Identification of Indoor Air Contamina

Laurel G. Jackson, J. R. Wells, Jason Ham
National Institute for Occupational Safe

Abstract

On average, U.S. citizens spend 80% or more of their daily lives indoors, whether at home, work, or in other commercial buildings. Over the last two decades, there has been increasing awareness regarding the potential impact of indoor air pollution on health. These studies use an *in vitro* monitoring system called VitroCell which employs the air/cell interface allowing for direct contact between cells and components of a test atmosphere to assess chemicals found in the indoor air environment. The structurally similar dicarbonyls diacetyl, 4-oxopentanal, glyoxal, glutaraldehyde, and methyl glyoxal were selected for use in this system. Exposure to these volatile organic compounds (VOC), which are formed from reactions with ozone and are found in the indoor environment, has been suggested to contribute to adverse health effects. The VitroCell module was used to evaluate immune-related gene expression after a pulmonary epithelial A549 cell line was exposed to these aerosolized chemicals. A low density real-time PCR gene array, screening 84 immune-related genes, was used to investigate the exposure effects of these compounds. Alterations in the inflammatory cytokines Il-1α, Il-8, GM-CSF, and TNF were identified after exposure to these compounds. The identified cytokines can potentially be used as biomarkers to screen contaminated indoor air environments. These studies may provide an *in vitro* method for identification and characterization of chemical hazards including indoor air pollutants in work environments such as office buildings, allowing for the reduction of worker illness and more specifically reducing respiratory consequences of exposures to allergens and irritants.

Introduction

Of the 89 million people in the U.S. 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 $20-70 billion annually (Mendell *et al.* 2002). Investigators searching for specific causes of these increasing complaints have ascribed the effects to both biological (e.g. fungi or endotoxin) and chemical (VOC) exposures (Brightman and Moss 2000). Thus, research in exposure to indoor gas-phase chemistry is being conducted to describe indoor work environments. 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. We have previously identified several structurally similar dicarbonyl compounds that are produced by the reaction of O_3 or –OH with α-terpineol (Figure 1 and Table 1). Research has shown that exposure of human lung cells to oxidized atmospheric environments causes an increase in Il-8 mRNA which is associated with an enhanced inflammatory response (Sexton *et al.* 2004). Studies exposing animals to products of chemical reactions such as ozone 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). 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). The results described above highlight that VOC present in the indoor environment can be transformed into oxidized organic reaction products (Figure 1) and biological systems can be affected by exposure to these compounds. However, at the present time there is no detection method for these VOC reaction products to determine if they are responsible. These studies identify changes in inflammatory cytokine expression in a pulmonary epithelial cell line after exposure to the structurally similar ozone reaction products (diacetyl, 4-oxopentanal, glyoxal, methyl glyoxal and glutaraldehyde) individually and in a reaction mixture.

Figure 1

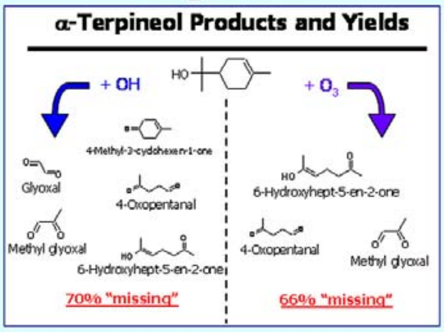


Figure 2

Materials and Methods

TEST ARTICLE: Methyl glyoxal (CAS 78-98-8), glyoxal (CAS 107-22-2), diacetyl also known as 2, 3-butanedione (CAS 431-03-8), and glutaraldehyde (CAS 111-30-8) were all purchased from Sigma Aldrich Chemical Company (St. Louis, MO). 4-Oxopentanal was synthesized by Richman Chemical Inc. (Lower Gwynedd, PA).

BAG PREPARATION:
Teflon chambers (FEP 500, American Durafilm, Hollston, MA) were constructed to facilitate cell exposure via the VitroCell apparatus to gas-phase chemicals. Chemicals were injected into a 50% relative humidity air stream through a heated ¼ inch stainless steel tee into the 70–100 liter Teflon chambers. For these cell exposure experiments typical concentrations of the pertinent species were 65 ppm (1.6 x 10 15 molecule cm^{3}) oxygenated organic compound (test articles) and ~ 1 ppm (2.5 x 1013 molecule cm^{3}) α-terpineol. For the reaction product experiments, ozone was produced by photolyzing air with a mercury pen lamp (Jelight, Irvine, CA) in a separate Teflon chamber and transferred using a gas-tight syringe. Ozone concentration (~ 100 ppb) was measured with a UV photometric ozone analyzer (model 49C or 49i, Thermo Fisher Scientific, Inc., Waltham, MA)

VITROCELL EXPOSURES:
Human epithelial lung cells, A549, were cultured (250,000 cells/4.67 cm^2) in 10% FCS DMEM on removable permeable Transwell inserts. The media was exchanged for serum-free media 24±4 hours prior to exposures in the VitroCell chambers. Immediately before exposures, the inserts were washed twice with sterile PBS and placed in the wells of the VitroCell chambers, to which has been added a predetermined volume of serum-free media to sustain the basal surface of the cells during exposures. The chambers were attached to a circulating 37°C water bath for the duration of the experiment. The contents of the prepared reaction bags were pulled across the apical surface of the cells for 2 or 4 hours at a constant rate of 3.0 ml/min (Figure 2). Post-exposure, the Transwell inserts were placed in a 6-well plate with 10% FCS DMEM and allowed recovery periods ranging from 2-6 hours in a 37°C incubator with 5% CO_2.

