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Evaluation of the Contact and Respiratory Sensitization Potential of Volatile Organic Compounds Generated by Simulated Indoor Air Chemistry

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Up to 60 million people working indoors experience symptoms such as eye, nose and throat irritation, headache, and fatigue. Investigations into these complaints have ascribed the effects to volatile organic compounds (VOCs) emitted from building materials, cleaning formulations, or other consumer products. New compounds can result when the VOCs react with hydroxyl or nitrate radicals or ozone present in indoor environments. Several oxygenated organic compounds, such as glyoxal, methylglyoxal, glycolaldehyde, and diacetyl, have been identified as possible reaction products of indoor environment chemistry. Although research has previously identified diacetyl and glyoxal as sensitizers, additional experiments were conducted in these studies to further classify their sensitization potential. Sensitization potential of these four compounds was assessed using quantitative structure-activity relationship (QSAR) programs. Derek for Windows and National Institute for Occupational Safety and Health logistic regression predicted all compounds to be sensitizers, while TOPKAT 6.2 predicted all compounds except for methylglyoxal. All compounds were tested in a combined irritancy and local lymph node assay (LLNA). All compounds except for glyoxal were found to be irritants and all tested positive in the LLNA with EC3 values ranging from 0.42 to 1.9%. Methylglyoxal significantly increased both the $B220^+$ and IgE^+B220^+ cell populations in the draining lymph nodes and total serum IgE levels. The four compounds generated by indoor air chemistry were predicted by QSAR and animal modeling to be sensitizers, with the potential for methylglyoxal to induce IgE. The identification of these compounds as sensitizers may help to explain some of the health effects associated with indoor air complaints.

Key Words: **respiratory sensitizers; hypersensitivity; LLNA; indoor air chemistry.**

Of the 89 million people in the United States working in indoor office environments, between 35 and 60 million have one or more weekly building-related symptoms such as eye, nose and throat irritation, headache, and fatigue (Mendell *et al.*, 2002). The estimated costs due to illness or performance losses range from $20 to 70 billion annually (Mendell *et al.*, 2002). Investigators, searching for specific causes of these increasing complaints, have ascribed the effects to both biological (for example, fungi or endotoxin) and chemical (volatile organic compounds [VOCs]) exposures (Brightman and Moss, 2000). Thus, research in exposure to indoor gas-phase chemistry is being conducted to describe indoor work environments.

Studies exposing animals to products of chemical reactions such as ozone mixed with limonene demonstrated that the reaction products had a significant impact on the breathing rate of the exposed animals when compared to the animals exposed to the reactants separately (Rohr *et al.*, 2003; Wilkins *et al.*, 2001). Exposure of human lung cells to oxidized atmospheric environments caused an increase in interleukin-8 mRNA, which is associated with an enhanced inflammatory response (Sexton *et al.*, 2004). Epidemiological studies have also found workers exposed to diacetyl (an oxygenated organic compound) to have twice the expected rates of physician-diagnosed asthma (Kreiss *et al.*, 2002; Mendell *et al.*, 2002). One possible connection between reaction products and health effects is that oxygenated organic compounds reduce the release of an epithelium-derived relaxing factor which can lead to a damaged and thus more susceptible epithelium (Fedan *et al.*, 2004). At the cellular level, it has been shown that glyoxal (HC(=O)C(=O)H) is capable of inducing cellular damage and may also lead to the increased concentration of advanced glycation end products that may be toxic to the cell. In fact, the term ''carbonyl stress'' has been suggested for this type of cellular stress (Kasper *et al.*, 2000). The compounds investigated in these experiments, methylglyoxal, diacetyl, glyoxal, and glycolaldehyde (dimer), all have the structural similarity of being dicarbonyls. A recently developed asthma hazard assessment tool based on chemical structure concluded that chemicals with bifunctional reactivity (for instance, two carbonyl

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groups) were strongly associated with occupational asthma (Jarvis *et al.*, 2005).

Recent reformulation of many household cleaners to include more ''green'' and plant-derived compounds such as α and β-pinene, α-terpineol, citronellol, geraniol, and β-irisone is likely to cause increases in the concentrations of terpenes, terpene alcohols, and ethers in indoor office environments. Investigations of the gas-phase chemistry of the chemicals mentioned above have identified oxygenated organic compound reaction products such as the α,β-dicarbonyls glyoxal, diacetyl, methylglyoxal, and the simple sugar glycolaldehyde (Calogirou *et al.*, 1999; Fick *et al.*, 2003; Forester *et al.*, 2006; Hakola *et al.*, 1994; Ham *et al.*, 2006; Jaoui and Kamens, 2003; Wells, 2005; Yu *et al.*, 1998).

The results described above highlight that VOCs present in the indoor environment can be transformed into oxidized organic reaction products, and biological systems could be affected after exposure to these compounds. The focus of the present study is to further define the possible health effects by evaluating the sensitization potential of VOCs. Quantitative structure-activity relationship (QSAR) modeling and *in vivo* models were used to evaluate contact and respiratory sensitization potential of the four structurally similar compounds, methylglyoxal, diacetyl, glyoxal, and glycolaldehyde.

MATERIALS AND METHODS

Animals. Female BALB/c mice, 8–12 weeks old, were purchased from Taconic (Hudson, NY). Mice were quarantined for 1 week upon arrival and maintained under conditions specified by National Institutes of Health (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°C and 26°C and 25–70% relative humidity with light-dark cycles at 12-h intervals (Light: 6:00 A.M.–6:00 P.M.). Cages were cleaned and sanitized weekly. The National Institute for Occupational Safety and Health (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. Methylglyoxal (CAS 78-98-8), glyoxal (CAS 107-22-2), diacetyl also known as 2,3-butanedione (CAS 431-03-8), and glycolaldehyde dimer (CAS 23147-58-2) were all purchased from Sigma Aldrich Chemical Company (St Louis, MO). Heating the glycolaldehyde dimer in solution (10 min at 65°C) was performed to produce the glycolaldehyde monomer (CAS 141-46-8) (Magneron *et al.*, 2005). Alpha-hexylcinnamaldehyde (HCA) (CAS 101-86-0) and toluene 2, 4-diisocyanate (TDI) (584-84-9) were purchased from Aldrich Chemical Company, Inc (Milwaukee, WI).

