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Health Effects of Exposures to VOC's, Ozone and Stress

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ABBREVIATIONS

CEF	controlled environment facility
CI	chemical odor intolerance
CMH	Cochran-Mantel-Haenszel
FEF	forced expiratory flow
FEV	forced expiratory volume
FVC	forced vital capacity
GEE	general estimating equations
ICC	intraclass correlation coefficient
IL	interleuken
MCA	masked clean air
MCS	multiple chemical sensitivity
NA	negative affect
NL	nasal lavage
NSBRI	non-specific building related illness
PBS	phosphate buffered saline
PEFR	peek expiratory flow rate
PMN	polymorphonuclear leukocytes
POL	performance-on-line
ppb	parts per billion
RAST	radioallergosorbant test
TLV	threshold limit value
VOC	volatile organic compounds
VOC+O	volatile organic compounds + ozone

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ABSTRACT

Epidemiologic investigations of building-related health complaints document that volatile organic compounds (VOCs), stressors, and individual characteristics (e.g., female gender) contribute to the reported non-specific symptoms. The purpose of the proposed study was to determine the effects of a psychological stressor and the individual difference variables, negative affect and odor intolerance, on responses of women to a mixture of volatile organic compounds with and without ozone. One hundred and thirty healthy women (mean age = 27.2 years; mean education = 15 years) participated in a 2 (Negative Affect - high & low) x 2 (Odor Intolerance - high & low) x 2 (Stressor) x 3 (Exposures: masked clean air (MCA), VOCs, VOCs with ozone (VOC + O) repeated measures design. All subjects completed each exposure condition. One-half of the subjects were randomly assigned to exposure conditions with the stressor while the remaining subjects completed the exposures without the stressor. During each 3 hour exposure condition, health effects measured before, during and after exposure included: symptom questionnaires, neurobehavioral performance, salivary cortisol, nasal inflammation (PMN, albumin, IL-6, IL-8), and lung function (FEV 1). Secondary stable products of the ozone-VOC reaction were measured during the VOCs + O sessions only.

Among healthy women, exposure to concentrations of VOCs with and without ozone did not result in significant subjective or objective health effects. Combining VOCs with ozone at a low ventilation rate (~ 2 air exchanges per hour) was successful in producing aldehydes, organic acids and submicron particles primarily through the interaction of ozone with terpenes (283 ppb). Despite the addition of these potentially irritating products, subjects' ratings of health symptoms, environmental qualities, and odor were indistinguishable between the VOCs + O and the VOCs condition. Although numerous epidemiologic studies suggest that symptoms are significantly increased among workers in

buildings with poor ventilation and mixtures of VOCs (e.g., Mendell et al., 2002; Mendell, 1993; Sieber et al., 1996), the current controlled acute exposure to similar chemical mixtures did not validate these epidemiologic findings. Similar to previous studies, however, subjects in the present study found the air quality and odor to be poorer in the VOCs and VOCs + O than in the MCA condition. Even though the MCA condition was intentionally "spiked" with the odor of the VOC mixture, subjects perceived this odor to be of lesser strength and the air of better quality than for the VOCs and VOCs + O conditions. Although symptoms were not significantly increased, subjects rated the VOCs and VOCs + O conditions as more annoying if these conditions were present in their daily work environment. Psychological stress, on the other hand, significantly increased salivary cortisol for all subjects and ratings of anxiety particularly among women high in negative affect. In fact, psychological stress overwhelmed any effect of exposure on symptoms of anxiety for subjects high in negative affect. In contrast, subjects low in negative affect reported greater anxiety following the psychological stressor and during the VOCs + O condition. However, the addition of psychological stress did not exacerbate other health symptoms for any subject group. Objective markers including lung function, nasal inflammation, and neurobehavioral performance were also not affected by exposure to VOCs with or without ozone. Contrary to the hypothesis, subjects high in chemical intolerance did not report more symptoms or show elevation on markers of nasal inflammation in response to VOCs with ozone. In conclusion, the present study suggests that indoor air complaints may be driven by annoyance with unpleasant odors. The effects of psychological stress may be operating independently to increase symptoms of anxiety particularly among individuals who are prone to negative affect. These symptoms may be mistakenly attributed to poor indoor air quality.

SIGNIFICANT FINDINGS

1. Terpenes and ozone, both found in indoor environments, interact to create aldehydes, organic acids and submicron particles when ventilation rates are low (~two air exchanges per hour).
2. Despite the production of irritating compounds, health symptoms, markers of nasal inflammation, neurobehavioral performance, and lung function were not adversely affected by exposure to VOCs with and without ozone relative to a clean air condition with low level VOC odor.
3. Subjects rated the VOC exposures with and without ozone as stronger, the odor as more intense and less pleasant, and the air quality poorer with a greater need to ventilate the room than a clean air with low level VOC odor. Moreover, subjects reported after being exposed to VOCs both with and without ozone that they would find a similar work environment more annoying than the one in which clean air was spiked with the odor of VOCs.
4. Psychological stress during exposure increased symptoms of anxiety but not other health symptoms.

USEFULNESS OF FINDINGS

Potentially irritating gases and particles will be produced in buildings with low ventilation rates, mixtures of volatile chemicals and ozone. However, the odors associated with these mixtures appear to be the most salient factor in the complaints of building occupants. These odors are rated as unpleasant and intense but health symptoms were not increased above those observed when the odor of the volatile chemicals was present but at a very low concentration. Therefore, workers are

probably not at increased health risk but probably will find their working environment more unsatisfactory and annoying. Furthermore, psychological stress contributes to symptoms of anxiety, which may be mistaken for symptoms associated with poor indoor air.

OVERALL OBJECTIVE:

To assess the health effects of psychological stress and exposure to a mixture of volatile organic compounds (VOCs) with (VOCs+O) and without ozone at concentrations comparable to those encountered in poorly ventilated buildings. Health effects of these exposure conditions will be compared between women high and low in negative affect and chemical odor intolerance.

SPECIFIC AIMS:

Aim 1: To assess, in two separate repeated measures designs, the symptomatic, neuroendocrine, neurobehavioral, inflammatory, and lung function effects of controlled exposures to masked clean air (MCA), VOCs, and VOCs + O. Each exposure condition is presented with or without a psychological stressor to four groups of women high and low in the self-reported personal traits of negative affect (NA) and chemical odor intolerance (CI).

Design: 2 (Negative Affect) x 2 (Chemical Odor Intolerance) x 2 (Stressor) x 3 (Exposure) repeated measures.

Aim 1.1 Symptoms: To assess symptoms associated with VOCs, ozone, and stress.

Hypothesis 1: (Exposure Main Effect): Subjects will report significantly more symptoms in the VOCs and VOCs + O condition than the masked clean air condition with significantly higher levels of symptoms in the VOCs + O condition.

Hypothesis 2: (Exposure x NA x CI Interaction): High NA, high CI subjects will report significantly more symptoms than low NA, low CI subjects in response to VOCs and VOCs + O relative to MCA. Subjects who are high NA, low CI and low NA, high CI will report significantly more symptoms than low NA, low CI but significantly less symptoms than high

NA, high CI in response to both VOCs exposure conditions. The VOCs + O will produce significantly more symptoms of irritation than the VOCs or MCA conditions.

Hypothesis 3: (Exposure x Stressor Interaction): VOCs and VOCs + O with the stressor will produce significantly more symptoms than VOCs and VOCs + O without the stressors and than MCA with and without the stressor.

Hypothesis 4: (Exposure x NA x Stressor Interaction): Regardless of CI, high NA subjects will report significantly more symptoms than low NA subjects when exposed to VOCs and VOCs + O with the psychological stressor than when those exposure conditions do not include the stressor.

Aim 1.2: Neuroendocrine: To assess salivary cortisol as a neuroendocrine indicator of hypothalamic- pituitary-adrenal axis activation in response to exposures with and without the psychological stressor.

Hypothesis 1: (Exposure Main Effect): Baseline to post-exposure changes in salivary cortisol will be significantly elevated post-exposure to VOCs and VOCs + O relative to MCA.

Hypothesis 2: (Exposure x NA x CI Interaction): Relative to low NA, low CI subjects, high NA, high CI subjects will show significantly higher baseline to post-exposure cortisol changes in the VOCs and VOCs + O conditions than in the MCA condition.

Hypothesis 3: (Exposure x NA x CI x Stressor Interaction): Relative to low NA, low CI subjects, high NA, high CI subjects will show significantly greater baseline to post-exposure cortisol changes in the VOCs and VOCs + O conditions with the stressor than in these conditions without the stressor and than in MCA with or without the stressor.

Aim 1.3 Neurobehavioral: To assess neurobehavioral performance on a computerized vigilance task.

Hypothesis 1: (Exposure x CI Interaction): Regardless of NA, subjects high in CI will perform significantly worse than low CI subjects on the neurobehavioral vigilance task following exposures to VOCs and VOCs + O versus MCA.

Hypothesis 2: (Exposure x NA x CI x Stressor Interaction): High NA, high CI subjects will perform significantly worse than low NA, low CI subjects on the neurobehavioral vigilance task following exposure to VOCs and VOCs + O that include the psychological stressor versus exposures without the psychological stressor.

Aim 1.4 Inflammation: To assess polymorphonuclear leukocytes (PMN), albumin, Il-6, and Il-8 as markers of nasal inflammation in response to VOCs and VOCs + O.

Hypothesis 1: (Exposure Main Effect): Markers of nasal inflammation will be significantly higher in the VOCs + O exposure condition relative to VOCs alone and MCA.

Hypothesis 2: (Exposure x CI Interaction): Regardless of NA and relative to low CI subjects, high CI subjects will have significantly higher levels of PMN, albumin, Il-6, and Il-8 in response to VOCs and VOCs + O versus MCA.

Aim 1.5 Lung Function: To assess changes in lung function in response to exposure.

Hypothesis 1: (Exposure Main Effect): Spirometrically measured flow (FEV1, FEF_{25-75}, FEF_{75}, PEFR) will be significantly less in the VOCs and VOCs + O conditions relative to MCA with VOCs + O condition showing the most significant reduction in lung function.

Aim 2: To measure the major secondary stable products of ozone-VOCs reactions and to assess the effect of the interaction of VOCs and ozone on indicators of inflammation. The secondary products include gas-phase formaldehyde, other aldehydes and ketones, gas-phase carboxylic acids, and fine and ultra-fine particles. Reactive intermediates such as hydroxyl radical and Crieege biradicals cannot be measured though they may contribute to health effects.

Hypothesis 1: (Exposure Main Effect): Secondary products from indoor ozone reactions with olefinic VOCs will produce a complex mixture of aldehydes, other organic compounds in gas-phase, and fine particles, and extremely reactive intermediates such as the hydroxyl and other free radicals. This exposure mixture will produce significantly more symptoms of irritation and higher levels of nasal inflammatory markers than VOCs alone or than MCA.

BACKGROUND AND SIGNIFICANCE

Mixtures of volatile organic compounds (VOCs) and ozone pollute indoor environments(Fan et al., 2003) and VOC mixtures have been associated in office buildings with complaints of mucosal irritation and non-specific symptoms such as headache (Mendell et al., 2002; Mendell, 1993). In comparison to clean air conditions, healthy men and women exposed to mixtures of VOCs, similar to those found in office buildings, have shown increased symptoms of sensory and nasal irritation and poorer ratings of air quality (Hudnell et al., 1992 ; Prah et al., 1998). Relative to healthy controls, individuals, self-identified as having sick building syndrome, also reported significantly more symptoms in response to a VOCs mixture (Molhave et al., 1986). The number of symptoms reported in controlled exposure studies, however, are relatively few and of mild intensity in comparison to the ongoing complaints of office workers in field investigations of buildings. Thus, several investigators have implicated the interaction of ozone with VOCs and psychosocial risk factors such as stress and negative affect or neuroticism as contributory to building related complaints (Mendell et al., 2002; Bachman and Meyers, 1995; Bauer et al., 1992; Hoppe et al., 1995; Wolkoff et al., 1997; Wolkoff et al., 2000). The purpose of the present study was to assess symptoms, odor ratings, neurobehavioral performance, markers of nasal inflammation and lung function in response to the following exposure conditions, each presented with and without psychological stress: volatile organic compounds (VOCs), VOCs with ozone (VOCs + O), and clean air with a one minute spike of VOCs (MCA).

Based on several surveys, between 800,000 and 1.2 million buildings in the United States may be associated with sick building syndrome or building related illnesses and thus, between 30 and 70 million workers are exposed to potentially unhealthy working conditions (Woods, 1989; Kreiss, 1990). Investigations focus on characterizing the air quality and ventilation systems of "problem

buildings" and the symptoms of affected workers (Apter et al., 1994; Mendell and Smith, 1990). Physical factors such as inadequate or lower ventilation rates, mechanical air handling systems, particularly those with air conditioning, and humidification have been associated, in a number of NIOSH building investigations and in several cross-sectional studies, with an increase in symptoms (Apter et al., 1994; Mendell and Smith, 1990; Nordstrom et al., 1994; Zweers et al., 1992; Sundell et al., 1991). Investigators manipulated these factors, i.e., outdoor air supply and ventilation, and found a correspondence between symptoms and air quality (Menzies and Bourbeau, 1997; Menzies et al., 1996). Specifically, Menzies et al. (1996) reported more mucosal symptoms with increased total VOC concentrations, nitrogen dioxide, and total contaminant load while systemic symptoms and eye symptoms were associated with higher dust levels. When health symptoms and exposure measures were collected simultaneously in non-problem buildings, Hodgson et al. (1991) also found the concentration of VOCs and length of time spent at the desk along with lighting were significant predictors of complaints.

While air contaminants and physical factors clearly play an important role in non-specific building related illness (NSBRI), a parallel literature addresses the effect of individual differences and psychological stress in response to these environmental variables (Ryan and Morrow, 1992; Norback et al., 1990). Consistently, the highest prevalence of symptoms occur in women with up to a 3 fold difference reported (Stenberg and Wall, 1995; Skov et al., 1989). Bachman and Myers (1995) found gender and psychological symptoms to be significant predictors of symptoms in two problem and one non-problem building. Temperature, uncomfortable humidity, and reported odors were also associated with symptoms in these buildings. Therefore, based on the consistent evidence of higher

prevalence of NSBRI in women, the current study investigated the effects of exposure and individual difference characteristics in women.

The individual and psychosocial variables investigated in studies of NSBRI include those related to work stress (e.g., work load and control), job satisfaction, interpersonal relationships at work, non-specific hyperreactivity and atopy, non-work demands (e.g. children), and psychological symptoms (Ryan and Morrow, 1992; Norback et al., 1990; Bauer et al., 1992; Stenberg et al., 1994). For example, Bauer et al. (1992) in a cross-sectional comparison between workers in sick versus a control building, found an increased number of psychological symptoms reported on the MMPI and SCL-90 among workers in the sick building. However, these measures did not distinguish between workers who did and did not meet their criteria for NSBRI. The authors suggested that sick buildings produce psychological consequences, but that these psychological symptoms did not account for the occurrence of NSBRI. Because all previous investigations have been retrospective, the impact of pre-exposure personality on the occurrence of NSBRI has not been addressed.

The personality trait of negative affect is defined as the tendency to experience negative, distressing emotions and is associated with a number of somatic illnesses whose symptoms overlap with NSBRI. For example, a higher degree of negative affect is documented for individuals with irritable bowel syndrome, chronic fatigue syndrome, and chronic pain, also characterized as syndromes with relatively non-specific symptoms (Watson and Pennebaker, 1989; Lumley et al., 1996; Lumley et al., 1997; Wise and Mann, 1994; Deshields et al., 1995). To date, no study of NSBRI has evaluated the contribution of negative affect as a personality construct on the expression of NSBRI symptoms in

exposure conditions. Therefore, the current study assessed the effect of high and low negative affect, on symptomatic responses to the proposed controlled exposure conditions.

