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Identification of Phenolic Dermal Sensitizers in a Wound Closure Tape

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A latex-allergic patient presented with a severe local reaction to a non-latex wound closure bandage following surgery. Extracts of the bandage were analyzed by gas chromatograph-electron impact-mass spectrometry (GC EI-MS) in the total ion monitoring mode. Components were identified by their ion mass fingerprint and elution time as a corresponding standard from the GC column. The chemicals identified were 4,4′-thiobis-(6-tert-butyl-m-cresol) (TBBC), 6-tert-Butyl-m-cresol (BC), 2,4-di-tert-butylphenol (BP) and erucamide (EA). Sensitization potential of these chemicals was evaluated using two quantitative structure-activity relationship (QSAR) programs. The phenol 2,6-di-tert-butyl-4-(hydroxymethyl)phenol (BHP) was also included in the test series. It was initially thought to be present in the bandage but detectable levels could not be confirmed. The potential for TBBC to induce a sensitization response was predicted by both Derek for Windows and TOPKAT 6.2. The potential for BC and BP to induce a sensitization response was predicted by Derek for Windows, but not TOPKAT. BHP and EA were not predicted to be sensitizers by either QSAR program. Local lymph node assay (LLNA) analysis of the chemicals identified TBBC, BP, and BC as potential sensitizers with EC3 values between 0.2 and 4.5%. None of the animals exhibited body weight loss or skin irritation at the concentrations tested. In agreement with the toxicological modeling, BHP did not induce a sensitization response in the LLNA. Following a positive LLNA response, TBBC, BP, and BC were further characterized by phenotypic analysis of the draining lymph nodes. A positive LLNA result coupled with a lack of increase in B220$^+$IgE$^+$ cell and serum IgE characterize these chemicals as Type IV sensitizers. These studies used a multidisciplinary approach combining clinical observation, GC-EI-MS for chemical identification, QSAR modeling of chemicals prior to animal testing, and the LLNA for determination of the sensitization potential of chemicals in a manufactured product.

Keywords dermal sensitizers, hypersensitivity, wound closures

INTRODUCTION

These studies were undertaken following the reaction of a patient to a latex-free wound closure bandage. Within 36 hr after surgery, significant swelling and discharge was present at the surgical sites. Removal of the wound closure bandages revealed a large area of erythema and pustular dermatitis, associated with discomfort and swelling, wherever there had been contact with the steri-strips. The patient's reaction to the latex-free wound closure bandage suggests that a chemical component present in the bandage was responsible for the sensitization. These studies attempted to identify the responsible agent.

Sensitization to chemical compounds is of public health importance. It can result in increased costs to both employers and workers due to time off work and decreased productivity (Phillips et al., 1999; Horwitz et al., 2001); therefore, it is important to identify chemicals that may induce a sensitization response. This can be a difficult task for products that contain complex mixtures not identified in the ingredients. Although mixtures can be tested in accepted skin sensitizer models, not all responsible agents can be identified unless all ingredients are known. To properly identify the active sensitizing chemicals in manufactured products, it is necessary to identify the components.

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Initial concerns on the sensitization from a wound closure bandage surfaced from a report regarding a latex-allergic patient. The patient exhibited a severe local reaction to a non-latex wound closure tape following surgery. Other studies of sensitization resulting from bandage use revealed an increase in allergic responses associated with several types of bandages. For example, ointment bases (Gallenkemper et al., 1998), phenoethanol (Gallo et al., 2003), and benzoyl peroxide (Greiner et al., 1999) present in bandages have been reported to cause allergic contact dermatitis in humans. Skin occlusion from the bandage may lead to increased sensitization (Zhai and Maibach, 2001).

These studies involved isolation of identified chemical components of the wound closure bandage in question, and screening for their sensitization potential using an animal model. Several phenolic compounds were identified and predicted to be sensitizers using two commercially available QSAR software programs. The sensitization potential of these phenolic compounds was confirmed in the LLNA. The process described in this manuscript provides a method for the detection and testing of potential sensitizers from a manufactured product.