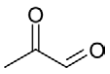
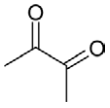
Structural activity relationship modeling. Two commercial software packages, TOPKAT 6.2 (Accelrys, Inc, San Diego, CA) and Derek for Windows version 9.0.0 (Lhasa Limited, Leeds, UK), together with the NIOSH logistic regression model (Fedorowicz *et al.*, 2005) were used to estimate the skin sensitization potential of tested chemicals. TOPKATs trinary classification scheme (negative [−], indeterminate [+/−] and positive [+]) was also applied to the NIOSH logistic regression model instead of the originally proposed binary one (Fedorowicz *et al.*, 2005). Predictions of indeterminate are near chance (0.5) for an assessment to be meaningful. To some extent, this category might be regarded as a transition between clearly defined sensitizer and nonsensitizer categories. After thorough consideration, the authors decided that chemicals in this category should be considered as potential skin sensitizers although with lowered significance. In the early stages of the hazard identification and the risk assessment process, it is very important to identify all potential hazardous materials; therefore, a pragmatic decision was made to use a conservative approach and classify indeterminates as possible skin sensitizers. Because glycolaldehyde exists in an equilibrium between monomer and dimer forms in solution, both were used to estimate the skin sensitization activity of this chemical. The skin permeation coefficients for tested chemicals were calculated using the Frasch model of skin permeation (Frasch, 2002) as implemented in the online skin permeation calculator (http://www.cdc.gov/niosh/topics/skin/skinPermCalc.html). The calculator is an online tool that implements three well-known models of skin permeation: the Potts&Guy, modified Robinson, and Frasch (Frasch, 2002; Potts and Guy, 1992; Wilschut *et al.*, 1995).

Range finding and toxicological studies. Range finding studies were performed to select the concentration of methylglyoxal and glycolaldehyde to be used for dermal exposures. Range finding studies were not conducted using diacetyl and glyoxal because their EC3 values had previously been published (Patlewicz *et al.*, 2002; Roberts *et al.*, 1999). Briefly, mice were exposed topically to a 4:1 acetone/olive oil mixture (AOO) and increasing concentrations of test articles on the dorsal surface of each ear (25 μl per ear) for three consecutive days. Animals were allowed to rest for 2 days following the last exposure and then weighed and examined for signs of toxicity including loss of body weight and ruffled fur. AOO is an accepted vehicle for the LLNA and was selected based on solubility of the compounds (NIEHS, 1999). The highest soluble concentrations were selected for these studies in an attempt to identify toxicity. For these studies, the chemicals were tested at the following concentrations, methylglyoxal (0.1–30%), glyoxal (0.35–1.5%), diacetyl (1.25–24%), and glycolaldehyde (0.8–30%). Maximum concentrations were selected for the subsequent studies that were soluble in the vehicle and did not cause toxicity (NIEHS, 1999). After the 6-day period, the mice were sacrificed by CO_2 asphyxiation, weighed, and examined for gross pathology. The following organs were removed, cleaned of connective tissue and weighed: liver, spleen, kidneys, and thymus.

Irritancy measurement. Irritancy measurements were 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). BALB/c mice were exposed to 25 μl of AOO or test article for 3 days. Ear thickness measurements were taken 24 h following the final exposure. The mean percentage of ear swelling was calculated based on the following equation: ([mean postchallenge ear thickness − mean prechallenge ear thickness]/mean prechallenge thickness) × 100. For these studies, the chemicals were tested at the following concentrations: methylglyoxal (0.1–1%), glyoxal (0.35–1.5%), diacetyl (1.25–5%), and glycolaldehyde (0.8–3.2%).

Local lymph node assay. The local lymph node assay (LLNA) was performed following the method described in the Interagency Coordinating Committee on the Validation of Alternative Methods Peer Review Panel report (NIEHS, 1999) with minor modifications. Briefly, mice were exposed topically to AOO, increasing concentrations of test articles, 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 days following the last exposure. On day 6, mice were injected iv via the lateral tail vein with 20 μCi ^{3}H-thymidine (Dupont NEN, Waltham, MA; 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% trichloracetic acid, samples were counted using a Packard Tri-Carb 2500TR liquid scintillation analyzer. Stimulation indices (SIs) were calculated by dividing the mean disintegrations per minute (DPM) per test group by the mean DPM for the vehicle control group. EC3 values (concentration of chemical

TABLE 1
Experimental EC3 Values and Predicted Skin Sensitization Activity

Chemical name	Chemical structure	EC3	TOPKAT (Binary) activity	TOPKAT (6.2) severity	Derek for Windows	NIOSH logistic regression
Methylglyoxal		0.42	−	Inactive	+	+
Glyoxal		0.74	+	Severe	+	+
Glycolaldehyde	 Monomer	1.8	−	Severe	+	+/−
Diacetyl		1.9	+/−	Severe	+	+/−

Note. +/− represents indeterminate results.

required to induce a threefold increase over the vehicle control) were calculated based on the equation from Basketter *et al.* (1999). For these studies, the chemicals were tested at the following concentrations: methylglyoxal (0.05–30%), glyoxal (0.35–1.5%), diacetyl (1.25–24%), and glycolaldehyde (0.8–3.2%).

Phenotypic analysis. To determine if the chemicals were likely T-cell–mediated (type IV) or IgE-mediated hypersensitivity (type I) chemical sensitizers, the DLNs were analyzed for IgE+B220+ cells after dermal exposure to the compounds. For the phenotyping analysis, methylglyoxal was tested at concentrations between 0.1 and 30%, glyoxal was administered at concentrations between 0.35 and 1.5%, diacetyl was administered at concentrations up to 24%, and glycolaldehyde was administered at concentrations between 0.8 and 3.2%. Lymph node cell phenotypes were analyzed using flow cytometry as described by Manetz and Meade (1999). Mice were exposed to AOO or increasing concentrations of test articles topically on the dorsal surface of each ear (25 µl per ear) for four consecutive days. Animals were allowed to rest for 6 days after the final exposure and then euthanized on day 10 by CO_2 inhalation. DLNs were collected (two nodes per animal per tube) in 2-ml phosphate-buffered saline (PBS) and were dissociated using 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 Fc 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 suspended in staining buffer and analyzed with a Becton Dickinson FACSVantage flow cytometer using PI viability gate.

