most vivid in the purple gloves. Post-permeation thickness measurements (before reconditioning) on all four glove types in the moving and still robotic hand experiments revealed a significant difference in thicknesses (P<0.05). This is due to the accumulation of the low-volatile solvent (limonene) within the glove. However, the swelling was reversible after reconditioning. The post-
permeation measurements on blue, purple, sterling, and lavender gloves after reconditioning from dynamic and static whole glove experiments indicated no significant differences in glove thickness.

**Permeation Results**

Table 2 and 3 present permeation parameters of limonene through blue, purple, sterling, and lavender disposable nitrile whole gloves for moving and still hand conditions, respectively. All glove specimens (blue, purple, sterling, and lavender) showed similar standardized breakthrough times (SBRT) and steady-state permeation periods (SSPP) whether the hand moved or not. However, the steady-state permeation rate (SSPR) and the diffusion coefficient ($D_1$) differed for all gloves relative to hand movement.

Blue gloves in both moving and still hand experiments showed an average standardized breakthrough time of 30 ±10 min (mean ± standard deviation). The earliest SBRT was observed in lavender gloves at 5±5 min. The highest and lowest SSPP was observed in blue and lavender gloves at 40-80 min and 10-40 min, respectively.

Lavender gloves represented the highest steady-state permeation rate values as opposed to blue ones with the lowest rates. The average calculated SSPR in moving and still hand lavender gloves was 0.490±0.029 μg/cm²/min and 0.423±0.031 μg/cm²/min, respectively (significant at $P≤0.05$). The corresponding values for blue gloves were 0.012±0.001 μg/cm²/min for moving hand and 0.010±0.001 μg/cm²/min for static hand.

Infrared reflectance analysis for both moving and still hand blue gloves showed a stronger absorbance on the outer surface of the gloves with the C-H bend at 888 cm⁻¹ and the C-H stretch at 2,800-3,000 cm⁻¹, which is a good

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**Table 1.** Average Thicknesses and Weights for Blue, Purple, Sterling, and Lavender Gloves for the Moving and Still Robotic Hand Before and Just After Permeation (M: moving, S: still). Values are representing arithmetic mean (µ) ± standard deviation (SD).

| Glove Material | Thickness before challenging (µm) | Thickness after challenging (µm) | Weight before challenging (g) | Weight after challenging (g) |
|----------------|----------------------------------|---------------------------------|------------------------------|-----------------------------|
| Blue M         | 121 ± 3                          | 135 ± 2                         | 6.66 ± 0.05                  | 7.02 ± 0.10                 |
| Purple M       | 106 ± 3                          | 124 ± 3                         | 5.96 ± 0.20                  | 6.84 ± 0.40                 |
| Sterling M     | 74 ± 4                           | 86 ± 5                          | 3.94 ± 0.01                  | 4.76 ± 0.06                 |
| Lavender M     | 59 ± 3                           | 73 ± 3                          | 3.17 ± 0.02                  | 4.11 ± 0.20                 |
| Blue S         | 122 ± 3                          | 136 ± 2                         | 6.56 ± 0.03                  | 7.07 ± 0.20                 |
| Purple S       | 105 ± 2                          | 122 ± 4                         | 5.94 ± 0.10                  | 6.71 ± 0.50                 |
| Sterling S     | 74 ± 3                           | 85 ± 5                          | 3.93 ± 0.05                  | 4.65 ± 0.15                 |
| Lavender S     | 58 ± 2                           | 72 ± 4                          | 3.17 ± 0.02                  | 4.20 ± 0.40                 |

**Table 2.** Permeation Parameters of Limonene through Blue, Purple, Sterling, and Lavender Disposable Nitrile Whole Gloves for the Moving Hand.

| Glove Material | Replicates | SBRT (min) | SSPR (µg/cm²/min) | $D_1×10^{-5}$ (cm²/min) | SSPP (min) |
|----------------|------------|------------|-------------------|-------------------------|------------|
| Blue           | 3          | 30 ± 10    | 0.012             | 9.9                     | 40-80      |
| Purple         | 3          | 15 ± 5     | 0.078             | 7.9                     | 20-60      |
| Sterling       | 3          | 15 ± 5     | 0.104             | 10.5                    | 20-60      |
| Lavender       | 3          | 5 ± 5      | 0.490             | 21                      | 10-40      |

**Table 3.** Permeation Parameters of Limonene through Blue, Purple, Sterling, and Lavender Disposable Nitrile Whole Gloves for the Still Hand.