RNA ISOLATION & GENE EXPRESSION ANALYSIS:
RNA was isolated from the cells according to the TRIzol protocol with QIAGEN clean-up. RNA concentration was determined using ND-1000 spectrophotometer (NanoDrop). Reverse transcription (2 μg) was performed using the high capacity cDNA reverse transcription kit (Applied Biosystems) according to manufacturer's instructions. RNA was quantified (CCL2, CCL5, GM-CSF, Il-1α, Il-β, Il-6, Il-8, TNF, and TGFβ1) using real-time polymerase chain reaction on a 7500 Fast Real-Time PCR system (Applied Biosystems) using TaqMan reagents as prescribed by manufacturer's instructions. Data is expressed as relative fold increase over control (clean air or ozone), calculated by the following formula: $2^{\Delta\Delta Ct}$, where ΔΔCt = ΔCt (sample) – ΔCt (Control). The ΔCt = Ct GAPDH) – Ct (Target), where Ct = cycle threshold as defined by manufacturer's instructions.

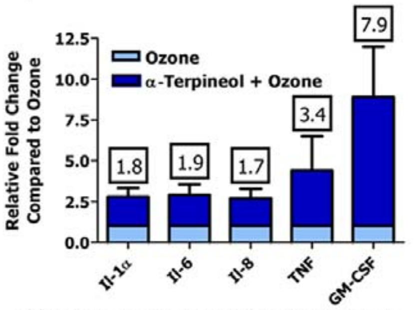
nts Using an *in Vitro* Exposure System

, Albert Munson, and Stacey E. Anderson
ty and Health, Morgantown, WV 26505

Results

Figure 3

Alterations in Gene Expression after Exposure to Ozone Reaction Products



A549 cells were exposed to ozone or α-terpineol + ozone for 2 or 4 hours with a 2-6 hour recovery. Results represent the mean standard error from four experiments. Numbers above bars represent relative fold change in gene expression compared to ozone control.

Table 2

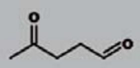
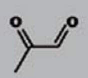
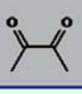
Alternations in Gene Expression after VitroCell Exposure of A549 Cells to Aerosolized VOC

	GM-CSF	IL-1α	IL-1β	IL-6	IL-8	TNF
4-Oxopentanal	1.7	2.1	3.1	3.2	2.4	3.6
Glyoxal	1.4 ± 0.58	1.6 ± 0.49	1.4 ± 0.22	1.2 ± 0.12	1.2 ± 0.22	1.6 ± 0.16
Methyl glyoxal	14.1 ± 7.6	1.3 ± 0.17	1.7 ± 0.59	2.9 ± 0.89	2.1 ± 0.03	2.1 ± 0.11
Glutaraldehyde	1.0 ± 0.17	1.1 ± 0.07	2.1 ± 1.1	1.2 ± 0.24	1.0 ± 0.17	1.7 ± 0.78
Diacetyl	1.1 ± 0.46	1.1 ± 0.02	1.1 ± 0.01	1.1 ± 0.03	1.1 ± 0.03	0.8 ± 0.27

Values represent the mean ± standard error of the relative fold change in gene expression compared to clean air exposure control. The results for 4-OPA represent a single experiment. A549 cells were exposure for 2 or 4 hours to each specific aerosolized chemical or clean air control and given a 2-6 hour recovery.

Table 1

Structure of Dicarbonyls Found in the Indoor Air Environment

Chemical	Structure
4-Oxopentanal	
Glutaraldehyde	
Methyl glyoxal	
Glyoxal	
Diacetyl	

Summary and Conclusions

- An *in vitro* exposure system using the VitroCell module (Figure 2) has been developed to investigate the adverse health effects associated with exposure to polluted indoor air environments.

- Exposure to VOC, generated as individual compounds and as ozone reaction products, caused alterations in inflammatory cytokine gene expression (Table 2 and Figure 3). These changes were similar between the individual chemicals and the reaction products.

- While the concentrations of the chemicals in the reaction bags are higher than those likely to be found in most indoor environments, it is important to consider the cumulative effect of these structurally similar indoor contaminants (Table 1).

- The increased expression in inflammatory cytokines (Il-1α, Il-6, Il-8, TNF and GM-CSF) was greater for the VOC reaction products (α-terpineol + ozone) than ozone. No change in gene expression was observed when the A549 cells were exposed to ozone alone (data not shown).

- Studies examining the effects of α-terpineol and α-terpineol + ozone are currently underway.

- The missing chemicals generated from indoor air "reactions" (Figure 1) still need to be identified and investigated. These compounds may also contribute to the observed alterations in inflammatory cytokine expression.

- Exposure to these VOC reaction products is currently being investigated in a more complex tissue system that consists of normal, human-derived tracheal/bronchial epithelial cells which have been cultured to form a pseudo-stratified, highly differentiated model which closely resembles the epithelial tissue of the respiratory tract.

- This system may help to clarify the cause of Sick Building Syndrome and the diverse health complaints of those working in indoor environments.