Total serum IgE. Blood samples were collected via cardiac puncture from the animals used in the phenotypic assays. Sera were collected, 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. 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 h 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 standard (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 h. The plates were washed three times with PBS/Tween 20, and biotin-conjugated rat anti-mouse IgE (clone R35-92) was added in a 100-µl volume and incubated at room temperature for 1 h. The plates were washed three times with PBS/Tween 20, and streptavidin-alkaline phosphatase (Pharmingen Cat# 554065) was added (100 µl of a 1:400 dilution) and plates were incubated for 1 h 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 for up to 30 min at room temperature or until the optical density 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.

Statistical analysis. Statistical analysis was performed using Graph Pad Prism version 3.0 (San Diego, CA). All data were analyzed by a 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 * ($p \leq 0.05$) and ** ($p \leq 0.01$).

RESULTS

QSAR Modeling of Indoor Air Chemistry Reaction Products

Methylglyoxal, glyoxal, diacetyl, and glycolaldehyde were evaluated for sensitization potential using three QSAR programs: TOPKAT, Derek for Windows, and the NIOSH logistic regression model (Table 1). The potential for all the chemicals to induce a sensitization response was predicted by both Derek for Windows and the NIOSH logistic regression model. Skin sensitization potency was also predicted for all chemicals except methylglyoxal when TOPKAT 6.2 skin sensitization models were used for analysis. However, the TOPKAT binary (sensitizer/nonsensitizer) model only recognized glyoxal as a skin sensitizer. Glycolaldehyde and methylglyoxal were identified as inactive, and diacetyl was classified into an indeterminate category (+/−). Methylglyoxal was the only chemical identified as inactive by both TOPKAT models. Glycolaldehyde, although classified as inactive by the binary model, was identified as severe skin sensitizer by the TOPKAT 6.2 model. Because these two TOPKAT models are independent identities, it would be prudent in the hazard identification process to classify this chemical as a potential skin sensitizer. Both forms of glycolaldehyde were predicted to be sensitizers

by the TOPKAT binary model and Derek for Windows (data not shown). The NIOSH logistic regression model identified methylglyoxal and glyoxal as sensitizers, although both glycolaldehyde forms and diacetyl were labeled indeterminate. The expert system of Derek for Windows classified all chemicals as potential skin sensitizers. Methylglyoxal, glyoxal, and diacetyl were identified due to the presence of 1,2-dicarbonyl group –C(=O)C(=O)–. Glycolaldehyde was identified due to the presence of aldehyde group in the monomer or aldehyde precursor group in the dimer. The calculated skin permeation coefficients of these chemicals are identical (log Kp ~ − 4.86), suggesting that there should not be any difference in skin sensitization potency between these chemicals due to physical process of skin permeation.

Toxicity

Overt clinical toxicity was not observed after exposure to the highest concentrations of methylglyoxal, glyoxal, diacetyl, and glycolaldehyde (data not shown). Table 2 shows organ weights (as a percent of body weight) and the change in body weights after exposure of female BALB/c mice to methylglyoxal, glyoxal, diacetyl, or glycolaldehyde. No significant changes in body, spleen, thymus, kidney, and liver weights were observed after exposure to glyoxal, diacetyl, or glycolaldehyde for any of the treatment groups compared to the vehicle. Animals exposed to methylglyoxal (0.1 and 0.5%) did show a significant increase in spleen weight compared to vehicle after exposure. This is not considered biologically significant due to the low spleen weight recorded for the vehicle control group. This increase was not observed when this experiment was repeated (data not shown).

Irritancy as Indicated by Ear Swelling

Since both IgE- and T-cell–mediated sensitizers may induce an irritancy response that may confound LLNA interpretation, irritancy was evaluated. Methylglyoxal, diacetyl, and glycolaldehyde induced a significant increase in ear swelling 24 h after final chemical exposure (Fig. 1). TDI (2.5%) was used as a positive control for irritancy studies and resulted in an averaged significant increase of 60% ear swelling after exposure for all experiments.

Sensitization Potential Determined by the LLNA

Since QSAR predictions have not been accepted as replacements for validated animal models for allergic sensitization potential, methylglyoxal, glyoxal, diacetyl, and glycolaldehyde were evaluated using the LLNA. The concentrations selected

TABLE 2
Change in Body Weight and Organ Weight as a Percent of Body Weight after Dose-Dependant Exposure to Methylglyoxal, Glyoxal, Diacetyl, and Glycolaldehyde

Dose group	Kidney (% of body weight)	Spleen (% of body weight)	Liver (% of body weight)	Thymus (% of body weight)	Change in body weight (grams)
AOO[a]	1.31 ± 0.04	0.39 ± 0.02	4.77 ± 0.12	0.34 ± 0.03	0.37 ± 0.32
Methylglyoxal					
0.1%	1.29 ± 0.04	0.53 ± 0.4*	5.14 ± 0.08	0.39 ± 0.02	0.34 ± 0.18
0.5%	1.24 ± 0.03	0.49 ± 0.02*	5.13 ± 0.2	0.37 ± 0.02	0.65 ± 0.24
1%	1.23 ± 0.05	0.44 ± 0.12	4.99 ± 0.07	0.34 ± 0.04	0.77 ± 0.14
AOO[a]	1.31 ± 0.01	0.46 ± 0.01	4.98 ± 0.14	0.31 ± 0.12	0.70 ± 0.18
Glyoxal					
0.35%	1.20 ± 0.04	0.45 ± 0.03	4.92 ± 0.12	0.32 ± 0.03	0.77 ± 0.11
0.7%	1.22 ± 0.13	0.51 ± 0.03	4.95 ± 0.44	0.32 ± 0.03	1.12 ± 0.29
1.5%	1.26 ± 0.23	0.49 ± 0.02	5.15 ± 0.07	0.35 ± 0.02	0.14 ± 0.57
AOO[a]	1.31 ± 0.01	0.47 ± 0.01	4.98 ± 0.14	0.31 ± 0.02	0.43 ± 0.19
Diacetyl					
1.25%	1.23 ± 0.03	0.49 ± 0.04	5.01 ± 0.08	0.29 ± 0.03	0.89 ± 0.15
2.5%	1.29 ± 0.02	0.50 ± 0.02	5.04 ± 0.17	0.32 ± 0.02	0.52 ± 0.07
5.0%	1.18 ± 0.03	0.48 ± 0.03	4.81 ± 0.08	0.31 ± 0.02	0.67 ± 0.12
AOO[a]	1.31 ± 0.01	0.47 ± 0.01	4.98 ± 0.14	0.31 ± 0.02	0.49 ± 0.15
Glycolaldehyde					
0.8%	1.19 ± 0.04	0.46 ± 0.01	5.0 ± 0.08	0.31 ± 0.02	0.40 ± 0.04
1.6%	1.21 ± 0.04	0.46 ± 0.01	4.96 ± 0.11	0.32 ± 0.02	0.60 ± 0.29
3.2%	1.24 ± 0.05	0.42 ± 0.03	4.88 ± 0.17	0.27 ± 0.03	0.69 ± 0.43