MATERIALS AND METHODS

Case History

Following diagnosis of invasive carcinoma by biopsy of the right breast, a 48-year-old female physician with known latex allergy presented to surgery for excisional lumpectomy and sentinel node biopsy. The surgical sites on the breast and right axillae were dressed post operatively with non-latex steri-strips. The patient was treated in a latex-safe operatory.

Within 36 hr post-operation, she began to complain of pruritis and obvious swelling and discharge from the surgical sites. She called her surgeon and was instructed to remove the steri-strips. Removal of the steri-strips revealed a large area of erythema and pustular dermatitis wherever there had been contact with the steri-strips. This was associated with marked discomfort and swelling. Although the surgeon elected to treat this initially as a cellulitis with a broad spectrum cephalexin, the patient's condition continued to deteriorate over the next 48 hr with confluence of the pustular area, blistering and weeping of the blisters. There was persistence of the marked swelling and discomfort. The patient remained afebrile, although she did complain of shaking chills. Antibiotics were discontinued and she was started on a topical steroid cream, which did not alleviate symptoms. She was then started on 50 mg of prednisone burst and improved within 48 hr of initiating of oral prednisone.

Animals

Female BALB/c mice, 8–12 weeks old, were purchased from Taconic (Hudson, NY). Mice were quarantined in the NIOSH Animal Facility for 1 week upon arrival and maintained under conditions specified by NIH guidelines. Animals were fed a modified NIH-31 6% irradiated rodent diet (Harlan Teklad #7913) and provided tap water *ad libitum*. Animal facilities were maintained between 18 and 26°C and 25–70% relative humidity with light–dark cycles at 12-hr intervals (6:00–18:00). Cages were cleaned and sanitized weekly. The NIOSH Animal Facility is an environmentally controlled barrier facility fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International. Mice were weighed, tail-marked for identification, and assigned to homogeneous weight groups (n = 5) before each experiment.

Chemicals

4,4′-Thiobis (6-tert-butyl-m-cresol) (CAS 96-69-5), 6-tert-butyl-m-cresol (CAS 88-60-8), 2,4-di-tert-butylphenol (CAS 96-76-4), 2,6-di-tert-butyl-4-(hydroxymethyl) phenol (CAS 88-26-6), toluene 2,4-diisocyanate (CAS 584-84-9), alpha-hexylcinnamaldehyde (CAS 101-86-0), and acetone (CAS 67-64-1) were purchased from Sigma Chemical Company (St. Louis, MO). All chemicals were prepared in acetone at concentrations presented in Table 1.

Gas Chromatograph-Electron Impact-Mass Spectrometry (GC-EI-MS) Analysis

Compounds were assessed by GCMS-full scanning mode, using a 5972 series GCMS, (Agilent Technologies, Palo Alto, CA). Electron impact (EI, 70eV) MS was used to analyze samples, standards, and blanks. Data acquisition and processing was performed within the ChemStation software suite (Agilent Technologies, Palo Alto, CA). The GC column was an HP-5MS

TABLE 1
Concentration of chemicals used in the irritancy, LLNA, phenotypic analysis for IgE/B220, and serum IgE studies

Chemical	Acronym	Concentrations tested
4,4′-Thiobis(6-tert-butyl-m-cresol)	TBBC	0.1, 0.5, 1.0, 5.0, 10.0, 12.5, 25, 50 (% w/v)
6-tert-Butyl-m-cresol	BC	1, 3.1, 5, 6.3, 10, 12.5, 25, 50 (% v/v)
2,4-di-tert-butylphenol	BP	1.0, 5.0, 10.0, 12.5, 25, 50 (% w/v)
2,6-Di-tert-butyl-4-(hydroxymethyl)phenol	BMP	12.5, 25, 50 (% w/v)
a-hexylcinnamaldehyde	HCA (Positive control)	30.0 (% v/v)
toluene 2,4-diisocyanate	TDI (Positive control)	2.5 (% w/v)
Acetone	VH (Vehicle)	100 (% v/v)

(30 m× 0.25 mm, 0.25 μm film thickness) fused silica capillary (J&W Scientific, Folsom, CA). Analytes were eluted using ultra high purity (UHP) helium as the carrier gas at 1 ml/min. Following the 1 μl, splitless injection, the GC oven was held at 50°C for 2 min; the temperature was then increased by 5°C/min to a final temperature of 310°C and held for 1 min (for a total run time of 55 min).