Bad or unpleasant odors and sensitivity to odors is also reported in studies of NSBRI. Malodors are documented to increase symptom reports though not to decrease performance on cognitive tests of concentration (Knasko, 1993; Knasko et al., 1990). Moreover, olfactory evoked potentials in response to pleasant and trigeminal stimulants suggests that evoked potentials may differ as a function of emotional information associated with the odors (Kobal et al., 1992). In cases of NSBRI, unpleasant odors are associated with symptom reports (Bachmann and Myers, 1995). Individuals are known to respond differentially to unpleasant odors, which may be partially explained by biologic differences in the ability to detect and discriminate odors, but also by differential responses to the hedonics of odors. Generally, women have lower odor detection thresholds (Cowart, 1989) and are also more likely to be hypersensitive or intolerant of odors (Bell et al., 1993; Bell et al., 1995; Bell et al., 1996). Bell et al. (1995;1996) has repeatedly shown that psychological factors do not fully account for chemical odor intolerance. Further, the symptoms reported by individuals identified as odor intolerant overlap with those reported by patients with NSBRI. Thus, the current study included identification of individuals with high and low chemical odor intolerance to test the effect of this individual difference on responses to the proposed exposure conditions.

Nasal Inflammatory Responses to Acute Exposure:

In the first controlled human exposure studies, Molhave et al., 1986 demonstrated that exposure to a mixture of 23 VOCSs at a concentration of 25 mg/m^3 ("Molhave mixture") induced sensory irritation symptoms in subjects identified as having NSBRI (Molhave, Back, & Pedersen 1986; Kjaergaard,

Molhave, & Pedersen 1991). Other studies have demonstrated sensory irritation from unblinded exposure to a similar mixture of 22 VOCs (Prah 1998; Hudnell et al. 1992). Using PMNs in nasal lavage fluid (NL) as an objective marker of nasal inflammation, controlled studies using similar exposures have yielded contradictory results(Pappas et al. 2000). Koren et al.(1992) observed a 2-fold increase in nasal lavage PMNs immediately following a 4-hour exposure at 25 mg/m^3 (Koren, Graham, & Devlin 1992), whereas Pappas et al., 2000 found no significant increase in PMNs in 15 subjects after similar exposures at 25 mg/m^3 and 50 mg/m^3(Pappas et al. 2000).

Some investigators hypothesize that the irritation potency of VOCs may be enhanced by oxidation reactions that occur in indoor air (Wainman et al. 2000; Weschler 2000). Some unsaturated VOCs, such as terpenes, can be rapidly oxidized in indoor air. Low-level ozone, generated by office equipment or infiltrating from outdoors, reacts with terpenes in indoor air to form a complex mixture of potentially irritating products that include hydroxyl radicals, aldehydes, carboxylic acids, and fine particles (Wainman, Zhang, Weschler, & Lioy2000; Wolkoff et al. 2000). Animal inhalation studies have demonstrated that these reaction products are considerably more irritating than their precursor compounds (Rohr et al. 2002; Wilkins et al. 2001), but no controlled studies of human exposure to VOCs-ozone reaction products have been published.

In summary, the current study assessed the effects of specific individual differences on subjective (i.e., symptoms) and objective (i.e., markers of inflammation, neurobehavioral performance, lung function) indicators of health effects. In addition, the current study assessed objective (neuroendocrine) and subjective (symptoms) responses to the interaction of chemical exposures and

stressors similar to those experienced in an office environment. Finally, the current study added an untested exposure dimension created by combining VOCs with ozone.

METHOD

Subjects (Table 1): One-hundred and thirty healthy, non-smoking women with a mean age of 27 and an average education of 15 years were recruited from the community, using advertisements in local newspapers and on radio stations. Subjects with any of the following health conditions were excluded: neurologic disease or brain injury, stroke or cardiovascular disease, serious pulmonary disease including asthma, liver or kidney disease, serious gastrointestinal disorders, known endocrine disease, pregnancy or lactation, and major psychiatric conditions to include psychoses, bipolar disorder, alcoholism or drug abuse, and multiple chemical sensitivities (MCS) with significant illness behavior or disability.

Table 1: Demographics and Pretest Characteristics

	Mean (SD)	Range
Age	27.2 (8.0)	20-45
Years Education	15.2 (1.9)	12-22
Chemical Odor Intolerance	8.3 (3.1)	4-19
Negative Affect	17.8 (5.9)	10-38
Stress Rating of Life Events-Past Year	8.0 (0.8)	0-42
Marlowe Crowne Social Desirability	8.1 (2.9)	0-16

Race:	Percent (Frequency)
Caucasian	56 (73)
Black	10 (13)
Hispanic	8 (10)
Asian	20 (26)
Other	6 (8)

Individual Difference Scales: To assess negative affect (NA), the PANAS-X (Watson and Clark, 1994) was administered. Negative affect is associated with the personality dimensions of neuroticism, measures of psychopathology, and somatic complaints (Clark and Watson, 1991; Watson and Clark, 1994; Clark et al., 1994). The Chemical Odor Intolerance Index was used as an index of illness associated with chemical odors; it has documented reliability and factorial validity in healthy adult samples (Bell et al., 1995; Bell et al., 1996; Baldwin et al., 1997; Szarek et al., 1997). To control for the effects of external life events on exposure related health effects, the PERI Life Events Scale was administered to assess the occurrence and stress rating for a standard list of negative life events during the past year (e.g., employment or family problems) (Dohrenwend et al., 1978).

Vigilance: A vigilance task was used to maintain subject alertness without inducing reactivity and has been used in our previous study (Fiedler et al., 2000). This is a simple task in which the subject must observe the display on the computer screen and count quietly to herself the number of times a bar display appears on the screen. At the end of the task the subject is asked to report how many bars she counted.

Stressor - Public Speaking Task: This task required the subject to construct and deliver a 4 minute speech after a 4 minute silent preparation period. The speeches were videotaped and the subject was told that three staff members of the laboratory would evaluate tapes of her performance; she received an additional stipend of $10. if she performed well. In addition, the experimenter listened to the speech while the subject was delivering it. Three distinct scenarios were used as described in al'Absi et al. (1997).

Symptom Questionnaire, Environmental Quality, Odor Ratings, Exposure Evaluation, Distress Rating of Experimental Procedures (Table 2): A questionnaire identifying the following symptom clusters was used: 1) VOC general symptoms; 2) VOC cognitive symptoms; 3) eye irritation; 4) anxiety; 5) upper respiratory; 6) lower respiratory; 7) general somatic. Symptoms associated with VOCs were those used in other studies and reflect general symptoms such as headache, cognitive disturbance, and mucosal irritation (Molhave et al., 1986; Hudnell et al., 1992), whereas symptoms of anxiety were those reflective of state anxiety, tension, and mood change. Symptoms associated with ozone were upper and lower respiratory symptoms (Horstman et al., 1990). General somatic symptoms were considered "control" symptoms that were not specifically associated with exposure (Dalton, 1997). The scale used to rate symptoms was developed by Green et al. (1996) to approximate a ratio scale (0 = no sensation to 100= strongest imaginable). Environmental Qualities were each rated on a one to five scale. Subjects rated their confidence in "guessing" the exposure condition (1= not at all confident to 5= completely confident), the strength of the exposure (1= very weak to 5= very strong), their annoyance with the physical working conditions and symptoms during exposure (1= not at all annoying to 5= extremely annoying), and their distress with the experimental procedures all on a 5 point scale (1= not at all stressful to 5= very stressful).

Table 2: Symptom Clusters, Environmental Quality, Odor Ratings, Exposure Evaluation and Distress Ratings

VOC GENERAL	VOC COGNITIVE	EYE IRRITATION
headache	difficulty concentrating	eye irritation(burning, dryness, or itching)
fatigue	disoriented/confused	runny/watery eyes
lightheaded	dizzy	
drowsy		
nausea		

ANXIETY	UPPER RESPIRATORY	LOWER RESPIRATORY
feel jittery in body	sneeze	short of breath
feel nervous	nasal congestion	wheezy
heart palpitations	choking	chest tightening
feel tense	throat irritation (burning or dryness)	chest pain
worried	nose irritation, dryness or itching	coughing

SOMATIC CONTROL	ENVIRONMENTAL QUALITY
skin irritation or dryness	lighting
stomachache	noise level
numbness/tingling	room temperature
ear ringing	humidity
leg cramps	air movement
back pain	air quality
sweating	odor level
body aches	ventilation

ODOR RATING	EXPOSURE EVALUATION	DISTRESS RATINGS
intensity	confidence in guess	chemical exposures
unpleasantness	strength of exposure	stressor task
irritation	physical work conditions	neurobehavioral task
	symptoms during exposure	odor of exposure
		typing task
		vigilance task
		questionnaires
		saliva samples
		nasal washing
		breathing test

Neurobehavioral: This computerized divided-attention test of cognitive performance, Performance On-Line (POL), (Mills et al., 1996) offered five different levels of complexity. The test was validated in alcohol dosing trials and was developed explicitly for use in repeated measures studies of alcohol and drug effects. POL included a central task in which the subject was presented with two lanes of traffic, divided by a double yellow line. Four conditions of "headlights" and "taillights" appeared on any one trial. The subject was instructed to press the space bar only when a "safe" condition (i.e., left lane, white headlights and right lane, red tail lights) existed. The peripheral task required the subject to respond with one of four arrow keys (up, down, left, right) in the direction of the critical stimulus (red octagon among other shapes). Task difficulty increased by increasing the number of distracting stimuli in the peripheral display to a random assortment of different colored circles, squares, and triangles. For the divided-attention display, the subject responded to both central and peripheral critical stimuli. A composite performance score, composed of 7 component scores to include hits, misses, false positives, response latency, and responses to targets at varying levels of visual angle, was the performance variable measured.

Cortisol assays: An extensive literature documents the significant (r>=.90) association between salivary and plasma cortisol (Kirschbaum and Hellhammer, 1989;1994). Salivary flow, which may be affected by anxiety, is not documented to affect the concentration of salivary cortisol, (Dirks et al., 1988; Kahn J-P et al., 1988) though circadian rhythm affects cortisol production (Walker et al., 1984). Thus, subjects were tested at the same time of day to control for the well-known circadian effect on cortisol production. Kirschbaum & Hellhammer (1989;1994) also reported that while a highly significant correlation is shown between salivary and plasma cortisol, absolute values vary significantly. However, in the present study, relative change scores

rather than absolute values were evaluated. Hormonal fluctuations also affect cortisol levels. Therefore, salivary estradiol was measured at baseline before each exposure session to account for the effects of ovulation on salivary cortisol.

Consistent with Kirschbaum and Helhammer, (1989) samples were collected by using the "Salivette" (Sarstedt Inc., Rommelsdorf, FRG) method. The subject was asked to chew on a cotton swab for 60 seconds and then place the swab into a salivette holder and affix the cap. The samples were centrifuged at 3000 rpm for 10 minutes which produced 0.5 to 1 ml. The saliva was frozen though no alteration of salivary cortisol values were shown after storage of samples at room temperature for up to 2 weeks. Samples were analyzed, blind to exposure condition and subject characteristics, in one of the co-investigators laboratories (JO). Samples were run in duplicate and equal numbers of samples from each group were run in the same assays.

Controlled Environment Facility and Exposure Generation:

The EOHSI Controlled Environment Facility (CEF) is a 2.2 m high by 4.1 m wide by 2.7 m deep stainless steel room with a total volume of $25m^3$. To simulate a poorly ventilated office building and to allow sufficient time for the formation of ozone-alkene reaction products, the air flow rate through the chamber was reduced to 1.8 ± 0.2 air exchanges per hour for all the exposures conducted in this study. The air supply enters the chamber through two diffusers in the ceiling and exits through the perforated stainless steel floor to the exhaust vents. All controls were computer interfaced to maintain constant conditions in the chamber. Small brushless (to prevent unwanted particle generation from brush degradation) fans were used in the partitioned area to control the air exchange rate within the CEF and to ensure that the air was well mixed. A Teflon partition separated the subjects work stations, which were equipped with a computer and typing

stand on a stainless steel table. The technician communicated with the subject through headphones and could view subjects at all times through a 2-way window.

Introduction of chemicals at desired concentrations: The composition and relative weight of the VOCs mixture used are similar to previous indoor air studies and are shown in Table 3 (Otto et al. 1992; Molhave et al. 1986; Kjergaard et al. 1991). D-limonene, the most frequently identified terpene in indoor air, was also added to the mixture. A flask containing the liquid mixture of these 23 compounds was heated to 250°C by a hot plate, flash evaporated and injected into the clean air stream that delivered the VOCs mixture into the CEF. The total VOCs concentration in the air of the indoor environment was approximately 26 mg/m^3 with the concentration of each compound (see Table 3) below the threshold limit value (TLV) recommended for occupational exposure. The desired concentrations of VOCs in the indoor environment was achieved by adjusting the flow rate of the delivering air using a mass flow controller.

Table 3. The Mixture of VOCs

No.	Compound	Relative weight	Concentration (mg/m^3)
1	n-Butylacetate	10	8.25
2	p-Xylene	10	8.25
3	n-Butanol	1	0.825
4	n-Decane	1	0.825
5	1-Decane	1	0.825
6	1,1,-Dichloroethane	1	0.825
7	d-Limonene	1	0.825
8	Enthylbenzene	1	0.825
9	Ethoxyethylacetate	1	0.825
10	n-Hexanal	1	0.825
11	n-Hexane	1	0.825
12	n-Nonane	1	0.825
13	α-Pinene	1	0.825
14	2-Butanone	0.1	0.083
15	Cyclohexane	0.1	0.083
16	3-Methyl-2-butanone	0.1	0.083
17	4-Methyl-2-pentanone	0.1	0.083
18	n-Pentanal	0.1	0.083
19	Isopropanol	0.1	0.083
20	n-propylbenzene	0.1	0.083
21	1,2,4-Trimethylbenzene	0.1	0.083
22	n-Undecane	0.1	0.083
23	1-Octene	0.01	0.008
Total			26.330

Masked clean air was generated by introducing a one minute spike of the VOCs mixture at 0.7 ppm or approximately 10% of the exposure concentration for the VOCs exposure condition.

Ozone generated *in situ* by an ozone generator was delivered into the indoor environment. A steady-state concentration of 40 ppb of ozone was maintained in the indoor environment. This was accomplished by adjusting the power level of the ozone generator and/or the flow rate of

delivering air. This is consistent with the intention to examine the effect of ozone/VOCs by-products rather than ozone, itself. Temperature and humidity were maintained at in a range from 73° –78° F (23° - 26°C) and 24-49%, respectively, as done by Otto et al. (1992).

Nasal lavage (NL): Nasal lavage with normal saline was performed before and after exposures using a nasal spray technique described by Peden (Noah et al. 1995). Briefly, a nasal metered dose inhaler (100 uL per spray) was used to wash out one nostril while the other was held occluded. After 5 sprays, the subject gently exhaled through the washed nostril, expelling the lavage fluid into a cup. This was repeated 8 times in each nostril for a total of 8 ml of saline, of which approximately 50% was recovered. The sample was immediately placed on ice and processed within 2 hours.