Note. Values represent the ± SE derived from a mean of 5.
[a]Represents AOO vehicle.
*Significantly different from AOO controls, $p \leq 0.05$.

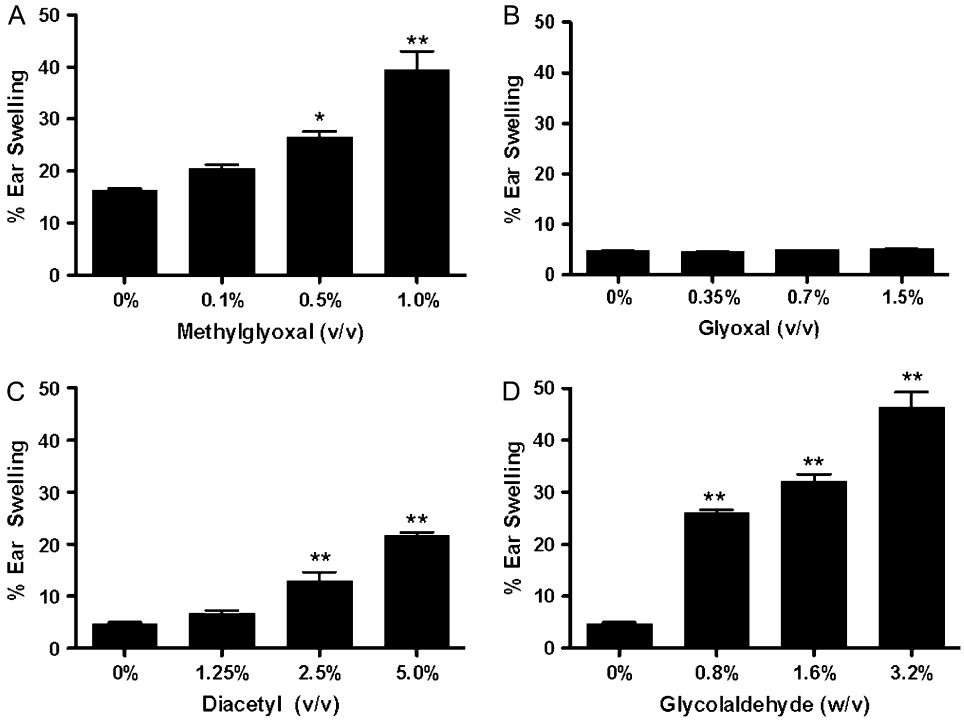


FIG. 1. Ear swelling as a result of dermal exposure. Analysis of irritation after exposure to methylglyoxal (A), glyoxal (B), diacetyl (C), or glycolaldehyde (D). Bars represent means ± SE of five mice per group. Levels of statistical significance are denoted as $*p \leq 0.05$, $**p \leq 0.01$ as compared to VH.

were determined by range-finding studies (data not shown). No overt toxicity was observed following exposure to the highest soluble concentrations of methylglyoxal (30%) and glycolaldehyde (3.25%); therefore, these concentrations were used as the high dose for the sensitization studies. Methylglyoxal was initially tested at a range of 7–30%. Since all of these concentrations generated a response greater than a threefold SI (Table 3), the experiment was repeated using concentrations between 0.1 and 1% in order to calculate an EC3 value (Fig. 2 and Table 3). Published concentrations ranges used to determine EC3 values for glyoxal (0.35–1.5%) and diacetyl (6–24%) were used to evaluate these chemicals. Diacetyl was initially tested at a range of 6–24% (Table 3) based on the published EC3 value, but the study was repeated at concentrations spanning 1.25–5% to generate an EC3 value for these studies (Fig. 2 and Table 3). Glycolaldehyde was tested at a range of 0.8–3.25% due to limits of solubility. Methylglyoxal, glyoxal, diacetyl, and glycolaldehyde had positive LLNA responses with EC3 values calculated at 0.42, 0.74, 1.9, and 1.8%, respectively (Fig. 2). HCA (30%) was used as a positive control for the LLNA and resulted in an average SI value of 15.3 for all experiments.