Structural Activity Relationship Modeling

Two commercial software packages, TOPKAT 6.2 (Accelrys, Inc., San Diego, CA) and Derek for Windows version 9.0.0 (Lhasa Limited, Leeds, UK) were used to estimate the skin sensitization potential of tested chemicals.

Range Finding and Toxicological Studies

Range finding studies were performed to select the concentration of chemicals to be used for sensitization studies. For these studies mice (three per group) were dosed with acetone vehicle (VH), 12.5%, 25%, and 50% of the test article(s) on the dorsal surface of each ear (25 μl per ear) for three consecutive days. Animals were allowed to rest for 2 d following the last exposure and then weighed and examined for signs of toxicity including loss of body weight and ruffled fur. At the end of the study, mice were sacrificed by CO_2 asphyxiation. The highest soluble concentrations were selected for these studies in an attempt to identify toxicity. For the subsequent studies, maximum concentrations were selected that were soluble in the vehicle and did not cause toxicity (NIEHS, 1999).

Irritancy Measurement

Evaluation of irritancy measurements was performed as previously described (Woolhiser et al., 1998). Briefly, before the first chemical administration, the thickness of the right and left pinnae of each mouse was measured using a modified engineer's micrometer (Mitutoyo Co., Japan). Mice were exposed to 25 μl of VH or test article for three consecutive days. Ear thickness measurements were taken 24 hr following the final exposure. The mean percentage of ear swelling was calculated based on the following equation: [(mean post-challenge ear thickness − mean pre-challenge ear thickness)/mean pre-challenge thickness] × 100. For these studies TBBC was tested at a range of 0.1–10%, BC was tested at a range of 3.1–12.5%, BP was tested at a range of 1–10% and BHP was tested at a range of 12.5–50%.

Local Lymph Node Assay

The local lymph node assay (LLNA) was performed following the method described in the ICCVAM Peer Review Panel report (NIEHS, 1999) with minor modifications. Briefly, mice (five per group) were exposed topically with VH, increasing concentrations of test article(s), or positive control (30% HCA) on the dorsal surface of each ear (25 μl per ear) for three consecutive days (Table 1). Animals were allowed to rest for 2 d following the last exposure. On Day 6, mice were injected intravenously via the lateral tail vein with 20 μCi [^{3}H]-thymidine (Dupont NEN; specific activity 2 Ci/mmol). Five hours after [^{3}H]-thymidine injection, animals were euthanized via CO_2 inhalation, and the left and right cervical draining lymph nodes (DLNs) located at the bifurcation of the jugular vein were excised and pooled for each animal.

Single cell suspensions were made and following overnight incubation in 5% trichloroacetic acid (TCA), samples were counted using a Packard Tri-Carb 2500TR liquid scintillation analyzer. Stimulation indices (SI) were calculated by dividing the mean disintegrations per minute (DPM) per test group by the mean DPM for the VH control group. EC3 values (concentration of chemical required to induce a 3-fold increase over the VH control) were calculated based on the equation from Basketter and colleagues (Dearman et al., 1999). For the LLNA the chemicals were tested at the following concentrations, TBBC (0.1–10%), BC (3.1–12.5%), BP (1–10%) and BHP (12.5–50%).