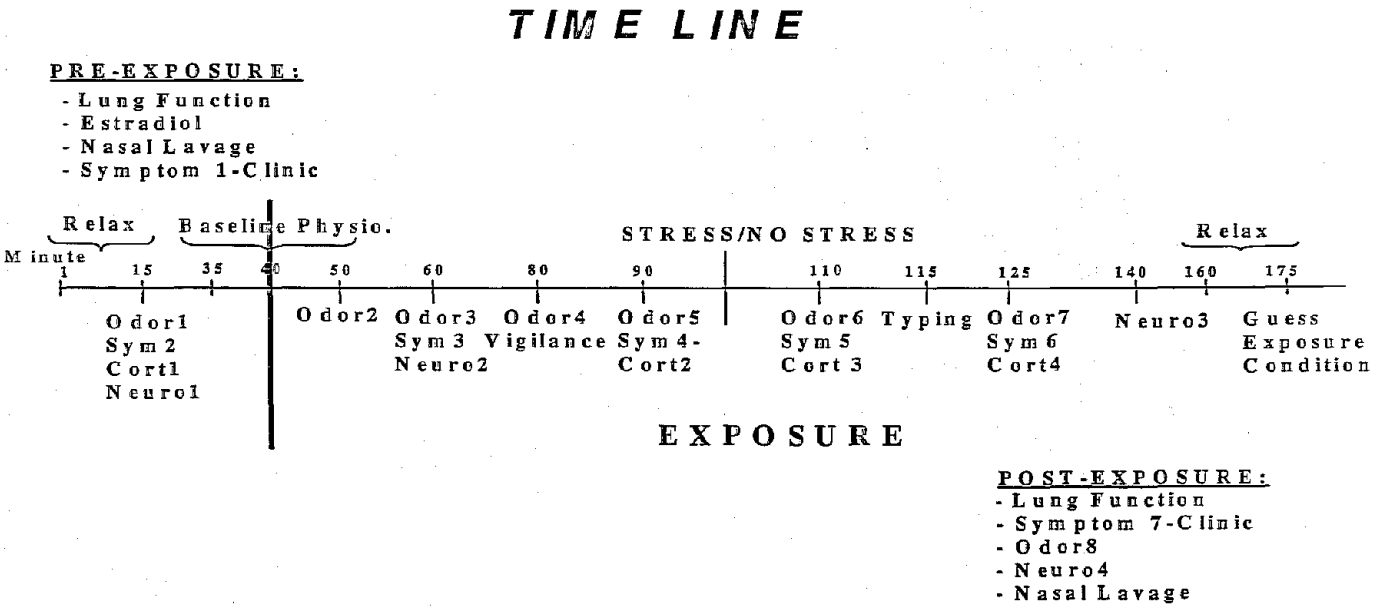
Processing of NL fluid: NL samples were filtered through a 60 μm nylon mesh syringe filter to remove large particles and solids, prior to centrifugation at 400x g for 5 minutes. The supernatant was removed, aliquotted into labeled cryovials (0.4 ml) and stored at -80°C until used for cytokine and protein assays. Cells were resuspended in 2 ml phosphate buffered saline (PBS) and centrifuged at 400 x g for 5 minutes, then resuspended in 0.5-1.5 ml of PBS, pH 7.4. A 100 μl aliquot was taken for counting in the Coulter counter. 100 μl of cell suspension were centrifuged in the Shandon cytospin for 8 minutes at 800 rpm. Slides containing the cells were stained with Wright's-Giemsa quick stain for 1 minute and washed in distilled water for 2 minutes. The slides were air dried and 200 cells were counted.

Analysis of Soluble Proteins: IL-6 and IL-8 levels were measured using ELISA kits purchased from R&D systems (Minneapolis, MN), following the manufacturer's instructions. Protein

levels were determined using a centrifugal chemical analyzer and reagents purchased from Roche Laboratories, following instructions from the manufacturer.

Procedure (See Figure 1):

FIGURE 1



Prior to receiving a complete physical examination including a medical history review and routine blood chemistries, subjects who met inclusion criteria were given informed consent according to the UMDNJ-IRB. To reduce practice and novelty effects, subjects were shown the Controlled Environment Facility (CEF) and trained to perform the following procedures: the Symptom Questionnaire, the speech and the neurobehavioral tasks, salivary cortisol, spirometry, and nasal lavage. The study was double blind and subjects were randomly assigned to the order of exposure conditions with or without the stressor.

Each experimental session was 3 hours in duration and occurred in the morning. On the day before and on the day of the testing session, subjects were asked not to use caffeine or alcohol. Subjects also could not have an active upper respiratory illness (either infection or allergy) nor use medication for allergies or other respiratory conditions for one week prior to each exposure session.

Two subjects were tested at the same time. On the day of the first exposure session, a pregnancy test was given. A spirogram was performed for baseline assessment prior to each exposure session. Subjects completed the symptom questionnaire, practiced the POL and performed baseline nasal lavage and salivary cortisol (estradiol).

Subjects were escorted to the CEF where they were seated at a table and asked to rest quietly for 15 minutes after which they completed the symptom questionnaire, environmental quality and odor ratings, collected salivary cortisol, and performed the neurobehavioral task. The exposure period began 40 minutes after the beginning of the session. During the initial 20 minutes of the exposure, subjects were allowed to read quietly. During the next 20 minute phase, subjects completed the symptom questionnaire, environmental quality and odor ratings, and the neurobehavioral task. Each subject then completed the 10 minute vigilance task after which salivary cortisol samples and the symptom questionnaire, environmental quality and odor ratings were collected. For those subjects assigned to the exposure conditions with the psychological stressor, the public speaking task was then administered followed by salivary cortisol collection, environmental quality and odor ratings, and the symptom questionnaire. Subjects assigned to exposure conditions without the stressor completed simple arithmetic problems for this period of

10 minutes and then completed salivary cortisol collection, the symptom questionnaire, environmental quality and odor ratings. After this period, all subjects typed a standard text for 10 minutes after which they completed the symptom questionnaire, environmental quality and odor ratings, the neurobehavioral task, and post-stress salivary cortisol. After completing the tasks, subjects were asked to guess the exposure condition, to rate their confidence in the guess, the strength of exposure and their annoyance due to the physical working conditions and symptoms during exposure. Subjects were escorted to the Clinical Center where they performed spirometry, completed the final symptom questionnaire and environmental quality ratings, rated their distress in response to each of the experimental tasks, performed the neurobehavioral task and post-exposure nasal lavage.

STATISTICAL ANALYSIS

Symptoms: The effects of exposure, stress, NA, CI, and time on both symptom severity and presence/absence of any symptom were analyzed. For presence/absence, if a subject reported any symptom at all, then a "yes" was recorded; otherwise a "no" was recorded.

For presence/absence, a hierarchical logistic regression (Davidian and Giltinan, 1995 and McCulloch and Searle, 2000) modeled the log-odds of no symptoms being reported for assessment at minute 15 (baseline) through minute 185 (after removal from exposure chamber). For symptom severity, data were analyzed using a hierarchical Poisson regression model. In both cases, generalized estimating equations were used that accounted for correlations between repeated measurements on the same individual (Liang and Zeger, 1986). Tests of the exposure effects were conducted using Type 3 score tests (Liang and Zeger, 1986) of the interaction between exposure and time. Time was entered into the model as a categorical variable.

Contrasts were used to test whether individual changes in symptoms from baseline (minute 15) to minute 60 through minute 185 (post-exposure) differed between exposures. The mean odds of reporting symptoms or the mean severity of symptoms at baseline were assumed to be the same for all three exposures. This analysis was first completed for the total symptoms and then for each classification of symptoms including VOC general, VOC cognitive, eye irritation, anxiety, upper respiratory, lower respiratory and somatic control symptoms. Results were based on the 130 subjects who received all three exposures including the MCA condition. Uncorrected alpha values are reported with the alpha level after Bonferroni correction noted for each group of multiple comparisons.

Negative Affect, Chemical Intolerance, and Exposure Interaction: Negative Affect (NA) and Chemical Intolerance (CI) scores for all subjects were approximately divided into tertiles. In particular, the first tertile of NA scores was composed of scores from 0 through 13, the second tertile of scores from 14 through 18, and the third tertile of scores that were 19 and above. For Chemical Intolerance, the tertiles ranged from 0 through 6, 7 through 9, and 10 and above. As expected, an individual's classification into tertiles based on these two measurements was associated (chi-square=20.43, d.f.=4, p-value=0.0004). When evaluating the effects of NA and CI, it was expected that unless the upper and lower tertiles were compared directly, there would not be enough differentiation for powerful comparisons. Thus the middle tertiles were dropped in these analyses. As such, analyses that considered NA and CI separately contained 93 and 86 subjects, respectively. A combined "NACI" classification was created such that "low/low" corresponds to those who were classified as low on both scales, "high/high" corresponds to those who were classified as high on both scales and "mid" to correspond to participants who were

classified as high on one scale and low on the other. Analyses with this combined "NACI" classification included 64 subjects. Using tertiles, NA, CI and the combined NACI may then be used in the models as the categorical predictors.

Formal analyses of the interaction between exposure and NA/CI scores were based on the same general estimating equations (GEE) models used for assessing the main effect of exposure. These models included the main effect terms representing exposure and NA/CI ranking as well as the interaction between the two predictors. Estimation and hypothesis testing was conducted using the SAS programming language (SAS Institute, 1999).

Nasal PMNs: Descriptive statistics calculated for the PMN concentrations included means and percentiles, both at baseline and post-exposure. Since the distributions of these concentrations were largely non-normal, robust estimates of the means and corresponding 95% confidence intervals were calculated based on geometric means. To calculate geometric means and associated confidence intervals, PMN concentrations were first transformed to the logarithmic scale. A value of 0.1 was added to PMN concentrations of zero before taking the log-transform. The means and confidence intervals of the log-concentrations were transformed back to the original scale to get the robust means and confidence intervals.

Differences between exposures were evaluated by using stratified Cochran-Mantel-Haenszel tests for ordinal responses with exposure as a nominal predictor (Landis, et al., 1998; SAS Institute Inc., 1999). Each subject represented one stratum since each subject participated in sessions for all three exposures. Four ordinal categories for the PMN concentrations were

created by dividing the baseline values into zero concentrations and non-zero concentrations where the latter were further divided into tertiles. Four ordinal categories provided adequate division of values to be informative about the magnitude of the effect while simultaneously providing enough information about each cell for accurate estimation. Linear models were inappropriate because of the extreme skewness of the raw PMN concentrations or of any response representing the change in PMN concentration and because of the large proportion of zero PMN concentrations (36.2% at baseline and 32.1% post-exposure).

The odds of PMN concentration increasing from baseline to post-exposure were calculated for each exposure using a proportional odds model (Ohman-Strickland and Lu, 2003; Agresti and Lang, 1993) in conjunction with the ordinal categories described previously. More precisely, the odds ratio that compared the odds of being in a specific category or higher for individuals who were in lower categories with the same odds for individuals that were already in the higher categories were estimated.

The above analyses of PMN concentrations were repeated for a subset of symptomatic "responders" and a subset of atopic individuals. Responders were defined as individuals who had larger increases in nasal irritation from baseline to minute 125 in either the VOCs or the VOCs + O exposure as compared to in the MCA exposure. Out of the 130 subjects, 49 were identified as "responders." Atopic individuals were defined as those with a RAST (radioallergo-sorbant test) class score of at least 2 for at least one of the five tested allergens (tenuis, cat dander, ragweed, Farinae, pteronyssinus). Of the 130 subjects, 51 were identified as "atopic" using this criterion.

Statistical Analysis of Soluble Proteins: A mixed linear model was used to examine the effect of exposure on the soluble measures of IL-6, IL-8 and total protein for all subjects, for atopic subjects, and for the responders. The change in the log concentrations of these proteins [log(post)-log(pre)=log(post/pre)] was normally distributed.

Statistical Analysis of Lung Function: Lung function variables were normally distributed. Therefore, mixed linear models were used to model the effect of exposure. In particular, the mixed linear model for each variable includes a single predictor (fixed effect) for exposure and a random effect for the subject that accounts for correlation between sessions within a subject. One mixed model is fitted for each of the response variables associated with lung function, the difference between pre- and post-exposure scores for FVC (change FVC), the percent change in FVC (% change FVC), absolute change in FEV1, percent change in FEV1, absolute change in FEV1/FVC, percent change in FEV1/FVC, absolute change in PEFR, percent change in PEFR, absolute change in FEF_{25-75}, and percent change in FEF_{25-75}.

Environmental Quality and Odor Ratings: A repeated measures linear model to account for correlation between responses from the same subject was used to examine the main and interaction effects of exposure, stress, negative affect (NA), chemical intolerance (CI), and NA/CI on intensity, irritation, and unpleasantness ratings of odor and on the following measures of environmental quality: lighting, air quality, air movement, noise, odor level, room temperature, humidity, and need to ventilate.

Evaluation of and Distress Ratings of Exposure and Experimental Procedures: Chi-square analysis was used to determine subjects' accuracy regarding the actual exposure during each session. A repeated measures analysis of variance followed by paired post-hoc tests was used to compare the effect of exposure on ratings of confidence of their guess, strength of the exposure condition and ratings of annoyance with the work conditions and symptoms, and ratings of distress related to the various experimental procedures (e.g., nasal lavage, exposure, speech task).

RESULTS

Aim 1: To assess, in two separate repeated measures designs, the symptomatic, neuroendocrine, neurobehavioral, inflammatory, and lung function effects of controlled exposures to MCA, VOCs, and VOCs + O. Each exposure condition was presented with or without a psychological stressor to four groups of women high and low in the self-reported attributes of negative affect (NA) and chemical odor intolerance (CI).

Aim **1.1 Symptoms**: To assess symptoms associated with VOCs, ozone, and stress.

Hypothesis 1: (Exposure Main Effect): Subjects will report significantly more symptoms in the VOCs and VOCs + O condition than the MCA condition with significantly higher levels of symptoms in the VOCs + O condition.

After controlling for baseline symptom reporting (minute 15), Table 4 summarizes the overall test of the exposure main effect for total symptoms and each symptom subscale. Based on these overall tests with Bonferroni corrections, Hypothesis 1 was not confirmed (Figure 2). However, marginal effects for presence/absence of VOC general symptoms and severity of lower respiratory symptoms were observed.

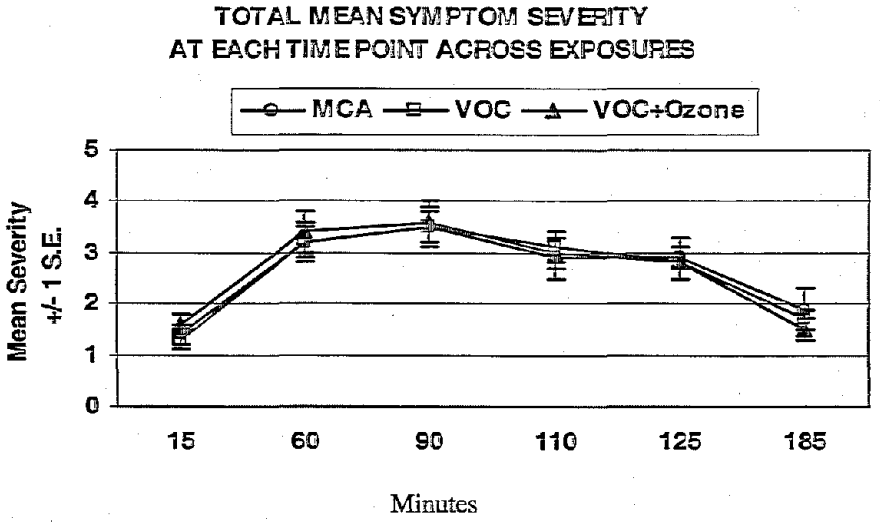
Table 4: Test statistics and p-values based on generalized estimating equation for each symptom subscale (All chi-square tests are based on 10 degrees of freedom).

Exposure Main Effect

	Presence/Absence		Severity	
	χ 2 Statistic	P-value	χ2 Statistic	P-value
Any Symptom	9.98	0.442	5.51	0.855
VOC General	18.01	0.054	5.83	0.829
VOC Cognitive	16.33	0.091	12.47	0.255
Eye Irritation	15.63	0.111	7.01	0.724
Anxiety	16.20	0.094	5.91	0.823
Upper Respiratory	11.13	0.347	13.49	0.197
Lower Respiratory	5.88	0.825	16.92	0.076
Somatic	9.87	0.452	14.98	0.133

Bonferroni Correction: $p<0.003$

FIGURE 2



To explore the marginal exposure effect for severity of lower respiratory symptoms (Figure 3), the effects of exposure at each time point for this symptom subscale were explored (Table 5). These results show that relative to the MCA condition, lower respiratory symptoms were more pronounced relative to baseline later during both the VOCs and VOCs + O exposures (Min 125).