Lymph Node Phenotyping and Analysis of Total Serum IgE

Phenotypic analysis of the DLNs of mice with exposure to glyoxal resulted in an increase in B220+ cells (24.4 ± 0.9%, 1.5%). Furthermore, there was no significant increase in the IgE+B220+ cells or total IgE (Table 3). The mild increase in IgE+B220+ (12.9 ± 2.1%) after exposure to 0.7% glyoxal was not considered biologically significant given that this effect was not dose responsive. This data provides further support for the LLNA identification of glyoxal as a contact sensitizer. Exposure of mice to diacetyl (1.5–5%) resulted in significant elevations in B220+ cell populations (21.7 ± 0.5%, 5%) in the DLNs with a dose-responsive significant increase in the IgE+B220+ cell populations (16.5 ± 0.6%, 5%). Exposure to these concentrations did cause an elevation in total serum IgE levels (Table 3). Although exposure to higher concentrations of diacetyl (24%) resulted in an elevation of total serum IgE (614 ± 42 ng/ml), this was not considered biologically significant due to the lack of a dose-responsive increase in the IgE+B220+ cell population. Phenotypic analysis of the DLNs of mice with exposure to glycolaldehyde resulted in increases in both the IgE+B220+ (10.1 ± 1.6%, 3.2%) and B220+ (18.4 ± 0.8%, 3.2%) but not in total serum IgE levels (Table 3). When exposed to methylglyoxal (7.5–30%), the DLNs had significant increases in IgE+B220+ (20.5 ± 4.2%, 15%) and B220+ cells (39.8 ± 1.4, 15%) along with significant elevations in total serum IgE (1059 ± 89 ng/ml, 15%) levels (Table 3). TDI (2.5%) was used as a positive control for phenotyping experiments and resulted in significant elevations of IgE+B220+ (23.4%) and B220+ (34.8%) cell populations. TDI (2.5%) was also used as a positive control for total IgE ELISA and resulted in a significant elevation of total IgE (~1500 ng/ml average for all experiments) when compared to vehicle.

TABLE 3
LLNA/Phenotypic Analysis and Total IgE Dose-Response Studies

Dose group	LLNA (DPM)	% IgE+ B220+	%B220+	Total IgE (ng/ml)
Methylglyoxal				
Experiment 2				
AOO[a]	1054 ± 138.4	0.6 ± 0.2	17.5 ± 1.0	312.1 ± 45.1
0.1%	1354 ± 262.3	0.9 ± 0.6	19.8 ± 2.4	369.2 ± 65.6
0.5%	3590 ± 744.1	0.5 ± 0.2	21.0 ± 1.4	237.8 ± 105.4
1%	5350 ± 690.4*	1.3 ± 0.3	26.1 ± 2.3	266.6 ± 78.2
Experiment 1				
AOO[a]	1271 ± 245	1.5 ± 0.8	19.4 ± 1.0	333 ± 17
7.5%	10279 ± 1848**	26.7 ± 1.3**	41.2 ± 1.2**	668 ± 44*
15%	30944 ± 3265**	20.5 ± 4.2**	39.8 ± 1.4**	1059 ± 89**
30%	41544 ± 5286**	18.1 ± 1.8**	42.9 ± 0.8**	1071 ± 90**
Diacetyl				
Experiment 2				
AOO[a]	587 ± 144.9	4.8 ± 0.2	2.9 ± 0.9	13.2 ± 0.9
1.25%	1260 ± 152.6	8.1 ± 1.7*	21.3 ± 0.9**	755.1 ± 75.5
2.5%	2417 ± 372.6**	10.5 ± 0.3**	18.2 ± 0.4**	797.1 ± 81.3
5%	6614 ± 137.8**	16.5 ± 0.6**	21.7 ± 0.5**	853.4 ± 57.3
Experiment 1				
AOO[a]	440 ± 107	1.5 ± 0.8	19.4 ± 1.0	333 ± 17
6%	5777 ± 267**	7.1 ± 1.5	35.1 ± 0.4**	387 ± 29
12%	9069 ± 981**	14.9 ± 4.3**	38.9 ± 1.6**	521 ± 33
24%	9899 ± 1347**	8.2 ± 1.2	39.2 ± 1.6**	614 ± 42*
Glyoxal				
AOO[a]	440.2 ± 107.8	2.9 ± 0.9	13.2 ± 0.9	767.7 ± 71.3
0.35%	491.4 ± 56.7	9.3 ± 2.1	22.1 ± 1.0**	797.7 ± 88.9
0.7%	1232 ± 454.6	12.9 ± 2.1**	24.3 ± 0.6**	965.9 ± 97.7
1.5%	2251 ± 503.7	9.6 ± 2.2	24.4 ± 0.9**	922.3 ± 102.8
Glycolaldehyde				
AOO[a]	1054 ± 138.4	2.9 ± 0.9	13.2 ± 0.9	767.7 ± 71.3
0.8%	2617 ± 425.5	5.3 ± 1.0	12.4 ± 0.6	807.8 ± 68.6
1.6%	3038 ± 326.4	6.9 ± 1.2*	16.9 ± 1.7*	942.9 ± 128.2
3.2%	4616 ± 563.9*	10.1 ± 1.6**	18.4 ± 0.8*	1065 ± 144.9

Note. Values represent the ± SE derived from a mean of 5.
[a]Represents acetone olive oil vehicle.
*Significantly different from AOO controls, $p \leq 0.05$.
**Significantly different from AOO controls, $p \leq 0.01$.

DISCUSSION

In this study, we utilized a multidisciplinary approach to characterize and test the sensitization potential of reaction products generated from chemical reactions of simulated indoor air chemistry samples. The compounds analyzed in these studies were produced from chemical reactions of parent compounds with oxygen species present in simulated indoor air chemistry samples. Many of these parent chemicals including α-terpineol, β-pinene, α-pinene, citronellol, and acrolein have been classified as high-volume chemicals with production exceeding one million pounds (EPA, 1990). Many of these compounds are used in the production of pesticide products, manufacturing of flotation agents, consumer products, and building materials or furnishings that contribute to indoor air pollution. Acrolein is ranked as one of the most hazardous compounds to ecosystems and human health. When released into the atmosphere, the dominate removal mechanism is expected to be the reaction of acrolein vapor with photochemically generated hydroxyl radicals (Ghilarducci and Tjeerdema, 1995). Products of this reaction include carbon dioxide, formaldehyde, and glycolaldehyde.

Sensitizing potential of all chemicals was first predicted by QSAR modeling and then confirmed using the LLNA. The LLNA was followed up by lymph node phenotyping and total serum IgE analysis in an attempt to further characterize the type of sensitizer. Although QSAR predictions cannot completely replace animal studies, utilizing several software packages with different predictive methodologies in concert may allow for a better classification and result in a very low false negative ratio. However, one should be careful using this approach and take into account the classification rates of methods used and common sense as all these predictive tools still require further improvements. The potential for all chemicals to induce a sensitization response was correctly predicted by both Derek for Windows and the NIOSH logistic regression model. TOPKAT 6.2 predicted all chemicals to have sensitization potential except for the most active one, methylglyoxal. However, the TOPKAT binary (sensitizer/nonsensitizer) model classified only glyoxal correctly, while glycolaldehyde and methylglyoxal were identified as inactive.