Phenotypic Analysis

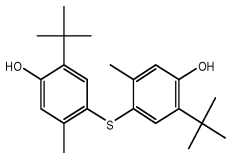
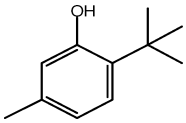
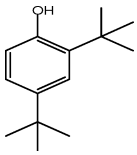
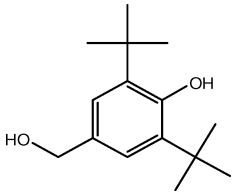
To determine if the chemicals were likely T-cell-mediated (Type IV) or IgE-mediated hypersensitivity (Type I) chemical sensitizers, after dermal exposure to the compounds the draining lymph nodes were analyzed for IgE^+B220^+ cells using flow cytometry as described by Manetz and Meade (1999). Mice (five per group) were exposed to VH or increasing concentrations of test article(s) topically on the dorsal surface of each ear (25 μl per ear) for four consecutive days (Table 1). Animals were allowed to rest for 6 days after the final exposure and then euthanized by CO_2 inhalation on Day 10. At the time of euthanization, blood was drawn by cardiac puncture for evaluation of total serum IgE. Draining lymph nodes were collected (two nodes/animal/tube) in 2 ml PBS (phosphate-buffered saline) and were dissociated between the frosted ends of two microscope slides. Cell counts were performed using a Coulter Counter (Z2 model, Beckman Coulter, Fullerton, CA), and 1×10^6 cells per sample were added to the wells of a 96-well plate.

Cells were washed using staining buffer (1% bovine serum albumin/0.1% sodium azide in PBS) and then incubated with F_c block (clone 2.4G2). The cells were then incubated with anti-CD45RA/B220 (PE, clone RA3-6B2) and anti-IgE antibodies (FITC, clone R-35-72) or the appropriate isotype controls, diluted in staining buffer, washed, and incubated with propidium iodide (PI). All antibodies and isotype controls were purchased from BD Pharmingen (San Jose, CA). After a final wash, cells were resuspended in staining buffer and analyzed with a Becton Dickinson FACSVantage flow cytometer gating on the non-PI staining lymphocyte population. For the phenotyping studies the chemicals were administered at the following concentrations: TBBC (0.1%, 0.5%, and 1.0%), BC (3.1%, 6.2%, and 12.5%) and BP (1%, 5%, and 10%).

Total Serum IgE

Blood samples were collected via cardiac puncture from the animals used in the phenotypic assays. Sera were collected,

TABLE 2
Experimental EC3 values and predicted skin sensitization activity

Chemical name	Chemical structure	ECS	TOPKAT Activity	TOPKAT Severity	DEREK for windows
TBBC		0.23	+	Non-Severe	+
BC		4.50	−	Inactive	+
BP		3.34	−	Inactive	+
BMP			−	Inactive	−
EA			−	Inactive	−

separated by centrifugation and frozen at −20°C for later analysis (within 2 weeks) of IgE by ELISA. All antibodies were purchased from BD Pharmingen (San Jose, CA). In brief, 96-well flat bottom plates (Dynatec Immulon-2) were coated with (2 μg/ml in PBS) purified monoclonal rat anti-mouse IgE antibody (clone R35-72), sealed with plate sealers, and incubated overnight at 4°C. The plates were washed three times with PBS/Tween 20 and then blocked for 1 hr with 2% Newborn Calf Serum (NCS) and 0.05% sodium azide at room temperature. Initial dilutions (1:10) were made from the serum samples and IgE control standards were prepared at 500 ng/ml. All dilutions were made in 2% NCS and 0.05% sodium azide. Serum samples and IgE control standards (mouse IgE anti-TNP, clone C38-2) were serially diluted (1:2), added to the coated plates in a 100 μl volume and incubated at room temperature for 1 hr.

The plates were washed three times with PBS/Tween 20, biotin-conjugated rat anti-mouse IgE (clone R35–92) was added in a 100 μl volume and samples were incubated at room temperature for 1 hr. The plates were washed 3 times with PBS/Tween 20, streptavidin-alkaline phosphatase (Pharmingen Cat# 554065) was added (100 μl of a 1:400 dilution) and plates were incubated for 1 hr at room temperature. p-Nitrophenyl phosphate (Sigma Cat# N-9389) was used as the alkaline phosphatase substrate and added to the plates in a 100 μl volume. The plates were allowed to develop at room temperature for up to 30 min or until the OD reading of the highest standard reached 3.0.