FIGURE 3

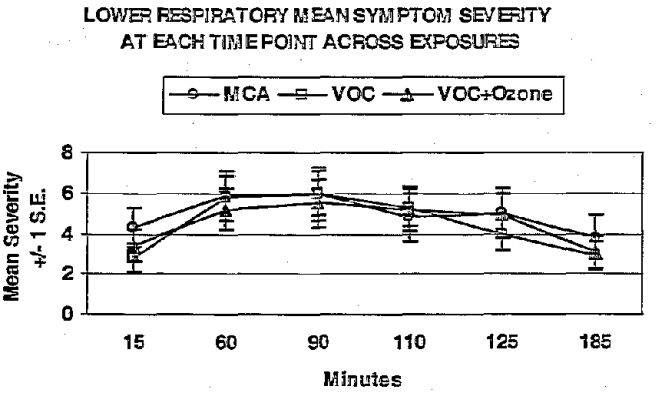


Table 5: P-values for comparing changes in symptom severity reports of **Lower Respiratory Symptoms.**

Exposure X Time Interaction

Change from Minute 15 (baseline) to	VOCs vs. MCA	VOCs + O vs. MCA
Minute 60	0.3437	0.2087
Minute 90	0.0348	0.0595
Minute 110 (post-stress)	0.0494	0.3087
Minute 125	0.0007*	0.0035*
Minute 185 (post-exposure)	0.0001*	0.0301

*Bonferroni Correction: $p < 0.005$

The marginal effect of presence/absence of VOC general symptoms was explored using contrasts. Specifically, the odds of reporting any VOC symptom were compared relative to baseline at each time point using odds ratios. Table 6 reveals that subjects show an increasing tendency to report some VOC general symptoms after VOCs or VOCs + O exposure onset (minutes 60, 90 and 110).

Table 6: Odds ratios (first row in each cell) compare the odds of reporting **VOC General Symptoms** versus not reporting symptoms for times after the start of exposure and baseline. P-values for testing the effect of exposure against MCA at each specific time (second row) are in italics. The overall p-value for the effect of exposure is 0.054 (chi-square=18.01 with 10 d.f.)

Exposure X Time Effect

Time	MCA	VOCs	VOCs + O
Min 60	1.943	3.160	**4.096**
	--	*0.022*	***0.002***
Min 90	2.219	3.845	3.321
	--	*0.029*	*0.014*
Min 110 (post-stress)	1.131	1.688	1.400
	--	*0.041*	*0.297*
Min 125	1.327	1.825	1.699
	--	*0.128*	*0.242*
Min 185 (post-exposure)	0.792	0.900	0.945
	--	*0.483*	*0.332*

Bonferroni Correction: $p<0.005$

Marginal effects of exposure on presence/absence of VOC cognitive and anxiety symptoms were also explored in the same manner and revealed an increasing tendency to report some VOC cognitive symptoms after the stressor (minute 110) in the VOCs and VOCs + O exposures relative to MCA. Anxiety symptoms were also more likely to be reported at minute 125 in the VOCs but not the VOCs + O exposure when compared to the MCA exposure (data not shown).

Hypothesis 2: (Exposure x NA x CI Interaction): High NA, high CI subjects will report significantly more symptoms than low NA, low CI subjects in response to VOCs and VOCs + O relative to MCA. Subjects who are high NA, low CI and low NA, high CI will report significantly more symptoms than low NA, low CI but significantly less symptoms than high NA, high CI in response to both VOC exposure conditions. The VOCs + O condition will produce significantly more symptoms of irritation than the VOC or MCA conditions.

Results from Table 7 do not confirm either hypothesis predicting that high NA/high CI subjects or either high NA/low CI or low NA/high CI subjects will report increased severity of symptoms or more likelihood of reporting any symptom than low NA/low CI subjects in response to exposure.

Table 7: Test statistics and p-values based on generalized estimating equations for each symptom subscale (All chi-square tests are based on 20 degrees of freedom.)

Exposure X NA/CI (upper/lower tertiles) Interaction

	Presence/Absence		Severity	
	X^2 Statistic	P-value	X^2 Statistic	P-value
Total Symptoms	Not Estimable	Not Estimable	19.63	0.4811
VOC General	20.57	0.4227	20.59	0.4219
VOC Cognitive	16.57	0.6804	13.45	0.8572
Eye Irritation	19.46	0.4923	14.84	0.7854
Anxiety	25.03	0.2001	18.52	0.5529
Upper Respiratory	14.55	0.8015	23.34	0.2725
Lower Respiratory	19.89	0.4651	17.06	0.6490
Somatic	21.06	0.3935	20.11	0.4511

Bonferroni Correction: $p<0.003$

Hypothesis 3: (Exposure x Stressor Interaction): VOCs and VOCs + O with the stressor will produce significantly more symptoms than VOCs and VOCs + O without the stressors and than MCA with and without the stressor.

Table 8 displays the overall test of the interaction of stress and time for presence/absence of symptoms and symptom severity. Subjects who were given the stressor reported significantly more symptoms of anxiety over time than those who did not. Furthermore, relative to baseline either before (minute 15) or after exposure onset (minutes 60 and 90), symptoms of anxiety were significantly greater after the stressor at minutes 110 and 125 (Table 9). However, the

hypothesis of an exposure X stressor interaction was not confirmed either for the presence/absence or severity symptom response (Table 10).

Table 8: Test statistics and p-values based on generalized estimating equation for each symptom subscale. (All chi-square tests are based on 5 degrees of freedom.)

Stress Main Effect

	Presence/Absence		Severity	
	χ^2 Statistic	P-value	χ^2 Statistic	P-value
Total Symptoms	7.46	0.189	10.48	0.063
VOC General	10.89	0.054	6.72	0.242
VOC Cognitive	5.38	0.372	2.73	0.741
Eye Irritation	2.21	0.819	5.81	0.325
Anxiety	24.33	0.0002	22.73	0.0004
Upper Respiratory	1.45	0.919	5.82	0.324
Lower Respiratory	4.55	0.474	4.17	0.525
Somatic	3.03	0.696	4.99	0.417

Bonferroni Correction: $p<0.003$

Table 9: Test statistics and p-values for comparing changes for **Anxiety Symptoms**

Stress X Time Interaction

	Presence/Absence*		**Severity****	
Change from Min. 15 (Baseline) to	X^2 Statistic	P-value	χ^2 Statistic	P-value
Min. 60	1.91	0.168	0.12	0.730
Min. 90	0.13	0.719	0.07	0.798
Min. 110 (post-stress)	21.07	<0.0001	15.17	<0.0001
Min. 125	5.93	0.015	0.15	0.701
Min. 185 (post-exposure)	1.35	0.246	0.01	0.941
Change from Min. 60 (after exposure onset) to				
Min. 90	1.49	0.222	0.65	0.421
Min. 110 (post-stress)	15.05	0.0001	12.52	0.0004
Min. 125	1.85	0.173	0.01	0.937
Min. 185 (post-exposure)	0.05	0.816	0.12	0.730
Change from Min. 90 (pre-stress) to				
Min. 110 (post-stress)	22.67	<0.0001	30.16	<0.0001
Min. 125	5.55	0.0185	0.62	0.431
Min. 185 (post-exposure)	0.53	0.469	0.01	0.911
Change from Min. 110 to				
Min. 125	11.00	0.0009	22.29	<0.0001
Min. 185 (post-exposure)	14.68	0.0001	11.64	0.0006
Change from Min. 125 to				
Min. 185	2.12	0.145	0.29	0.593

*Bonferroni Correction for Presence/Absence: $p<0.003$

**Bonferroni Correction for Severity: $p<0.003$

Table 10: Test statistics and p-values based on generalized estimating equations for each symptom subscale testing. (All chi-square tests are based on 10 degrees of freedom.)

Exposure X Stress Interaction

	Presence/Absence		Severity	
	X^2 Statistic	P-value	X^2 Statistic	P-value
Any Symptom	14.10	0.1684	8.50	0.5797
VOC General	9.50	0.4854	11.17	0.3445
VOC Cognitive	9.54	0.4821	4.63	0.9142
Eye Irritation	10.99	0.3584	6.66	0.7567
Anxiety	11.60	0.3128	10.63	0.3868
Upper Respiratory	10.48	0.3998	11.40	0.3272
Lower Respiratory	6.15	0.8029	8.34	0.5961
Somatic	7.22	0.7047	8.65	0.5658

Bonferroni Correction: $p<0.003$

Hypothesis 4: (Exposure x NA x Stressor Interaction): Regardless of CI, high NA subjects will report significantly more symptoms than low NA subjects when exposed to VOCs and VOCs + O with the psychological stressor than when those exposure conditions do not include the stressor.

In Hypothesis 4, chemical intolerance was ignored and only subjects who scored low and high on the NA scale were compared. In particular, subjects were divided into tertiles according to their score on NA. Subjects in the middle tertile were dropped from the analysis. Of the remaining 93 subjects, 48 and 45 were ranked in the lowest and highest tertiles, respectively. The hypothesis that high NA subjects would report more total symptoms than low NA subjects was not confirmed (Table 11). However, slight evidence exists that high NA subjects were more likely to report a symptom of anxiety than low NA subjects. Analysis of change in anxiety scores across time points (see Table 12) indicates that relative to the no exposure baseline (minute 15), high NA subjects were more likely to report a symptom of anxiety at minute 60 and 90 after the onset of exposure. Also, high NA subjects were more likely to report a symptom of anxiety at minutes 110 and 125 relative to minute 60.

Table 11: Test statistics and p-values based on generalized estimating equation for each symptom subscale. (All chi-square tests are based on 5 degrees of freedom.)

Main Effect of NA (upper/lower NA tertiles)

	Presence/Absence		Severity	
	χ^2 Statistic	P-value	χ^2 Statistic	P-value
Total Symptoms	5.46	0.362	5.82	0.324
VOC General	5.68	0.339	1.65	0.895
VOC Cognitive	2.35	0.800	3.71	0.592
Eye Irritation	3.62	0.606	1.72	0.886
Anxiety	16.04	0.007	5.00	0.416
Upper Respiratory	3.45	0.632	6.30	0.279
Lower Respiratory	1.68	0.891	6.50	0.261
Somatic	5.96	0.310	7.67	0.175

Bonferroni Correction: $p<0.003$

Table 12: Test statistics and p-values for comparing changes between two times for Anxiety Symptoms.

NA (upper/lower tertiles) X Time Interaction

	Presence/Absence	
Change from Min. 15 (Baseline) to	X^2 Statistic	P-value
Min. 60	7.04	0.008
Min. 90	3.05	0.081
Min. 110 (post-stress)	0.04	0.835
Min. 125	0.37	0.545
Min. 185 (post-exposure)	2.23	0.136
Change from Min. 60 to		
Min. 90	0.69	0.407
Min. 110 (post-stress)	7.23	0.007
Min. 125	14.17	0.0002
Min. 185 (post-exposure)	1.00	0.317
Change from Min. 90 to		
Min. 110 (post-stress)	3.59	0.058
Min. 125	6.62	0.010
Min. 185 (post-exposure)	0.10	0.749
Change from Min. 110 to		
Min. 125	0.26	0.607
Min. 185 (post-exposure)	2.41	0.120
Change from Min. 125 to		
Min. 185	5.14	0.023

Bonferroni Correction: $p<0.003$

The interaction of exposure and NA was not significant for any subset of symptoms (not shown) but the interaction of stress and NA was marginally significant for presence of anxiety and severity of somatic symptoms. (Again, they are not significant after application of a Bonferroni correction for multiple testing.) Table 13 summarizes the overall p-values for the NA-by-stress interaction, including all time points. Contrasts were used to look at individual time comparisons.

Table 13: Test statistics and p-values for the based on generalized estimating equation for each symptom subscale. (All chi-square tests are based on 5 degrees of freedom.)

NA (upper/lower tertiles) X Stress Interaction

	Presence/Absence		Severity	
	χ^2 Statistic	P-value	χ^2 Statistic	P-value
Total Symptoms	7.45	0.189	4.31	0.505
VOC General	6.56	0.256	3.28	0.656
VOC Cognitive	1.61	0.901	3.87	0.569
Eye Irritation	4.34	0.502	8.22	0.145
Anxiety	13.24	0.021	7.77	0.169
Upper Respiratory	6.41	0.269	4.11	0.534
Lower Respiratory	3.32	0.651	1.10	0.954
Somatic	3.24	0.663	11.18	0.048

Bonferroni Correction: $p<0.003$

Contrasts between time points suggest that high NA subjects who were in the stress condition were more likely to report an anxiety symptom after the onset of exposure (minute 60 and 90) relative to baseline (minute 15). Relative to anxiety symptoms before the stressor (minute 90), high NA subjects who were in the stress condition were also more likely to report an anxiety symptom after the stressor (minute 125) (Table 14) than were low NA subjects (Figure 4).

Table 14. Chi-square statistics and p-values for **Anxiety Symptoms**. (Chi-square tests are based on 1 d.f.)

Stress X NA (upper/lower tertiles)X Time Interaction

Difference between high and low NA in effect of stress	Presence/Absence	
Change from Min. 15 (baseline) to	$\chi 2$ Statistic	P-value
Min. 60	6.82	0.009
Min. 90	8.08	0.005
Min. 110 (post-stress)	0.59	0.443
Min. 125	0.09	0.760
Min. 185 (post-exposure)	1.14	0.286
Change from Min. 60 to		
Min. 90	0.26	0.609
Min. 110 (post-stress)	2.40	0.121
Min. 125	7.04	0.008
Min. 185 (post-exposure)	1.86	0.172
Change from Min. 90 to		
Min. 110 (post-stress)	3.75	0.053
Min. 125	8.50	0.004
Min. 185 (post-exposure)	3.06	0.080
Change from Min. 110 to		
Min. 125	0.53	0.468
Min. 185 (post-exposure)	0.04	0.833
Change from Min. 125 to		
Min. 185 (post-exposure)	0.72	0.395

Bonferroni Correction: $p<0.003$

FIGURE 4

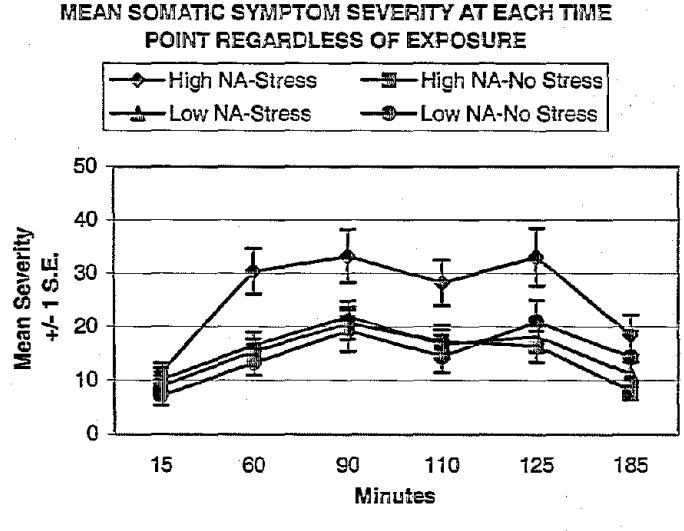


Table 15 contains similar statistics for investigating the interaction between stress and NA on severity of somatic symptoms. High NA subjects who were in the stress condition reported greater severity of somatic symptoms relative to baseline at minute 125 after the stressor. High NA subjects in the stress condition also reported more somatic symptoms after the stressor (minutes 110 & 125) relative to just before the stressor (minute 90).