Glyoxal and diacetyl are known sensitizers with published EC3 values. These compounds were evaluated in these studies for comparison purposes since, along with methylglyoxal and glycolaldehyde, they were observed as reaction products from simulated indoor environment gas-phase chemistry (Forester *et al.*, 2006; Ham *et al.*, 2006; Wells, 2005). Additional studies were also performed on these chemicals to begin to further classify them as likely T-cell–mediated or IgE-mediated sensitizers. The EC3 value obtained for glyoxal in this study is consistent with published data (Patlewicz *et al.*, 2002), while the EC3 value identified for diacetyl in these studies (1.9%) was approximately 10-fold lower than the EC3 value of 11.3% described in the published data (Roberts *et al.*, 1999). Explanation for the discrepancy could be the use of different mouse strains or vehicles. CBA/J mice were used in the Roberts and Patlewicz (2002) studies, while BALB/c mice were used in these studies.

The importance of research on α,β-dicarbonyl compounds is consistent with recent work on the development of health impacts from glutaraldehyde exposure (Azadi *et al.*, 2004; Cohen and Patton, 2006; Emmanuel *et al.*, 2005; Halatek *et al.*, 2003; Palczynski *et al.*, 2005; Pechter *et al.*, 2005; Takigawa and Endo, 2006). Glyoxal, methylglyoxal, and diacetyl belong to the chemical class of α,β-dicarbonyls, a group of potent and reactive chemicals, especially if one of the carbonyl groups is an aldehyde. Including the structurally similar glycolaldehyde, the skin sensitization activity of α,β-dicarbonyls is thought to be directly related to their ability to covalently bind with appropriate skin proteins through an electrophilic attack on the

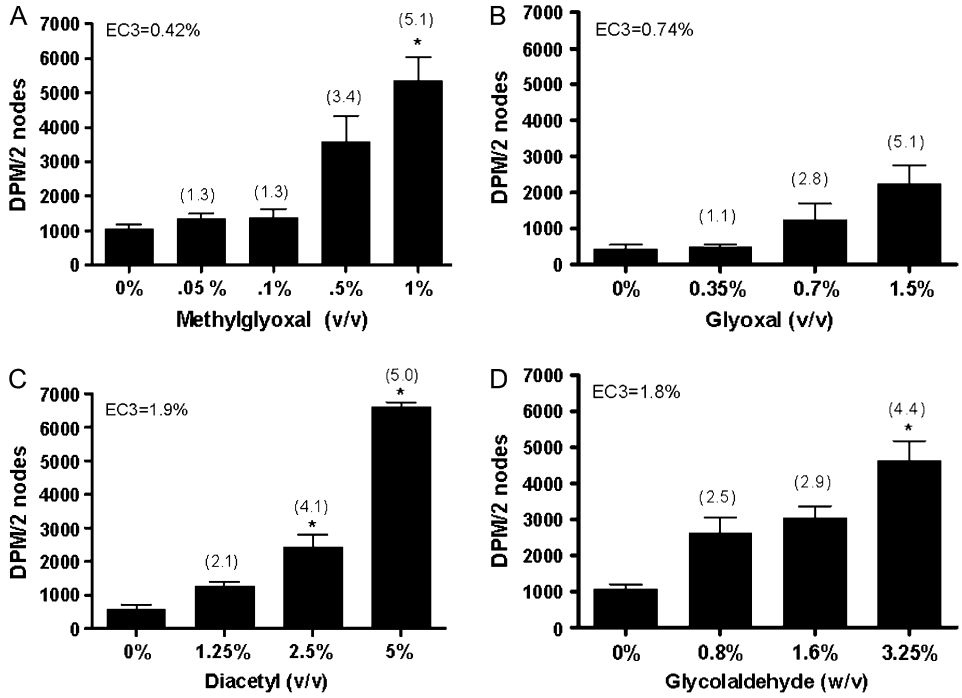


FIG. 2. Sensitization potential after dermal exposure. Analysis of the sensitization potential of methylglyoxal (A), glyoxal (B), diacetyl (C), or glycolaldehyde (D) using the LLNA. ^{3}H-thymidine incorporation into DLN cells of BALB/c mice following exposure to vehicle or concentration of VOC shown above. Bars represent means ± SE of five mice per group. Numbers appearing above the bars represent the SIs for each concentration tested. Levels of statistical significance are denoted as $*p \leq 0.05$, $**p \leq 0.01$ as compared to VH.

guanidine group of arginine residues by formation of heterocyclic products (Fig. 3) (Roberts and Patlewicz, 2002).

Based on this mechanism, one would predict the most reactive chemical species to be the stronger skin sensitizers. This suggests that α,β-dicarbonyls with aldehyde groups should be more reactive than those with only ketone groups. However, there are other factors that may additionally impact this process including skin permeability and the ability of protein adducts to be recognized as foreign by the immune system. The predicted skin permeation coefficient is identical for the studied chemicals thus ruling out this factor. Furthermore, it is surprising that methylglyoxal, containing one aldehyde and one ketone group, is a somewhat stronger sensitizer than glyoxal, containing two aldehyde groups. Permeability differences may be a possible explanation. As expected, diacetyl with two ketone groups and glycolaldehyde with only one aldehyde group were identified as weaker sensitizers.

The methods in this paper describe a screening process used to help classify sensitizers. QSAR modeling, LLNA, and phenotyping analysis predicted methylglyoxal, glyoxal, glycolaldehyde, and diacetyl as sensitizers. Although identified as sensitizers by the LLNA, dermal exposure of mice to glyoxal, glycolaldehyde, and diacetyl did not result in biologically significant elevations in the IgE+B220+ cell populations or serum IgE levels according to the criteria described by Manetz and Meade (1999). Based on the predictions of these screening tools, glyoxal, glycolaldehyde, and diacetyl would be classified as likely T-cell–mediated sensitizers.