Absorbance was determined using a Spectramax Vmax plate reader (Molecular Devices, Sunnyvale, CA) at 405–605 nm. Data analysis was performed using the IBM Softmax Pro 3.1 (Molecular Devices), and the IgE concentrations for each sample were interpolated from a standard curve using multipoint analysis. Serum IgE was evaluated after dermal exposure to the chemicals at the following concentrations: TBBC (0.1%, 0.5%, and 1.0%), BC (3.1%, 6.2%, and 12.5%) and BP (1%, 5%, and 10%).

Statistical Analysis

Statistical analysis was performed using Graph Pad Prism version 3.0 (San Diego, CA). All data were analyzed by a one-way analysis of variance (ANOVA). In the ANOVA, when significant differences were detected ($p = 0.05$), Dunnett's test was used to compare treatment groups with the appropriate control group. Statistical significance is designated by $^{*}p < 0.05$ and $^{**}p < 0.01$.

RESULTS

Identification of the Components of a Wound Closure Bandage

In order to identify and characterize the chemical components in the bandage, a sample was obtained from the same lot of the wound closure bandages, which caused the response in the initial report of adverse reaction. The wound closure tape was extracted in acetonitrile (10 ml/gram) and analyzed by GC-EI-MS in the total ion monitoring mode. Components were identified by their ion mass fingerprints and elution from the GC column at the same time as the respective standard chemical. Three phenolic compounds were identified: 4,4'-thiobis(6-tert-butyl-3-cresol) (TBBC), 6-tert-butyl-m-cresol (BC), and 2,4-di-tert-butylphenol (BP). The phenol 2,6-di-tert-butyl-4-(hydroxymethyl)phenol (BHP) was initially thought to be present in the bandage but detectable levels could not be confirmed. This compound was still included in the LLNA testing for QSAR modelingvalidation purposes. One aliphatic compound, erucamide (EA), was also isolated from the bandage. The relative concentrations of TBBC and BC were 4.27 and 1.65 μg/cm^2, respectively. BP was detected, but not quantified. EA, the aliphatic compound which was isolated, had a relative concentration of 0.34 μg/cm^2.

Toxicological Modeling of Extracted Chemicals

TBBC, BC, BP, BHP, and EA were evaluated for sensitization potential using two software packages, TOPKAT and Derek for Windows (Table 2). The potential for TBBC to induce a sensitization response was predicted by both Derek for Windows and TOPKAT 6.2. The potential for BC and BP to induce sensitization was predicted by Derek for Windows but was not predicted to be a sensitizer by TOPKAT. Neither program predicted BHP or EA as potential sensitizers.

Evaluation of Toxicity

TBBC, BC, BP, and BHP were evaluated for toxicity using a 6-day range finding study. BC caused >10% body weight loss as well as overt signs of toxicity at concentrations of 25% and 50%. Therefore, for further studies, concentrations of BC were 12.5% or lower. At the concentrations tested, no overt toxicity or body