Table 15. Chi-square statistics and p-values for testing the differences in severity of Somatic Symptoms. (Chi-square tests are based on 1 d.f.)

Stress X NA (upper/lower tertiles)X Time Interaction

Difference between high and low NA in effect of stress	Severity	
Change from Min. 15 (baseline) to	X^2 Statistic	P-value
Min. 60	2.41	0.120
Min. 90	1.01	0.316
Min. 110 (post-stress)	1.03	0.310
Min. 125	4.83	0.028
Min. 185 (post-exposure)	5.54	0.019
Change from Min. 60 to		
Min. 90	0.19	0.664
Min. 110 (post-stress)	0.15	0.696
Min. 125	2.38	0.123
Min. 185 (post-exposure)	2.68	0.102
Change from Min. 90 to		
Min. 110 (post-stress)	0.00	0.980
Min. 125	5.55	0.019
Min. 185 (post-exposure)	2.99	0.084
Change from Min. 110 to		
Min. 125	8.53	0.004
Min. 185 (post-exposure)	2.88	0.090
Change from Min. 125 to		
Min. 185 (post-exposure)	0.39	0.531

Bonferroni Correction: $p<0.003$

A linear model that included the interaction between exposure, NA and stress (and time) was used to assess significance of this interaction for the symptom subscales and comparisons at

individual time points. Table 16 reveals a marginally significant interaction between exposure, stressor and NA only for the anxiety subscale. Based on the results shown in Table 17 and depicted in Figures 5, 6, and 7, subjects high in NA who were given the stressor reported more symptoms in all exposure conditions, i.e., MCA, VOCs, VOCs + O, than low NA subjects and than high NA subjects without the stress. The significant 3-way interaction was revealed for low NA subjects. That is, low NA subjects who were given the stressor reported the most anxiety symptoms in the VOCs + O condition (Figures 5, 6 and 7).

Table 16: Test statistics and p-values based on generalized estimating equations for each symptom subscale using each scoring method.

Exposure X Stress X NA (upper/lower tertiles) Interaction
(All chi-square tests are based on 10 degrees of freedom.)

	Presence/Absence		Severity	
	X^2 Statistic	P-value	X^2 Statistic	P-value
Total Symptoms	NE*	NE*	11.57	0.3147
VOC General	NE*	NE*	10.32	0.4126
VOC Cognitive	4.78	0.9051	13.30	0.2072
Eye Irritation	8.38	0.5920	8.88	0.5436
Anxiety	12.53	0.2509	18.60	0.0456
Upper Respiratory	12.54	0.2507	10.36	0.4091
Lower Respiratory	12.02	0.2839	9.78	0.4602
Somatic	4.23	0.9365	10.85	0.3692

Bonferroni Correction: $p<0.003$
*NE = not estimable

Table 17: Chi-square statistics (first row) and p-values (second row in italics) for tests of changes in severity of **Anxiety Symptoms.**

Exposure X Stress X NA (upper/lower tertile) X Time Interaction

Change from Min. 15 (baseline) to	VOCs (relative to MCA)	VOCs + O (relative to MCA)
Min. 60	7.61 *0.0058*	8.09 ***0.0045***
Min. 90	7.86 *0.0051*	7.29 *0.0069*
Min. 110 (post-stress)	3.10 *0.0785*	4.55 *0.0329*
Min. 125	3.51 *0.0611*	4.78 *0.0288*
Min. 185 (post-exposure)	5.35 *0.0207*	10.97 ***0.0009***

Bonferroni Correction: $p<0.005$

FIGURE 5

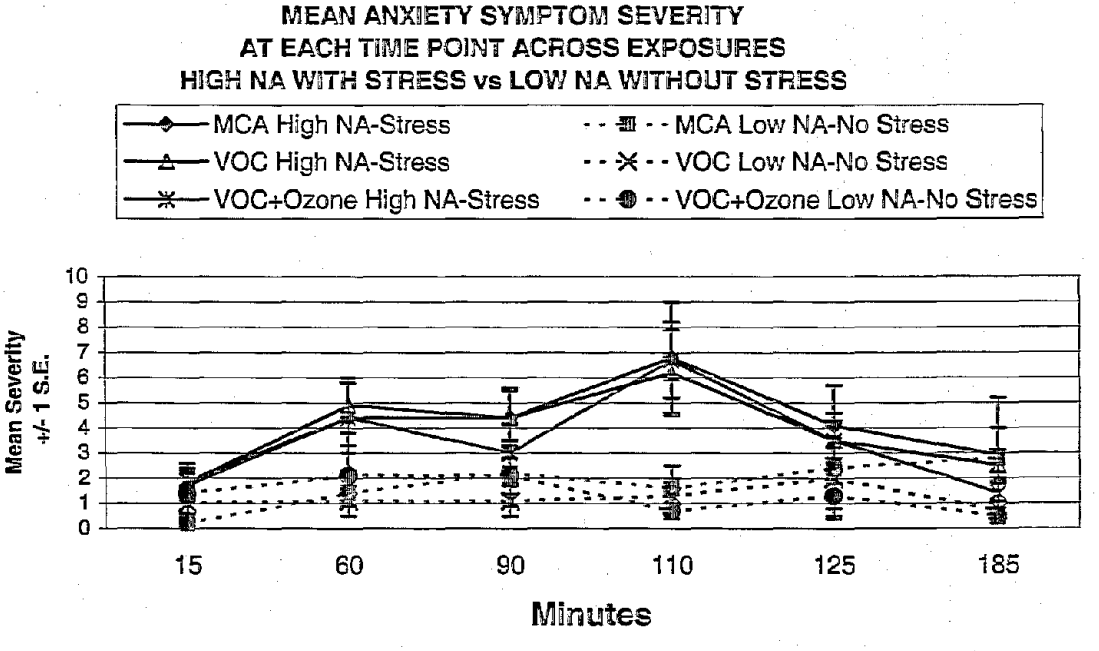


FIGURE 6

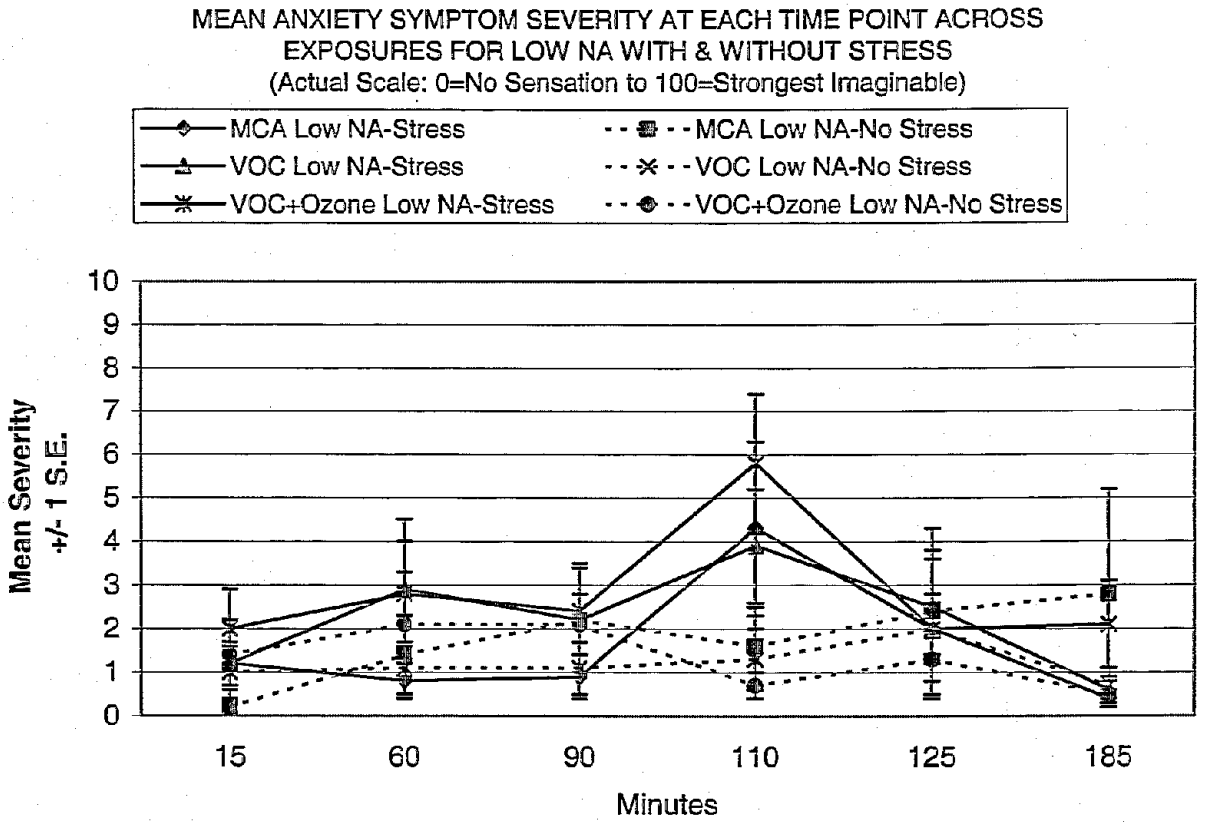
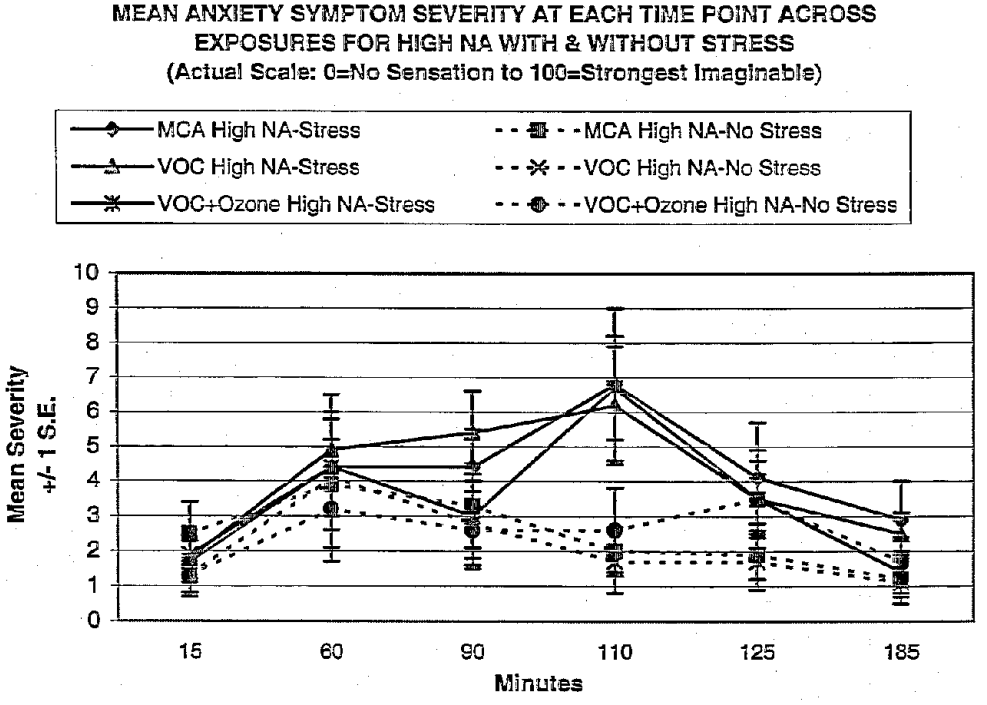


FIGURE 7



Exploratory Analysis of CI:

In addition to the effect of NA on changes over time, the effect of CI was also explored. Only those subjects who scored low and high on the CI scale were compared. The main effect CI analyses were based on the first and third tertiles, which included a total of 86 subjects. Subjects in the middle tertile were dropped from the analysis. Relative to low CI subjects, high CI subjects were marginally more likely to report a VOC general symptom after the onset of exposure (minutes 60 and 90) (Tables 18 and 19). However, the CI by exposure, CI by stress by time, and the exposure by stress by CI by time interactions were not significant (not shown).

Table 18: Test statistics and p-values based on generalized estimating equation for each symptom subscale using each scoring method. (All chi-square tests are based on 5 degrees of freedom.)

Main Effect of CI (upper/lower tertiles)

	Presence/Absence		Severity	
	χ^2 Statistic	P-value	χ^2 Statistic	P-value
Any Symptom	4.56	0.472	4.78	0.444
VOC General	10.75	0.057	4.15	0.529
VOC Cognitive	3.52	0.620	3.27	0.658
Eye Irritation	5.66	0.341	4.04	0.543
Anxiety	3.48	0.627	2.90	0.716
Upper Respiratory	7.17	0.209	7.04	0.218
Lower Respiratory	7.90	0.162	3.96	0.556
Somatic	2.66	0.753	7.61	0.179

Bonferroni Correction: $p<0.003$

Table 19: Test statistics and p-values for comparing changes for **VOC General Symptoms**

CI (upper/lower tertiles) X Time Interaction

	Presence/Absence	
Change from Min. 15 (Baseline) to	X^2 Statistic	P-value
Min. 60	3.33	0.068
Min. 90	6.06	0.014
Min. 110 (post-stress)	0.61	0.433
Min. 125	0.00	0.994
Min. 185 (post-exposure)	0.08	0.774
Change from Min. 60 to		
Min. 90	1.62	0.204
Min. 110 (post-stress)	2.10	0.147
Min. 125	5.30	0.021
Min. 185 (post-exposure)	2.95	0.086
Change from Min. 90 to		
Min. 110 (post-stress)	6.76	0.009
Min. 125	8.53	0.004
Min. 185 (post-exposure)	6.38	0.012
Change from Min. 110 to		
Min. 125	1.34	0.246
Min. 185 (post-exposure)	0.39	0.530
Change from Min. 125 to Min. 185	0.12	0.731

Bonferroni Correction: $p<0.003$

Aim 1.2: Neuroendocrine: To assess salivary cortisol as a neuroendocrine indicator of hypothalamic-pituitary-adrenal axis activation in response to exposures with and without the psychological stressor.

Cortisol is normally elevated during ovulation. If a woman was ovulating during a particular exposure session, this could attenuate her cortisol response to the stressor during that session. Therefore, cortisol data were analyzed both with and without data from the specific exposure session in which the subject's estradiol level was ≥ 5 μl/dl. Of the participants, 13 of the 130 (10%) subjects were ovulating during their MCA exposure, 13 out of 130 (10%) were ovulating during the VOCs exposure and 15 out of 130 (11.5%) who were ovulating during the VOCs + O

exposure. Upon comparison of the cortisol data analyses with and without ovulation sessions, no differences in outcome were noted. Therefore, sessions in which subjects were ovulating were included in the final analyses of cortisol.

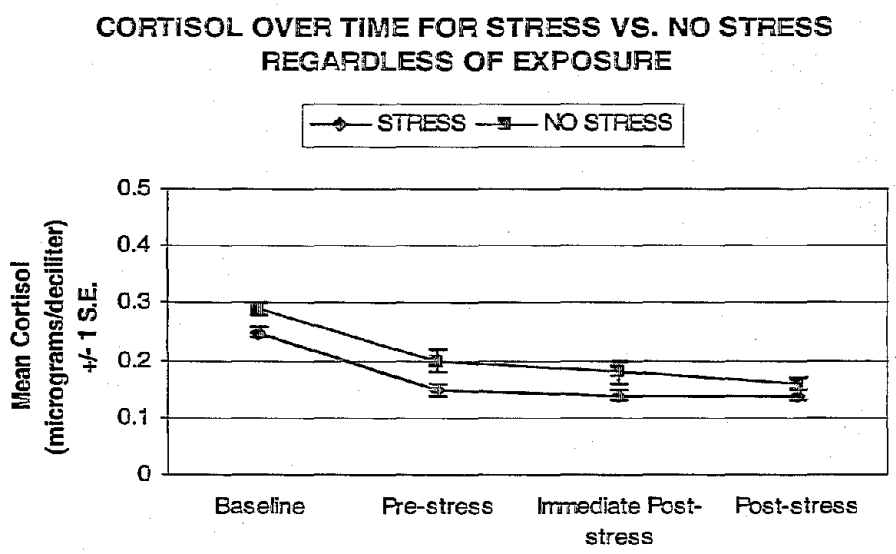
Given the continuous response, SAS PROC MIXED was used to analyze the fixed factor effects. The cortisol response was right skewed and, thus, for the linear models was transformed using a square root transformation in order to better satisfy the normality assumption required for the mixed linear model.