Exposure to IgE-mediated sensitizers has been found to cause a dose-responsive increase in the IgE+B220+ cell population in the DLN at the same test concentration that significantly elevates the B220+ population along with elevations in total serum IgE levels (Manetz and Meade, 1999). Exposure to concentrations from 7.5 to 30% methylglyoxal resulted in significant elevations of IgE+B220, B220+, and serum IgE levels. Elevations in IgE+B220+ may be an early marker for IgE-mediated sensitizers because IgE will initially bind the CD23 receptor before releasing into the serum. Although these methods only provide a screening technique, more extensive analyses are required for the confirmation of this result.

These results raise concern for the potential of methylglyoxal to be an IgE-mediated sensitizer. The LLNA is a test method that was developed and validated for the identification

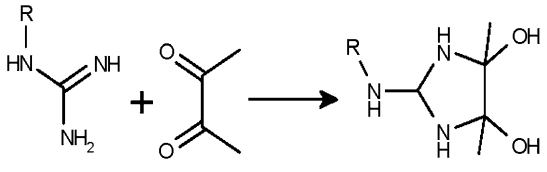


FIG. 3. Reaction of α,β-dicarbonyls with guanidine.

of contact sensitizers, and while chemical respiratory allergens such as TDI and TMA induce positive responses in the LLNA, not all LLNA-positive chemicals are capable of inducing respiratory allergy or asthma. Further analyses, including phenotyping and IgE analysis, were conducted to evaluate the potential for the chemicals testing positive in the LLNA to be respiratory sensitizers. It is often thought the most common route of exposure to respiratory allergens is inhalation, although published data have shown that dermal application may result in respiratory tract sensitization. Recent literature has shown that topical application is effective in sensitizing rats to TMA resulting in airway reactivity after inhalational challenge (Zhang *et al.*, 2004). Other literature has shown that dermal exposure of mice to natural rubber latex can induce latex-specific IgE and airway hyperreactivity upon respiratory challenge (Howell *et al.*, 2002).

In summary, the four compounds generated from simulated indoor air chemistry and tested in these studies were identified as sensitizers using QSAR modeling, LLNA, and phenotypic analysis with exposure to methylglyoxal resulting in elevated IgE levels. The high production volume of the parent chemicals increases the likelihood that VOCs may be generated in indoor air settings such as office spaces or janitorial closets. This raises the concern for human exposures with respect to allergy and asthma. These findings in animal models support the need for more extensive research into the adverse effect of indoor air exposure on human respiratory health.

REFERENCES

Azadi, S., Klink, K. J., and Meade, B. J. (2004). Divergent immunological responses following glutaraldehyde exposure. *Toxicol. Appl. Pharmacol.* **197,** 1–8.

Basketter, D. A., Lea, L. J., Dickens, A., Briggs, D., Pate, I., Dearman, R. J., and Kimber, I. (1999). A comparison of statistical approaches to the derivation of EC3 values from local lymph node assay dose responses. *J. Appl. Toxicol.* **19,** 261–266.

Brightman, H. S., and Moss, N. (2000). *Sick Building Syndrome Studies and the Compilation of Normative and Comparative Values.* McGraw-Hill, New York.

Calogirou, A., Larsen, B. R., and Kotzias, D. (1999). Gas-phase terpene oxidation products: A review. *Atmos. Environ.* **33,** 1423–1439.

Cohen, N. L., and Patton, C. M. (2006). Worker safety and glutaraldehyde in the gastrointestinal lab environment. *Gastroenterol. Nurs.* **29,** 100–104.

Emmanuel, E., Hanna, K., Bazin, C., Keck, G., Clement, B., and Perrodin, Y. (2005). Fate of glutaraldehyde in hospital wastewater and combined effects of glutaraldehyde and surfactants on aquatic organisms. *Environ. Int.* **31,** 399–406.

EPA. (1990). *1990 HPV Challenge Program Chemical List.* Environmental Protection Agency. Available at: http://www.epa.gov/HPV/pubs/update/hpv_1990.htm. Accessed April 25, 2007.

Fedan, J. S., Dowdy, J. A., Van Scott, M. R., Wu, D. X. Y., and Johnston, R. A. (2004). Hyperosmolar solution effects in guinea pig airways. III. Studies on the identity of epithelium-derived relaxing factor in isolated perfused trachea using pharmacological agents. *J. Pharmacol. Exp. Ther.* **308,** 30–36.

Fedorowicz, A., Singh, H., Soderholm, S., and Demchuk, E. (2005). Structure-activity models for contact sensitization. *Chem. Res. Toxicol.* **18,** 954–969.

Fick, J., Pommer, L., Nilsson, C., and Andersson, B. (2003). Effect of OH radicals, relative humidity, and time on the composition of the products formed in the ozonolysis of alpha-pinene. *Atmos. Environ.* **37,** 4087–4096.

Forester, C. D., Ham, J. E., and Wells, J. R. (2006). The gas-phase chemistry of dihydromyrcenol with ozone and OH radical: Rate constants and products. *Int. J. Chem. Kinet.* **38,** 451–463.

Frasch, H. F. (2002). A random walk model of skin permeation. *Risk Anal.* **22,** 265–276.

Ghilarducci, D. P., and Tjeerdema, R. S. (1995). Fate and effects of acrolein. *Rev. Environ. Contam. Toxicol.* **144,** 95–146.

Hakola, H., Arey, J., Aschmann, S. M., and Atkinson, R. (1994). Product formation from the gas-phase reactions of OH radicals and O_3 with a series of monoterpenes. *J. Atmos. Chem.* **18,** 75–102.

Halatek, T., Opalska, B., Swiercz, R., Palczynski, C., Gorski, P., Rydzynski, K., and Bernard, A. (2003). Glutaraldehyde inhalation exposure of rats: Effects on lung morphology, Clara-cell protein, and hyaluronic acid levels in BAL. *Inhal. Toxicol.* **15,** 85–97.

Ham, J. E., Proper, S. P., and Wells, J. R. (2006). The gas-phase chemistry of citronellol with ozone and OH radical: Rate constants and products. *Atmos. Environ.* **40,** 726–735.

Howell, M. D., Weissman, D. N., and Jean Meade, B. (2002). Latex sensitization by dermal exposure can lead to airway hyperreactivity. *Int. Arch. Allergy Immunol.* **128,** 204–211.