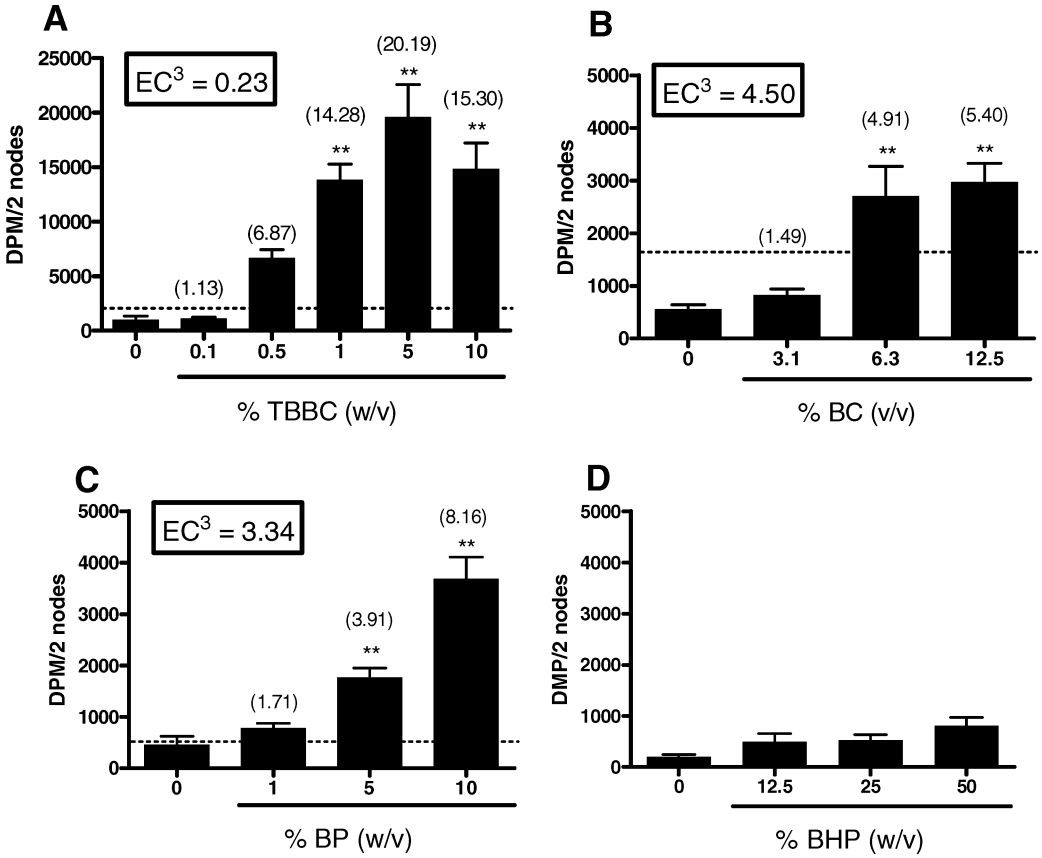


FIG. 1. Sensitization Potential after Dermal Exposure to TBBC, BC, BP, or BHP. Analysis of the sensitization potential of TBBC (A), BC (B), BP (C), or BHP (D) using the LLNA. [^{3}H]-Thymidine incorporation into draining lymph node cells of BALB/c mice following exposure to vehicle or test article shown above. Bars represent means ± SE of 5 mice per group. Numbers appearing above the bars represent the stimulation indices for each concentration tested. Levels of statistical significance are denoted as $^*p < 0.05$ or $^{**}p < 0.01$ as compared to VH.

TABLE 3
LLNA/Phenotypic analysis and total IgE dose response studies with TBBC, BC, and BP

Dose Group	DPM	% IgE+B220+	%B220+	Total IgE (ng/ml)
VH	968 ± 385	1.4 ±0.6	17.6 ±0.4	611 ±125
TBBC				
0.1%	1101 ±132	5.8 ±2.2	22.7 ±3.0	593 ± 228
0.5%	6660 ± 777	7.6 ±3.0	26.2 ±1.5**	895 ± 152
1%	13829 ±1477**	8.2 ± 3.7	29.3 ±1.0**	2214 ±1008
VH	549 ± 95	1.4 ±0.6	17.6 ±0.4	611 ±125
BC				
3.1%	820 ±118	5.2 ± 0.7	17.4 ±1.0	896 ±156
6.2%	2696 ± 574**	12.9 ±3.4	26.0 ±0.5**	1385 ±180
12.5%	2962 ± 367**	14.6 ±3.8	28.3 ±1.1**	784 ±121
VH	451 ± 167	1.1 ±0.1	18.5 ±0.9	650 ± 67
BP				
1%	772 ± 103	1.0 ±0.3	20.8 ± 0.7	483 ± 121
5%	1762 ±186**	3.6 ±2.4	24.1 ±1.3**	1278 ±139
10%	3682 ±430**	3.4 ±1.4	28.4 ±1.4**	781 ±78

Values represent the ± SE derived from a mean of 5.
*Significantly different from VH controls, $p < 0.05$.
**Significantly different from VH controls, $p < 0.01$.

weight loss was reported for any of the other test articles (data not shown). EA was not evaluated in range finding or additional studies due to limits of solubility in acceptable LLNA vehicles.