> **Hypothesis 1: (Exposure Main Effect)**: Baseline to post-exposure changes in salivary cortisol will be significantly elevated post-exposure to VOCs and VOCs + O relative to MCA.

First, the overall main effects of exposure, stress, NA, CI and NA/CI were tested for significance. As before, these overall main effects were assessed by testing the cumulative significance of all of the regression coefficients describing their interaction with time. Hypothesis 1 of an exposure main effect was not confirmed but the main effect of stress was significant, even after a Bonferroni correction for multiple testing (requiring p-value<0.00625). The significance of the main effect of stress was due to changes in cortisol levels (µl/dl) from before the stressor (minute 90) to after the stressor (minutes 110 and 125). Cortisol levels decreased more for the no-stressor condition (from 0.196 to 0.154) than for the stressor condition (0.150 to 0.147) when examining changes from minutes 90 to 125. When examining changes from minutes 110 to 125, on average, the cortisol levels decreased (0.178 to 0.154) for the no-stressor condition while increasing (0.140 to 0.147) for stressor conditions. These effects are

consistent with the expected circadian rhythms predicting a decline of cortisol over the morning hours when all testing took place.

FIGURE 8



Hypothesis 2: (Exposure x NA x CI Interaction): Relative to low NA, low CI subjects, high NA, high CI subjects will show significantly higher baseline to post-exposure cortisol changes in the VOCs and VOCs + O conditions than in the MCA condition.

Hypothesis 3: (Exposure x NA x CI x Stressor Interaction): Relative to low NA, low CI subjects, high NA, high CI subjects will show significantly greater baseline to post-exposure cortisol changes in the VOCs and VOCs + ozone conditions with the stressor than in these conditions without the stressor and than in MCA with or without the stressor.

Neither Hypothesis 2 nor 3 was confirmed. (See Tables 20 – 24)

Table 20. Test statistics and p-values using SAS Proc Mixed to model effect on Cortisol.

Main Effects

Main Effect	Sample Size	F-Statistic	Degrees of Freedom	P-value
Exposure	130	0.45	6, 387	0.843
Stress	130	5.93	3, 388	0.0006
NA (upper/lower tertiles)	93	1.40	3, 277	0.243
CI (upper/lower tertiles)	86	0.95	3, 256	0.416
NA/CI	64	0.67	9, 188	0.732

Table 21. T-statistics and p-values using SAS Proc Mixed to model effect on Cortisol.

Stress X Time Interaction

Changes in times	t-stat (d.f.=388)	p-value
Change from Min. 15 (Baseline) to		
Min. 90	-0.60	0.552
Min. 110	-0.08	0.936
Min. 125	1.78	0.075
Change from Min. 90 to		
Min. 110 (post-stress)	1.05	0.295
Min. 125	4.00	<0.0001
Change from Min. 110 to		
Min. 125	3.63	0.0003

Bonferroni Correction: $p<0.008$

Table 22. Test statistics and p-values using SAS Proc Mixed to model exposures interaction effects on Cortisol.

Exposure Interactions

Interaction between Exposure and	Sample Size	F-Statistic	Degrees of Freedom	P-value
Stress	130	1.07	6, 384	0.381
NA (upper/lower tertiles)	93	1.21	6, 273	0.301
CI (upper/lower tertiles)	86	1.84	6, 252	0.091
NA/CI	64	0.95	18, 180	0.523

Table 23. Test statistics and p-values to model stress interaction effects on Cortisol.

Stress Interactions

Interaction between Stress and	Sample Size	F-Statistic	Degrees of Freedom	P-value
NA (upper/lower tertiles)	93	1.98	3, 275	0.117
CI (upper/lower tertiles)	86	0.54	3, 254	0.654
NA/CI	64	1.12	9, 184	0.353

Table 24. Test statistics and p-values to model exposure X stress interactions on Cortisol.

Exposure X Stress Interactions

Interaction between Exposure, Stress and	Sample Size	F-Statistic	Degrees of Freedom	P-value
NA (upper/lower tertiles	93	0.51	6, 236	0.800
CI (upper/lower tertiles)	86	1.38	6, 215	0.226
NA/CI	64	0.79	18, 143	0.706

Aim 1.3 Neurobehavioral: To assess neurobehavioral performance on a computerized vigilance task.

Hypothesis 1: (Exposure x CI Interaction): Regardless of NA, subjects high in CI will perform significantly worse than low CI subjects on the neurobehavioral vigilance task following exposures to VOCs and VOCs + O versus MCA.

Using the mixed linear model hypothesis 1 was not confirmed. That is, none of the interactions between exposure and NA, CI or NA/CI had a significant effect on neurobehavioral performance. Table 25 gives the test statistics and p-values for the tests of these interactions. Also, there were no significant main effects of NA, CI, or NA/CI on neurobehavioral performance (not shown).

Table 25. Test Statistics and p-values using mixed linear model of Exposure interactions on neurobehavioral performance.

Exposure Interactions

Interaction of Exposure with	Sample Size	F-Statistic	Degrees of Freedom	P-value
NA (upper/lower tertiles)	93	0.47	6, 273	0.831
CI (upper/lower tertiles)	86	0.51	6, 252	0.802
NA/CI	64	0.90	18, 180	0.581

Using the mixed linear model (as above), none of the interactions between stress and NA, CI or NA/CI had a significant effect on neurobehavioral performance (not shown).

Hypothesis 2: (Exposure x NA x CI x Stressor Interaction): High NA, high CI subjects will perform significantly worse than low NA, low CI subjects on the neurobehavioral vigilance task following exposure to VOCs and VOCs + O that include the psychological stressor versus exposures without the psychological stressor.

Using the mixed linear model (as above), hypothesis 2 was not confirmed. None of the interactions between stress, exposure and either NA, CI or NA/CI had a significant effect on neurobehavioral performance. Table 26 gives the test statistics and p-values for the tests of these interactions (mean values in Table 27).

Table 26. Test Statistics and p-values using a mixed linear model for Exposure and Stress interactions on neurobehavioral performance.

Exposure X Stress Interactions

Interaction of Exposure with	Sample Size	F-Statistic	Degrees of Freedom	P-value
NA (upper/lower tertiles)	93	1.70	3, 269	0.168
CI (upper/lower tertiles)	86	0.42	3, 248	0.866
NA/CI	64	0.45	9, 174	0.909

Table 27. Differences in means for both the stress and no-stress conditions (standard errors in parentheses) on neurobehavioral performance.

Change in times	Stress	No-stress
Change from Min 15 (Baseline) to		
Min. 60 (pre-stress)	1.614 (0.169)	1.735 (0.180)
Min. 140 (post-stress)	2.053 (0.228)	1.377 (0.243)
Min. 185 (post-exposure)	2.152 (0.274)	1.993 (0.292)
Change from Min 60 to		
Min. 140 (pre-stress)	0.439 (0.191)	-0.358 (0.203)
Min. 185 (post-exposure)	0.538 (0.251)	0.258 (0.268)
Change from Min 140 to		
Min. 125 (post-exposure)	0.098 (0.215)	0.616 (0.229)

Aim 1.4 Inflammation: To assess PMN, albumin, Il-6, and Il-8 as markers of nasal inflammation in response to VOCs and VOCs + O.

Hypothesis 1: (Exposure Main Effect): Markers of nasal inflammation will be significantly higher in the VOCs + O exposure condition relative to VOCs alone and MCA.

Blood contamination

After centrifugation, the macroscopic appearance of red blood cells was noted in 379 out of 780 total nasal lavage samples (49%). One hundred and three of 130 (79%) subjects had at least one sample with macroscopic blood. The amount of blood in specimens could not be quantified. We

were concerned that PMNs entering the nasal cavity from hemorrhage would cause an artifactual increase in the NL PMN counts. If presence of blood was associated with exposure, this might lead to a differential effect on PMN counts. To evaluate these possibilities, we first analyzed the data to determine if there was an association between presence of blood and PMN counts regardless of exposure. Since the PMN data was not normally distributed, the data was categorized into 5 groups (zero values and remaining quartiles). Using a Cochran-Mantel-Haenszel (CMH) procedure, we found no significant general association between presence of blood and PMN counts. Next, using a CMH paired analysis, we found no significant association between exposure condition and blood.

Results of the analyses below indicate that the hypothesis was not confirmed for the total sample or for individuals defined as atopic or as symptomatic responders.

PMNs

The geometric means and associated confidence intervals for each exposure at baseline and post-exposure are given in Table 28, both for the total collection of subjects as well as for the subsets of atopic subjects and symptomatic "responders." The 95% confidence intervals for the geometric means have large overlap, not only from pre- to post-exposure but also between exposures. However, it is inappropriate to compare these confidence intervals directly due to the matched nature of the data, in which each subject received all three exposures.

Table 28: Summary Statistics for Pre- and Post-PMN concentrations (cells/ml), by Exposure for all subjects, atopic subjects, symptomatic responders

	Pre-/Post-Exposure	Geometric mean	95% C.I.
All Subjects (N=130)			
MCA	Pre-	80.5	(31.8, 203.5)
	Post-	94.4	(37.1, 240.0)
VOCs	Pre-	94.9	(37.4, 240.7)
	Post-	154.4	(64.9, 367.4)
VOCs + O	Pre-	94.6	(37.7, 237.1)
	Post-	155.0	(62.8, 382.6)
Atopic (N=51)			
MCA	Pre-	183.7	(45.0,749.5)
	Post-	91.5	(19.9, 419.7)
VOCs	Pre-	62.0	(13.8, 279.2)
	Post-	189.8	(44.1, 816.4)
VOCs + O	Pre-	57.6	(13.3, 249.7)
	Post-	100.8	(22.8, 445.7)
Symptomatic "Responders (N=49)			
MCA	Pre-	129.9.9	(28.8, 585.1)
	Post-	56.6	(11.8, 270.1)
VOCs	Pre-	116.4	(25.3, 535.9)
	Post-	203.0	(46.9, 878.5)
VOCs + O	Pre-	145.6	(34.0, 623.9)
	Post-	85.7	(17.4, 422.7)

The stratified Cochran-Mantel-Haenszel test was used to compare the change in the response across exposures. Four ordinal variables were created such that the first ranked category includes just zero values. The remaining PMN concentration values were divided into tertiles to create the second (values > 1441 PMN/ml), third (1442-10385 PMN/ml) and fourth (>10386) categories. For the combined sample, the stratified Cochran-Mantel-Haenszel test yielding a p-value of 0.8061 (chi-square=0.431 with 2 d.f.) gives no evidence for an effect of exposure.

Table 29 contains point and interval estimates of the odds ratios describing the odds of a person increasing their PMN concentrations as represented by the ordinal categories for both the total sample as well as the atopic and "responder" subgroups. An odds ratio equal to one would indicate that an individual is no more likely to have responded in a higher category after exposure than at baseline. Although the odds ratios for the VOCs and VOCs + O exposures are larger than for MCA for all subjects and the subgroups, the confidence intervals for the 3 exposure conditions are similar as expected based on the formal inference concluding no effect of exposure.

Table 29: Point and Interval Estimates for the Odds of Increasing in PMN Response Category, by Exposure

	Odds Ratio	95% Confidence Interval
All Subjects (N=130)		
MCA	1.11	(0.65, 1.90)
VOCs	1.43	(0.80, 2.57)
VOCs + O	1.41	(0.80, 2.49)
Atopic Subjects (N=51)		
MCA	0.71	(0.37, 2.02)
VOCs	4.60	(0.60, 6.30)
VOCs + O	1.55	(0.49, 3.00)
Symptomatic "responders" (N=49)		
MCA	0.53	(0.48, 1.20)
VOCs	1.40	(0.73, 1.83)
VOCs + O	0.73	(0.53, 1.43)

Soluble proteins

For IL-6, IL8 and total protein, the effect of exposure was not significant in any of the categories of subjects. Table 30 below summarizes the p-values.

Table 30: P-values summarizing significance of main effects of exposure

	Protein	IL-6	IL-8
All Subjects (N=130)	0.876	0.487	0.597
Atopic Subjects (N=51)	0.930	0.926	0.493
Symptomatic Responders (N=49)	0.632	0.907	0.947

Hypothesis 2: (Exposure x CI Interaction): Regardless of NA and relative to low CI subjects, high CI subjects will have significantly higher levels of PMN, albumin, Il-6, and Il-8 in response to VOCs and VOCs + O versus MCA.

A marginal modeling approach with generalized estimating equations was used for the estimation. In SAS, Proc Genmod was used to carry out the analyses. Table 31 shows the results of this analysis for all subjects combined, for responders and for atopic subjects. The main effect of CI was not significant in any category of subjects.

Table 31. Test statistics and p values using a marginal modeling procedure for PMNs.

CI Main Effect

	Chi-Square (d.f.=1)	*P-value*
All Subjects (N=86)	0.12	*0.725*
Atopic Subjects (N=34)	0.23	*0.633*
Symptomatic Responders (N=34)	*1.09*	*0.296*

The same approach as above was used for testing the interaction between CI and Exposure for each of the three categories of subjects. Hypothesis 2 predicting higher levels of PMNs, albumin, IL-68 and IL-8 for high CI subjects exposed to VOCs or VOCs + O was not confirmed.

Table 32 summarizes these results. The interaction was not significant for any of the categories of subjects.

Table 32. Test statistics and p values using a marginal modeling procedure for PMNs.

CI X Exposure Interaction

	Chi-Square (d.f.=2)	*P-value*
All Subjects (N=86)	1.67	0.434
Atopic Subjects (N=34)	1.11	0.573
Symptomatic Responders (N=34)	0.22	0.897

Soluble Proteins

A global test for the effect of CI and the interaction between CI and exposure was performed using the change of the log concentrations as the response within a mixed linear model. The results for the main effect of CI are included in Table 33.

Table 33. P values using a mixed linear model for Soluble Proteins. .

Main Effect of CI

	Protein	IL-6	IL-8
All Subjects (N=86)	0.798	0.113	0.234
Atopic Subjects (N=34)	0.453	0.328	0.872
Symptomatic Responders (N=34)	0.975	0.004	0.293

The results for the global test for interaction between CI and exposure are included in Table 34.

Table 34. P-values using a mixed linear model to test the interactions for Soluble Proteins.

CI X Exposure Interaction

	Protein	IL-6	IL-8
All Subjects (N=86)	0.676	0.623	0.384
Atopic Subjects (N=34)	0.666	0.617	0.530
Symptomatic Responders (N=34)	0.576	0.643	0.241

Aim 1.5 Lung Function: To assess changes in lung function as a function of exposure status.

Hypothesis 1: (Exposure Main Effect): Spirometrically measured flow (FEV1, FEF_{25-75}, FEF_{75}, PEFR) will be significantly less in the VOCs and VOCs + O conditions relative to MCA with VOCs + O condition showing the most significant reduction in lung function.

Table 35 summarizes the results for the F-tests of the effect of exposure for each of the ten lung function variables. The only significant lung function value was for percent change in FEF2575.