Jaoui, M., and Kamens, R. M. (2003). Gaseous and particulate oxidation products analysis of a mixture of alpha-pinene plus beta-pinene/O-3/air in the absence of light and alpha-pinene plus beta-pinene/NOx/air in the presence of natural sunlight. *J. Atmos. Chem.* **44,** 259–297.

Jarvis, J., Seed, M. J., Elton, R. A., Sawyer, L., and Agius, R. M. (2005). Relationship between chemical structure and the occupational asthma hazard of low molecular weight organic compounds. *Occup. Environ. Med.* **62,** 243–250.

Kasper, M., Roehlecke, C., Witt, M., Fehrenbach, H., Hofer, A., Miyata, T., Weigert, C., Funk, R. H. W., and Schleicher, E. D. (2000). Induction of apoptosis by glyoxal in human embryonic lung epithelial cell line L132. *Am. J. Respir. Cell Mol. Biol.* **23,** 485–491.

Kreiss, K., Gomaa, A., Kullman, G., Fedan, K., Simoes, E. J., and Enright, P. L. (2002). Clinical bronchiolitis obliterans in workers at a microwave-popcorn plant. *N. Engl. J. Med.* **347,** 330–338.

Magneron, I., Mellouki, A., Le Bras, G., Moortgat, G. K., Horowitz, A., and Wirtz, K. (2005). Photolysis and OH-initiated oxidation of glycolaldehyde under atmospheric conditions. *J. Phys. Chem. A* **109,** 4552–4561.

Manetz, T. S., and Meade, B. J. (1999). Development of a flow cytometry assay for the identification and differentiation of chemicals with the potential to elicit irritation, IgE-mediated, or T cell-mediated hypersensitivity responses. *Toxicol. Sci.* **48,** 206–217.

Mendell, M. J., Fisk, W. J., Kreiss, K., Levin, H., Alexander, D., Cain, W. S., Girman, J. R., Hines, C. J., Jensen, P. A., Milton, D. K., *et al.* (2002). Improving the health of workers in indoor environments: Priority research needs for a national occupational research agenda. *Am. J. Public Health* **92,** 1430–1440.

NIEHS. (1999). National Institute of Environmental Health Sciences (NIEHS); the Murine Local Lymph Node Assay: A test method for assessing the allergic contact dermatitis potential of chemicals/compounds. *Fed. Regist.* **64,** 14006–14007.

Palczynski, C., Walusiak, J., Krakowiak, A., Halatek, T., Ruta, U., Krawczyk-Adamus, P., Wittczak, T., Swiercz, R., Gorski, P., and Rydzynski, K. (2005). Glutaraldehyde-induced occupational asthma: BALF components and BALF and serum Clara cell protein (CC16) changes due to specific inhalatory provocation test. *Occup. Med.* **55,** 572–574.

Patlewicz, G. Y., Wright, Z. M., Basketter, D. A., Pease, C. K., Lepoittevin, J. P., and Arnau, E. G. (2002). Structure-activity relationships for selected fragrance allergens. *Contact Derm.* **47,** 219–226.

Pechter, E., Davis, L. K., Tumpowsky, C., Flattery, J., Harrison, R., Reinisch, F., Reilly, M. J., Rosenman, K. D., Schill, D. P., Valiante, D., *et al.* (2005). Work-related asthma among health care workers: Surveillance data from California, Massachusetts, Michigan, and New Jersey, 1993–1997. *Am. J. Ind Med.* **47,** 265–275.

Potts, R. O., and Guy, R. H. (1992). Predicting skin permeability. *Pharm. res.* **9,** 663–669.

Roberts, D. W., and Patlewicz, G. (2002). Mechanism based structure-activity relationships for skin sensitisation—The carbonyl group domain. *SAR QSAR Environ. Res.* **13,** 145–152.

Roberts, D. W., York, M., and Basketter, D. A. (1999). Structure-activity relationships in the murine local lymph node assay for skin sensitization: Alpha,beta-diketones. *Contact Derm.* **41,** 14–17.

Rohr, A. C., Weschler, C. J., Koutrakis, P., and Spengler, J. D. (2003). Generation and quantification of ultrafine particles through terpene/ozone reaction in a chamber setting. *Aerosol Sci. Technol.* **37,** 65–78.

Sexton, K. G., Jefferies, H. E., Jang, M., Kamens, R. M., Doyle, M., Voicu, I., and Jaspers, I. (2004). Photochemical products in urban mixtures enhance inflammatory responses in lung cells. *Inhal. Toxicol.* **16,** 107–114.

Takigawa, T., and Endo, Y. (2006). Effects of glutaraldehyde exposure on human health. *J. Occup. Health* **48,** 75–87.

Wells, J. R. (2005). Gas-phase chemistry of (alpha-terpineol with ozone and OH radical: Rate constants and products. *Environ. Sci. Technol.* **39,** 6937–6943.

Wilkins, C. K., Clausen, P. A., Wolkoff, P., Larsen, S. T., Hammer, M., Larsen, K., Hansen, V., and Nielsen, G. D. (2001). Formation of strong airway irritants in mixtures of isoprene/ozone and isoprene/ozone/nitrogen dioxide. *Environ. Health. Perspect.* **109**, 937–941.

Wilschut, A., ten Berge, W. F., Robinson, P. J., and McKone, T. E. (1995). Estimating skin permeation. The validation of five mathematical skin permeation models. *Chemosphere* **30,** 1275–1296.

Woolhiser, M. R., Hayes, B. B., and Meade, B. J. (1998). A combined murine local lymph node and irritancy assay to predict sensitization and irritancy potential of chemicals. *Toxicol. Methods* **8,** 245–256.

Yu, J. Z., Flagan, R. C., and Seinfeld, J. H. (1998). Identification of products containing -COOH, -OH, and -C=O in atmospheric oxidation of hydrocarbons. *Environ. Sci. Technol.* **32,** 2357–2370.

Zhang, X. D., Fedan, J. S., Lewis, D. M., and Siegel, P. D. (2004). Asthmalike biphasic airway responses in Brown Norway rats sensitized by dermal exposure to dry trimellitic anhydride powder. *J. Allergy Clin. Immunol.* **113,** 320–326.