Irritancy and Evaluation of Sensitization Potential

QSAR modeling has not been accepted as a replacement for validated animal models for analyzing sensitization potential, therefore TBBC, BC, BP, and BHP were evaluated for sensitization potential using the local lymph node assay (LLNA). Exposure to TBBC, BC, and BP evoked a positive LLNA responses with EC3 values calculated at 0.2, 4.5, and 3.3% respectively (Figure 1A-C). Confirming the QSAR prediction, BHP did not have a positive LLNA response at any concentration tested (Figure 1D). None of the chemicals tested induced a significant irritancy response as demonstrated by the absence of an increase in ear swelling 24 hr post-challenge (data not shown).

Lymph Node Phenotyping Analysis and Total Serum IgE Levels

Mice were dosed with TBBC, BC, and BP at increasing concentrations to evaluate the ability of the chemical sensitizers to induce an IgE response. Elevated serum IgE is commonly used as a marker of type I hypersensitivity responses. None of the phenols tested caused an increase in serum IgE (Table 3). To further exclude the chemicals as Type 1 sensitizers, the draining lymph nodes were analyzed for the load production of IgE by quantification of IgE^+B220^+ cells. TBBC, BC and BP all showed increases in $B220^+$ cells but not in IgE^+B220^+ (Table 3). TDI (2.5%) used as a positive control for these experiments resulted in significant elevations of IgE^+B220^+ (27%) and $B220^+$ (41%) cell populations. The increase in lymph node cellularity without an increase in serum IgE or $B220^+IgE^+$ suggests that these compounds are type IV (T-cell mediated) sensitizers.

DISCUSSION

The primary purpose of these studies was to identify and test potential chemical sensitizers isolated from a non-latex bandage found to cause a contact dermatitis reaction in a patient. In order to meet these goals, a multidisciplinary approach including chemical analysis and characterization by mass spectrophotometry, screening by toxicological computer modeling, and sensitization testing using a validated animal model was employed. Since bandages consist of an unknown mixture of chemicals, components of the tape strip bandage were isolated and evaluated by GC-EI-MS. The screening by GC-EI-MS identified several phenolic compounds that were the likely sensitizers in the tape strip bandage. The sensitization potential of these phenolic compounds was predicted by QSAR modeling and confirmed using the LLNA.

Computer modeling correctly predicted three compounds TBBC, BC, and BP as sensitizers in these studies while confirming BHP as a non-sensitizer. Although at this time QSAR modeling cannot completely replace animal studies, utilizing software packages in concert may allow for a better prediction of sensitization potential and decrease the number of animals needed for these types of studies. Goals set by both the ICCVAM (US) as well as the ECVAM (EU) for decreasing animal use in lieu of alternative methods could be furthered by utilizing part if not all of the proposed multidisciplinary methodology.

Animal studies cannot be removed from the methodology until predictions by QSAR programs increase in their reliability. There is a potential structural explanation for why the phenolic compounds examined in these studies are sensitizers. TBBC, BC and BP are phenols that can be easily oxidized in ortho or para positions by cytochrome P450. Phenolic compounds that have an OH group(s) in the ortho and/or para position are known skin sensitizers as they can be further oxidized to 1,2- or 1,4-quinones which subsequently form covalent bonds with proteins via nucleophilic Michael-type addition.