Table 35: F-statistics, degrees of freedom and p-values using a mixed linear model for **Lung Function**

Exposure Main Effect

Variable	F-statistic	D.F.	P-value
Absolute change FVC	0.75	2, 258	0.4721
Percent change FVC	0.68	2, 258	0.5065
Absolute change FEV1	1.92	2, 258	0.1491
Percent change FEV1	2.05	2, 258	0.1303
Absolute change FEV1/FVC	1.68	2, 258	0.1876
Percent change FEV1/FVC	0.92	2, 258	0.3984
Absolute change PEFR	0.86	2, 258	0.4223
Percent change PEFR	0.41	2, 258	0.6614
Absolute change FEF_{25-75}	2.05	2, 258	0.1308
Percent change FEF_{25-75}	3.37	2, 258	0.0358

Bonferroni correction: $p<0.005$

Comparisons of the least square means for FEF_{25-75} reveal that the differences between VOCs versus MCA and VOCs + O versus MCA were marginally significant with p-values of 0.0302 and 0.0215, respectively. In the MCA condition, the percent change in FEF_{25-75} was not significantly different from zero (p-value=0.9309), while the VOCs and VOCs + O conditions

demonstrated, on average, 5.015 and 5.313 percent increases in FEF_{25-75}. These percent changes were significantly different from zero (p-values= 0.0017 and 0.0009, respectively).

Aim 2: To measure the major secondary stable products of ozone-VOCs reactions and to assess the effect of the interaction of VOCs and ozone on indicators of inflammation. The secondary products include gas-phase formaldehyde, other aldehydes and ketones, gas-phase carboxylic acids, and fine and ultra-fine particles. Reactive intermediates such as hydroxyl radical and Crieege biradicals cannot be measured though they may contribute to health effects.

Hypothesis 1: (Exposure Main Effect): Secondary products from indoor ozone reactions with olefinic VOCs will produce a complex mixture of aldehydes, other organic compounds in gas-phase, and fine particles, and extremely reactive intermediates such as the hydroxyl and other free radicals. This exposure mixture will produce significantly more symptoms of irritation and higher levels of nasal inflammatory markers than VOCs alone or than MCA .

See Appendix A for the published results (Fan et al., Ozone-initated Reactions with Mixtures of Volatile Organic Compounds Under Simulated Indoor Conditions, 2003).

Supplemental Analyses

Environmental Quality and Odor Ratings: In a repeated measures linear model, the main effects of exposure, stress, and the interaction of exposure and stress on individual ratings of environmental quality were analyzed. Air quality, the level of odor, and the need to ventilate

were significantly different in the VOCs and VOCs + O conditions relative to the MCA condition (Table 36). No main effect of stress or interaction effect of stress and exposure on environmental quality ratings was observed. During exposure, odor level and air quality were rated as poorer and subjects reported an increasing need to ventilate the room (Tables 37 and 38; Figures 9, 10, and 11).

Table 36: Overall F-tests (p-values and degrees of freedom) using a repeated measures linear model.

Exposure and Stress Main Effects and Exposure X Stress Interaction

	Exposure	Stress	Exposure*Stress
Air Movement	0.40 0.9453 10, 9453	1.99 0.0773 5, 768	0.56 0.8461 10, 1526
Air Quality	2.48 0.0060 10, 1547	1.32 0.2515 5, 768	0.65 0.7690 10, 1525
Humidity	0.18 0.9978 10, 1538	0.51 0.7679 5, 768	0.18 0.9979 10,1526
Light	0.20 0.9963 10, 1538	0.18 0.9692 5, 768	0.18 0.9974 10, 1526
Noise	0.24 0.9917 10, 1537	0.11 0.9900 5, 768	0.19 0.9973 10, 1525
Odor Level	8.17 <0.0001 10, 1532	2.04 0.0714 5, 768	1.05 0.3945 10, 1520
Room Temperature	0.32 0.9766 10, 1537	0.58 0.7118 5, 768	0.19 0.9973 10, 1525
Ventilation	2.72 0.0026 10, 1537	1.56 0.1699 5, 768	0.28 0.9856 10, 1525

Bonferroni correction: $p<0.002$

Table 37: Least square means and standard errors of the odor level, air quality and ventilation ratings under each condition at each time point.

Table 37: Least Square Means and Standard Errors

Minutes	50	60	80	90	110	125
Odor Level						
MCA	1.85 (0.08)	3.67 (0.08)	2.73 (0.08)	2.39 (0.08)	2.41 (0.08)	1.86 (0.08)
VOCs	1.89 (0.08)	4.04 (0.08)	3.61 (0.08)	3.16 (0.08)	3.23 (0.08)	1.87 (0.08)
VOCs + O	2.04 (0.08)	4.07 (0.08)	3.80 (0.08)	3.36 (0.08)	3.30 (0.08)	1.91 (0.08)
Air Quality						
MCA	2.72 (0.07)	3.57 (0.07)	3.19 (0.07)	3.04 (0.07)	2.98 (0.07)	2.61 (0.07)
VOCs	2.78 (0.07)	3.81 (0.07)	3.68 (0.07)	3.28 (0.07)	3.42 (0.07)	2.58 (0.07)
VOCs + O	2.89 (0.07)	3.89 (0.07)	3.71 (0.07)	3.45 (0.07)	3.47 (0.07)	2.74 (0.07)
Ventilation						
MCA	3.05 (0.10)	4.12 (0.10)	3.62 (0.10)	3.40 (0.11)	3.29 (0.11)	2.72 (0.10)
VOCs	3.00 (0.10)	4.48 (0.10)	4.16 (0.11)	3.91 (0.11)	3.91 (0.11)	2.67 (0.10)
VOCs + O	3.06 (0.10)	4.44 (0.10)	4.22 (0.10)	3.94 (0.10)	3.97 (0.11)	2.72 (0.10)

Table 38: Overall F-tests (p-values and degrees of freedom) using a repeated measures linear model.

Exposure X Time Interaction

Minutes	60	80	90	110	125
***Odor Level** (d.f.=1, 1532)*					
VOCs	3.79 0.0518	24.30 <0.0001	18.77 <0.0001	20.66 <0.0001	0.03 0.8557
VOCs + O	1.60 0.2060	27.14 <0.0001	21.31 <0.0001	17.11 <0.0001	0.67 0.4140
***Air Quality** (d.f.=1, 1537)*					
VOCs	1.82 0.1770	10.02 0.0016	1.96 0.1617	7.80 0.0053	0.32 0.5736
VOCs + O	1.27 0.2605	6.41 0.0114	3.02 0.0826	5.41 0.0201	0.08 0.7784
***Ventilation** (d.f.=1, 1537)*					
VOCs	3.79 0.0517	8.04 0.0046	7.47 0.0064	10.02 0.0016	0.00 0.9707
VOCs + O	2.16 0.1418	8.00 0.0047	6.53 0.0107	10.07 0.0015	0.00 >0.9999

Bonferroni Correction: p<0.005 for odor level, air quality and ventilation

FIGURE 9

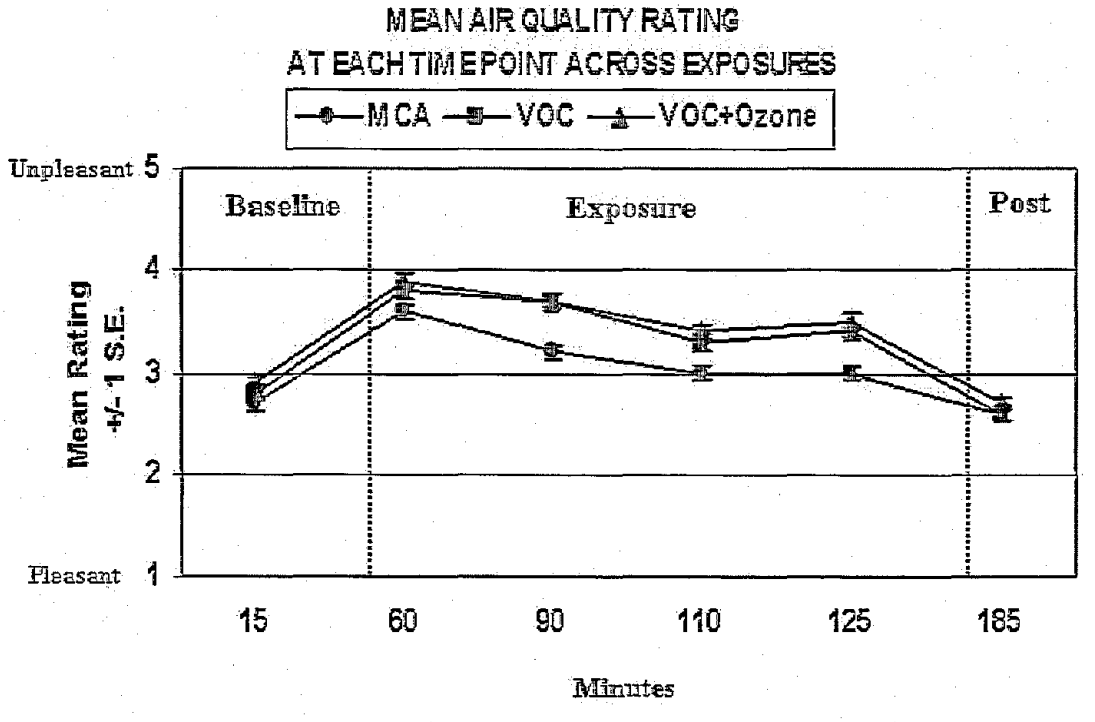


FIGURE 10

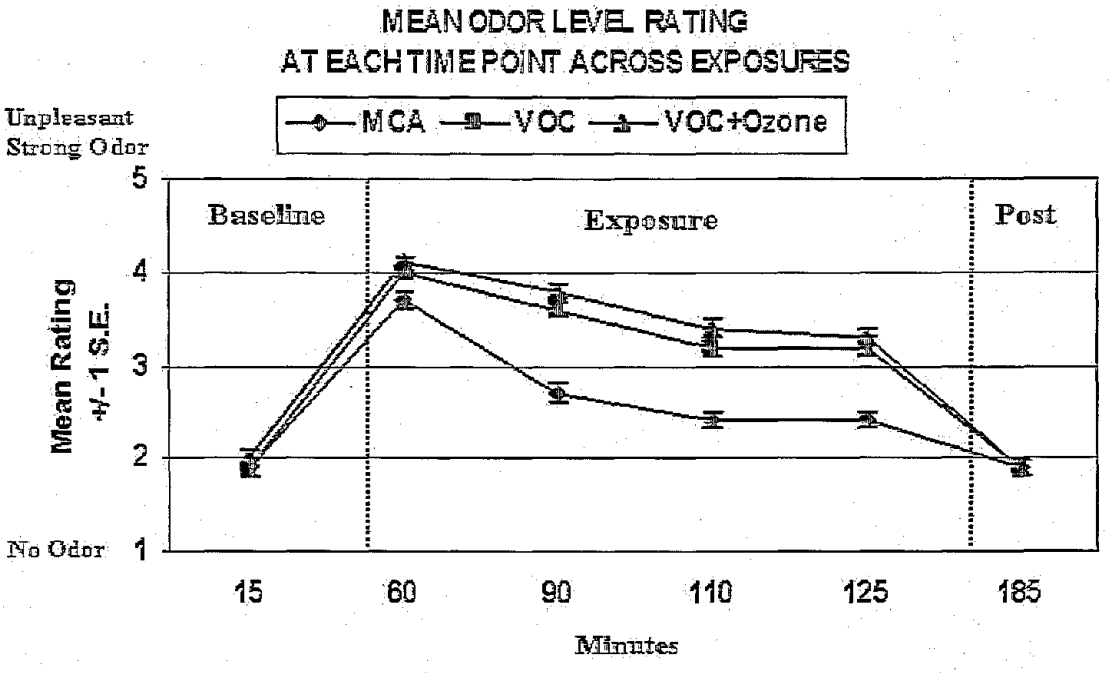
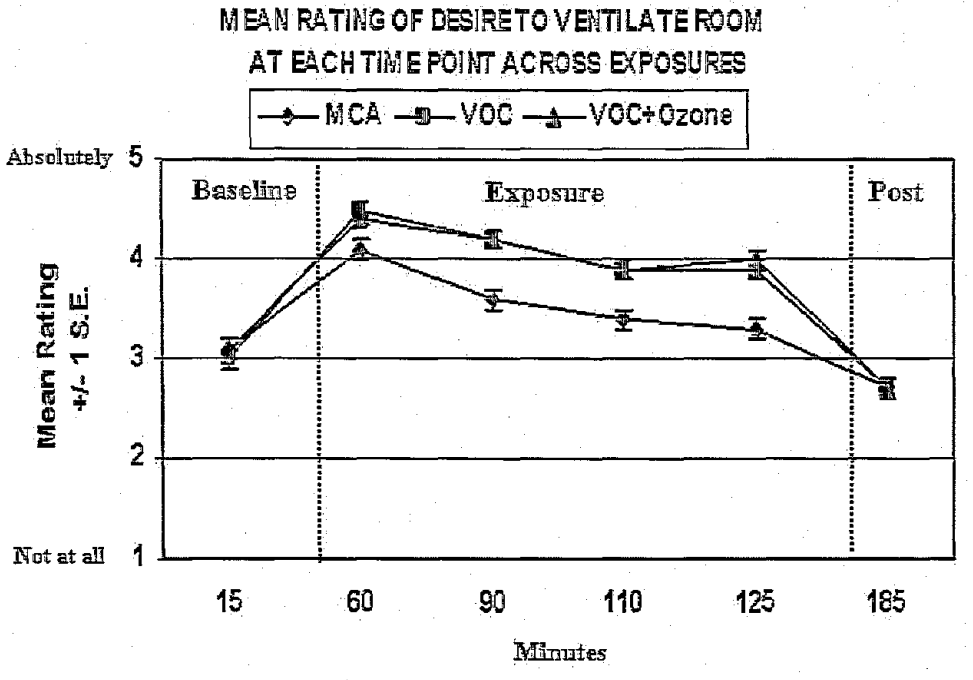


FIGURE 11



Using the same analytic strategy, the effect of exposure was significant for ratings of the intensity and unpleasantness of the odor (p-values <0.0001) (Table 39). No other main or interaction effects were observed (interactions not shown). Table 40 gives the least square means of the exposures for ratings of intensity at each time point. The mean ratings of intensity increased more from Minute 15 to Minute 50 during the VOCs and VOCs + O conditions than during the masked clean air condition (Table 41). In addition, the intensity ratings decreased from the peak at Minute 50 more quickly during the masked clean air condition than during the active exposures.

Table 39: Overall F-tests, p-values and degrees of freedom using a repeated measures linear model for odor ratings.

Exposure, Stress, NA, CI, and NA/CI Main Effects

	Exposure (N=130)	Stress/No Stress (N=130)	NA (N=93)	CI (N=86)	NACI (N=64)
Intensity	5.74 <0.0001 14, 3072	1.41 0.1983 7, 3080	0.94 0.4765 7, 2197	1.66 0.1146 7, 2030	0.91 0.5509 14, 1496
Irritation	1.17 0.2881 14, 3071	1.32 0.2372 7, 3079	0.42 0.8919 7, 2196	0.99 0.4389 7, 2029	0.46 0.9547 14, 1495
Unpleasantness	4.03 <0.0001 14, 3058	0.99 0.4397 7, 3066	1.67 0.1121 7, 2184	0.49 0.8413 7, 2020	0.76 0.7173 14, 1487

Bonferroni Correction: p<0.003

Table 40: Least Square means and standard error for mean ratings of odor intensity.

Minutes	15	50	60	80	90	110	125	185-Post
MCA	1.55 (1.42)	28.88 (1.42)	23.02 (1.42)	14.34 (1.42)	11.55 (1.42)	7.91 (1.42)	6.99 (1.45)	2.60 (1.42)
VOCs	1.81 (1.41)	36.15 (1.41)	34.30 (1.42)	27.27 (1.41)	25.27 (1.42)	20.00 (1.41)	19.45 (1.43)	2.38 (1.41)
VOCs + O	2.85 (1.41)	37.27 (1.43)	33.35 (1.41)	28.41 (1.42)	27.36 (1.42)	20.35 (1.42)	19.35 (1.41)	1.88 (1.41)

Table 41: F-statistics (d.f.=1, 3072) and p-values for odor intensity.