| Glove Material | Replicates | SBRT (min) | SSPR (µg/cm²/min) | $D_1×10^{-5}$ (cm²/min) | SSPP (min) |
|----------------|------------|------------|-------------------|-------------------------|------------|
| Blue           | 3          | 30 ± 10    | 0.010             | 9.2                     | 40-80      |
| Purple         | 3          | 15 ± 5     | 0.067             | 11.05                   | 20-60      |
| Sterling       | 3          | 15 ± 5     | 0.100             | 6.36                    | 20-60      |
| Lavender       | 3          | 5 ± 5      | 0.423             | 18                      | 10-40      |
indicator for residual limonene on glove surfaces. However, such differences were not found on the inner surfaces of the gloves. In lavender gloves, the texture was more impacted by limonene, as the infrared reflectance analysis indicated more changes with an intense increase in absorbance on both outer and inner surface of the glove with the C-H bend at 888 cm\(^{-1}\) and the C-H stretch at 2,800-3,000 cm\(^{-1}\). After subtracting the absorbance of exposed glove from the unexposed, traces of limonene were observed on the outer and inner surfaces. The lavender infrared spectra also indicated an intense reduction in the absorbance on both outer and inner surfaces of the glove materials through the whole spectra. The infrared spectra for blue and lavender gloves before and after permeation are presented in Fig. 2 and 3, respectively.

**Discussion**

The weight measurements on moving hand glove samples revealed an increasing trend. All blue, purple, sterling, and lavender glove samples in the still hand permeation experiments showed a statistically significant weight gain before reconditioning \((P<0.05)\). The weight gain for blue gloves was less than 10%. This was due to less swelling and changes in thickness in blue gloves compared to all other glove samples. However, the weight gain for rest of the glove samples still hand experiments was more than 10%. The weight measurement results were not surprising for a low-volatile solvent that is difficult to evaporate, and the weight data indicated limonene absorption and retention within the glove material even on reconditioning.

Thickness measurements on blue, sterling, and lavender gloves indicated a reverse trend between glove thickness and swelling in both whole glove moving and still hand experiences. This suggests that as the glove thickness increases, swelling moved in reverse trend. However, such trend was not observed in purple gloves.

A general overview of the SBRTs with the moving and static robotic hands (Table 2 and 3, respectively) indicated similar sensitivity in detecting the analyte during permeation. This was confirmed by the respective SBRTs and SSPPs for the static and dynamic robot hands. As can be seen, in all still and moving hand permeation experiments, the SSPPs were located in the same time ranges. This suggests that glove materials behaved similarly during the permeation process whether moving or non-moving.

Interestingly, the SSPR for the moving hand with the lavender glove (0.490 μg/cm\(^2\)/min) was significantly higher than for the still hand (0.423 μg/cm\(^2\)/min) in spite of the area of exposure being identical \((P<0.05)\). In contrast, the respective SSPRs for blue, purple, and sterling gloves were not significantly different \((P<0.05)\). For limonene, the lavender glove was just too thin and this was suggestive of a thickness threshold. Since the acrylonitrile content (percentage) for lavender gloves was similar to the sterling gloves, this parameter cannot explain the results. Moreover, the lavender glove infrared spectra indicated intense reductions in absorbance for the entire
wavelength range for both the outer and inner surfaces of the glove, which are signs of degradation. Such level of reduction was not observed in other glove specimens. Gloves with less thickness represented higher absorbance differential with higher permeation rates. This was probably due to loss of the coating on the challenge side of the glove during direct contact with limonene that was magnified by movement to cause increased permeation rate by movement.

Both moving and static hand results showed that the blue gloves provided the best performance with similar SBRTs. Kimberly-Clark Professional reported that the sterling glove shows a normalized breakthrough time of 105 min using the conventional permeation method (ASTM open-loop method) and classified it as excellent. In the present study on sterling gloves, the standardized breakthrough time for the moving and static hand was 15 ±5 min. This suggests that the moving and still robot hand models provided higher sensitivity and efficiency at least for limonene. Thus, whole glove permeation testing model represents a more realistic method for exposure to chemical at workplace comparing to ASTM model.

Mathews’s whole glove permeation study on cyclohexanol, revealed a faster SSPR in moving hand comparing to the still hand, although the difference was significant ($P<0.05$) only in sterling gloves. The same trend was observed in the present study for all glove samples, except that the SSPR was significantly faster ($P<0.05$) in lavender gloves with moving hand option.

The SBRTs and SSPPs for the static and dynamic robot hand models indicate that both methods have similar sensitivity in detecting the analyte during permeation, and the blue, purple, and sterling gloves behave similarly during the permeation process whether moving or non-moving. The SSPR for the moving robotic hand with the lavender glove was significantly higher than for the still hand ($P<0.05$) in spite of the area of exposure being identical. The lavender, sterling, and purple gloves should not be used as personal protective equipment for exposure to limonene, even for short period exposures. Although the blue gloves provided the best performance compared to the other gloves, they can only be used for no more than 20 minutes.

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Conflicts of interest: We have no conflict of interest regarding the manuscript to declare.

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