Quinones, well known skin sensitizers, include 1,4-benzoquinone (EC3 value of 0.01) whose plant derivative 3-n-alkylcatechol is contained in both urushiol (sensitizing oil in poison ivy), and gallates (Baer et al., 1967; Hausen and Beyer, 1992; Hausen et al., 1995). Both urushiol and gallates can be further metabolized to 1,2-quinone. TBBC, which already has hydroquinone-like structures with sulfur in the para position, could be considered a more potent sensitizer as both aromatic rings are more active and thus more susceptible to any oxidation. However, the fourth phenolic compound BHP is not a skin sensitizing agent. This could be explained by the substitution of all its ortho and para positions, which prevent any further oxidation that may lead to quinine like structures. In addition, the bulky t-butyl electron donor groups in both ortho positions could also hinder formation of a carbon active center on the phenyl ring.

Similarly structured phenolic compounds exist in rubber, latex, and acrylic bandages and have been implicated in dermal sensitization. Of the non-latex bandages, irritation is the most commonly reported symptom, however some people report contact dermatitis. For example, occupational exposure to phenol-resin in athletic tape has been linked to sensitization (Shono et al., 1991). Most reports indicate that colophony (pine resin) and colophony derivatives are involved in dermal sensitization by bandages but recent reports have shown that colophony-phenol-formaldehyde compounds may also be responsible (Sjoborg and Fregert, 1984; Hindson and Sinclair, 1988; Norris and Storrs, 1990; Shono et al., 1991; Daecke et al., 1993; Burden et al., 1994; Sasseville et al., 1997; Greiner et al., 1999). It is possible that the addition of the phenols could increase the sensitization to the resin compounds.

In the present study, the patient who reacted to the steristrips had skin lesions within 36 hr of exposure suggesting prior exposure to the sensitizing chemicals. There is potential for human exposure to phenols. Phenols are common antioxidants that are used in latex applications (Rich et al., 1991) and have also been reported to be present in non-latex (nitrile) gloves (Mutsuga et al., 2002). Phenols are also used in the production of polyethylene food containers (Scott, 1988; NTP, 1994; Kawamura et al., 1997; Marque et al., 1998; Skjevrak et al., 2005). Occupational as well as consumer exposure to phenolic antioxidants can occur both during manufacturing and product use.

The three identified sensitizers TBBC, BC, and BP are considered high production volume chemicals with production exceeding 1 million pounds per year (EPA, 1990). TBBC is considered more hazardous than most chemicals by the Environmental Protection Agency (EPA) and is used in at least four industries including petroleum, plastics, reinforced plastics and rubber. Its main use in these industries is as a primary antioxidant or stabilizer. TBBC is also used as a stabilizer for polyethylene in the production of food containers (NTP, 1994), which presents exposure potential of workers in the manufacturing process and the consumers who use the products.

The National Toxicology Program (NTP) has identified TBBC as a gastrointestinal or liver toxicant, an immunotoxicant, and a sensitizer (NTP, 1994). Consistent with the results described in this manuscript, TBBC has been identified as a sensitizer in occupational settings with a slightly positive result in human patch test experiments as early as the 1950s (Mallette and Von Haam, 1952). Latex studies have also identified TBBC as a possible sensitizer in gloves (Rich et al., 1991). Further, TBBC had also been shown to increase lymphocyte cell counts after oral ingestion (Munson et al., 1988).

Information regarding the health hazards, industrial, and consumer use of the two other phenolic compounds, BC and BP, is lacking. BP has been ranked by the EPA as more hazardous than most chemicals. It was identified to cause vitiligo or cutaneous depigmentation among employees who frequently handle rubber components required for the manufacturing of hydraulic pumps (O'Malley et al., 1988) and has thus been implicated in dermal sensitization.

In summary, this paper presents a method that can be used to identify potential sensitizers in manufactured products as well as mixtures where the ingredients are unknown that may have an effect of human health. The three phenolic compounds TBBC, BC, and BP, which were isolated from a wound closure bandage and tested in these studies were identified as potential Type IV sensitizers. Due to their high production volume and many sources, all three chemicals present the potential for both occupational and public exposure.

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