Exposure X Time Interaction

Minutes	50	60	80	90	110	125	185-Post
VOCs	6.13 0.0133	15.09 0.0001	19.98 <0.0001	22.51 <0.0001	17.43 <0.0001	18.19 <0.0001	0.03 0.8684
VOCs + O	6.35 0.0126	10.14 0.0015	20.27 <0.0001	26.18 <0.0001	15.43 <0.0001	15.03 0.0001	0.50 0.4788

Table 42 gives the least square means of the exposures at each time point for ratings of unpleasantness. The mean ratings of unpleasantness decreased more from minute 15 to minute 50 during the VOCs and VOCs+O conditions than during the masked clean air condition. In addition, the unpleasantness ratings increased from the lowest point at minute 50 more quickly during the masked clean air condition than during the active exposures (Table 43) (See Figures 12 and 13)

Table 42: Least Square means and standard error for mean ratings of unpleasantness.

Minutes	15	50	60	80	90	110	125	185-Post
MCA	5.34 (0.13)	3.84 (0.13)	3.81 (0.13)	4.43 (0.13)	4.67 (0.13)	4.88 (0.13)	4.94 (0.13)	5.31 (0.13)
VOCs	5.30 (0.13)	3.32 (0.13)	3.22 (0.13)	3.60 (0.13)	3.66 (0.13)	3.93 (0.13)	3.99 (0.13)	5.48 (0.13)
VOCs+O	5.16 (0.13)	3.43 (0.13)	3.33 (0.13)	3.64 (0.13)	3.57 (0.13)	3.92 (0.13)	3.98 (0.13)	5.53 (0.13)

Table 43: F-statistics (d.f.=1, 3058) and p-values for unpleasantness.

Exposure X Time Interaction

Minutes	50	60	80	90	110	125	185-Post
VOCs	3.33 0.0683	4.57 0.0326	9.22 0.0024	14.05 0.0002	12.34 0.0005	12.07 0.0005	0.59 0.4429
VOCs + O	0.78 0.3772	1.34 0.2470	5.40 0.0202	12.68 0.0004	8.98 0.0028	8.82 0.0030	2.37 0.1237

Bonferroni correction: $p<0.004$

FIGURE 12

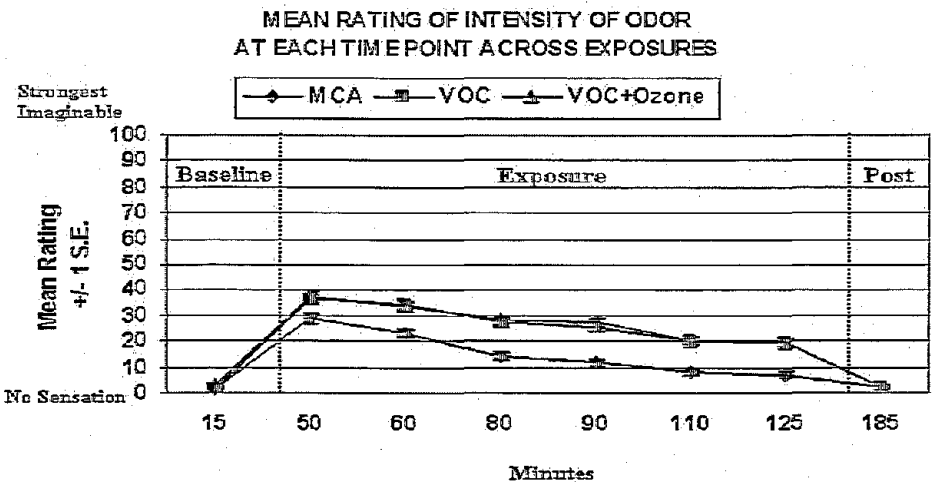
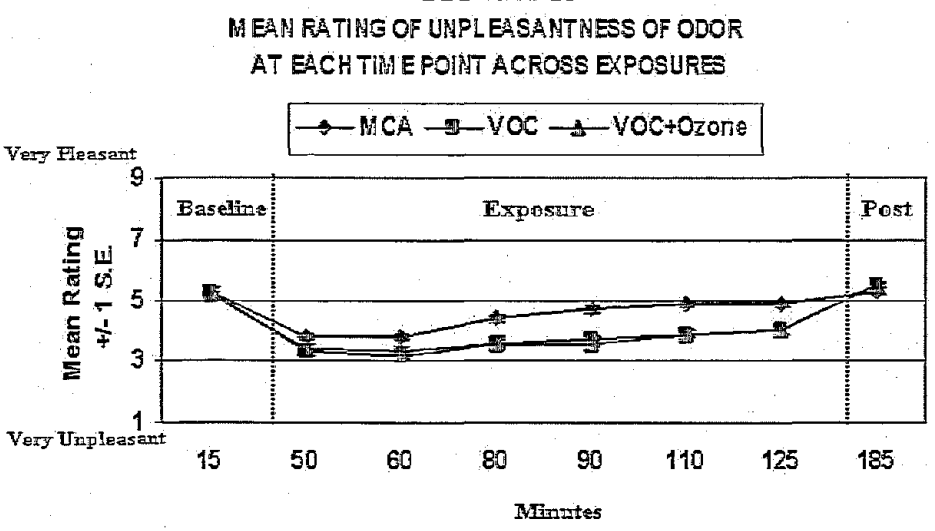


FIGURE 13



Exposure Evaluation and Distress Ratings of Experimental Procedures:

At the end of each session, subjects were asked to guess which exposure condition they had just experienced, to rate the confidence of their exposure "guess", and to rate the strength of the exposure. The first 82 subjects were given three choices (no exposure, mixture of indoor air contaminants (VOCs), mixture of indoor air contaminants (VOCs) with ozone while the remaining 48 subjects were given four choices (no exposure, mixture of indoor air contaminants (VOCs), mixture of indoor air contaminants (VOCs) with ozone, clean air with low level mixture of indoor air contaminants (VOCs). Ratings of confidence and strength were the same for all subjects.

Initially, a global test of the null hypothesis that guesses of exposure had no relationship to the actual exposure experienced was performed. This test was performed for two groups (those given three choices and those given four choices) separately. The Cochran-Mantel-Haenszel test, stratified by subject, was used to test independence. This test implicitly accounts for the possible association of responses by the same subject. In conducting this test, the exposure classifications for both the guesses and the actual exposure, were treated as categorical.

For the 82 subjects who were given three choices, the CMH statistic (7.4277, d.f.=4) yielded a p-value of 0.1149, indicating that their guesses about exposure reflected choices that were no better than might be expected by chance. Table 44 presents the percentage of guesses for each exposure within each actual exposure condition. So, for example, when subjects were exposed to the masked clean air condition, 2.5% guessed they had no exposure; 68.75% guessed they

were exposed to mixture of indoor air contaminants (VOCs); 28.75% guessed they were exposed to mixture of indoor air contaminants (VOCs) with ozone (VOCs +O).

Table 44: Percentage of each guess for actual exposure conditions

	Guessed Exposure (%)		
Actual Exposure	No Exposure	Mixture of Indoor Air Contaminants (VOCs)	Mixture of Indoor Air Contaminants (VOCs) with Ozone (VOC+O)
MCA (N=80)	2.50	68.75	28.75
VOCs (N=81)	3.70	65.43	30.86
VOCs + O (N=82)	1.22	53.66	45.12

It is evident that very few subjects (2, 3, and 1) respectively, thought that they had no exposure during the masked clean air condition, which had a single spike of VOCs at the beginning of the exposure session.

If the data based on the masked clean air condition is ignored, a test of the association between VOCs versus VOCs + O exposure using a CMH test yields a p-value of 0.0756 (CMH stat=5.1654, d.f.=2). This suggests that subjects had little ability to distinguish between the two exposure conditions. Examining the guess percentages in Table 44, subjects were more likely to guess they were exposed to VOCs than to VOCs + O, regardless of actual exposure. The percentage that guessed they were exposed to VOCs + O increased slightly from the VOCs exposure (30.86%) to the VOCs + O (45.12%), but remained below 50%.

Finally, subjects' confidence about their guesses, was examined for each exposure condition. Subjects rated their confidence about their guesses on a scale of 1 (not at all) to 5 (completely

confident). A stratified CMH test, examining whether average confidence ratings differed depending on which exposure a subject experienced, yielded a p-value of 0.0423 (CMH stat=6.3268, d.f.=2). This indicates a slightly significant difference in confidence about guesses depending on the exposure session. Table 45 presents the percentages of each confidence rating for each exposure. Subjects were at least twice as likely to be not at all confident about their guess in the masked clean air condition (17.5%) as in the VOCs (8.54%) and the VOCs + O (7.32%) conditions.

Table **45:** Percentages of each confidence rating about guesses for each exposure condition

	Confidence Rating (%)				
Exposure	**Not at all 1**	**2**	**Moderately 3**	**4**	**Completely 5**
MCA (N=80)	17.50	1.25	60.00	5.00	16.25
VOCs (N=81)	8.54	1.22	60.98	10.98	18.29
VOCs + O (N=82)	7.32	2.44	70.73	2.44	17.07

A simple analysis was conducted to determine the relationship between subjects' level of confidence and the probability that they guessed the correct exposure. It was assumed that if there were a relationship, it would be such that increased confidence would be associated with increased odds of making the correct guess about exposure. The test, based on generalized estimating equations adjusting for within-subject correlations, suggested there was no relationship between subjects' confidence in their guess and whether they correctly guessed the exposure (chi-square=0.11, d.f.=1, p=0.7427).

For the overall test of association between guesses and actual exposure when subjects were given four choices, the no exposure and the clean air with the low level of indoor air contaminants

(VOCs) guesses were combined. Using the stratified CMH test, a p-value of 0.0006 (CMH stat=19.6550, d.f.=4) indicates that subjects were able to distinguish between exposures. Table 46 presents the percentage of guesses for each exposure, including all four possible guesses, within each actual exposure condition. So, for example, when subjects were exposed to the masked clean air condition, 58.33% guessed correctly; 31.25% guessed they were exposed to VOCs; 8.33% guessed they were exposed to VOCs + O.

Table 46: Percentage of each guess for each actual exposure condition

	Guessed Exposure (%)			
Actual Exposure	**No Exposure**	**Clean Air with Low Level Mixture of Indoor Air Contaminants (VOCs)**	**Mixture of Indoor Air Contaminants (VOCs)**	**Mixture of Indoor Air Contaminants with Ozone (VOCs +O)**
MCA (N=48)	2.08	58.33	31.25	8.33
VOCs (N=48)	0	29.17	56.25	14.58
VOCs + O (N=48)	0	20.83	60.42	18.75

The significant deviation from chance exposure guesses appears to be due solely to the subjects' tendency to detect when they were exposed to the masked clean air condition versus the more active exposures. Of 48 subjects, 58.33% correctly guessed that they were exposed to the masked clean air condition during that exposure; whereas during the VOCs and the VOCs + O exposures only 29.17% and 20.83%, respectively, guessed that they were exposed to the masked clean air condition. However, subjects were not able to significantly distinguish between the two active exposure conditions, VOCs and VOCs + O. During the VOCs condition, 56.25% of the 48 subjects correctly guessed that exposure condition. However, during the VOCs + O condition, an even higher percentage, 60.42%, of subjects guessed they were exposed to just VOCs. Using the Mantel-Haenszel common Odds Ratio estimator, with a 95% confidence

interval, given subjects guessed one of the two active exposures, the odds of a subject guessing an exposure of just VOCs during the actual VOCs exposure are between 0.37 and 3.93 times, the same odds during the actual VOCs + O exposure. This interval includes a null value of 1, indicating it is plausible that the odds of guessing just VOCs are the same regardless of which active exposure subjects experienced.

Finally, subjects' confidence about their guesses for each exposure condition was examined. Subjects rated their confidence about their guesses on a scale of 1 (not at all) to 5 (completely confident). A stratified CMH test, examining whether average confidence ratings differed depending on which exposure a subject experienced, yielded a p-value of 0.9837 (CMH stat=0.0328, d.f.=2). This indicates no significant difference in confidence about guesses depending on the exposure session. Table 47 presents the percentages of each confidence rating for each exposure.

Table 47: Percentages of each confidence rating about guesses for each exposure condition

	Confidence Rating (%)				
Exposure	**Not at all 1**	**2**	**Moderately 3**	**4**	**Completely 5**
MCA (N=48)	8.33	0.00	79.17	0.00	12.50
VOCs (N=48)	2.08	6.25	81.25	0.00	10.42
VOCs + O (N=48)	8.33	6.25	66.67	6.25	12.50

With the addition of a fourth choice for exposure condition (clean air with low level mixture of indoor air contaminants), the distribution of confidence levels for that exposure is similar to the distributions for the other two exposures, with an overwhelming majority of subjects indicating a rating of "moderately" confident in their guess about masked clean air exposure.

The score test, based on generalized estimating equations adjusting for within-subject correlations, suggested there was no relationship between a person's confidence in their guess and whether they correctly guessed the exposure (chi-square=0.36, d.f.=1, p=0.5484).

Ratings of strength of exposure (1= very weak to 5= very strong) were compared using a repeated measures mixed linear model and revealed an overall significant effect of exposure ($F=18.15$; $df = 2$, $p < .0001$). Paired comparisons support that subjects were able to differentiate between the MCA condition and the VOCs ($t = -4.57$, $p < .0001$) and VOCs + O ($t = -5.69$, $p < .0001$) conditions but could not differentiate between the VOCs and VOCs + O conditions ($t = -1.11$, $p = 0.2661$).

Using a repeated measures mixed linear model with post-hoc comparisons, subjects' ratings of the exposure conditions in relation to their physical work conditions and of the symptoms they experienced (1= not at all annoying to 5= extremely annoying) indicated a significant overall effect of exposure (physical work: $F = 5.55$; $df = 2, 257$; $p = 0.0044$; symptoms: $F = 4.54$; $df = 2.257$; $p = 0.0115$). For physical work conditions and symptoms, subjects rated the MCA condition as significantly less annoying than the VOCs and the VOCs + O conditions.

Table 48: Mean Ratings of Subjects' Confidence of Guess of Exposure, Strength of Exposure and Comparison of Exposure Conditions to Work Environment

Exposure	MCA Mean (SD)	VOC Mean (SD)	VOC+O Mean (SD)
Confidence of Guess[a]	3.0 (1.1)	3.2 (1.0)	3.2 (1.0)
Rating of Strength of Exposure[b]	3.0 (1.1)	3.5 (1.0)	3.7 (0.9)
Think about conditions as your work environment & rate the following: [c]			
Physical work conditions	2.7 (1.0)	3.2 (1.1)	3.1 (1.1)
Symptoms	2.6 (1.1)	3.1 (1.2)	2.9 (1.1)

[a]1=Not at all confident; 3=Moderately confident; 5=Completely confident
[b]1=Very weak; 3=Moderate; 5=Very strong
[c]1=Not at all annoying; 2=A little bit annoying; 3=Moderately annoying; 4=Quite a bit annoying; 5=Extremely annoying

Ratings of the level of distress subjects experienced for each of the procedures were analyzed in the same manner (see Table 49). Subjects rated the odor of exposure as significantly more stressful for the VOCs and VOCs + O conditions than for MCA but did not differentiate between VOCs and VOCs + O conditions ($F = 4.52$; $DF = 2,254$; $p = 0.0118$). No significant differences in stress ratings were observed between exposure sessions for the chemical exposures, the stressor, the neurobehavioral task, typing task, vigilance task, questionnaires, saliva samples, nasal washings, or the breathing test.

DISCUSSION

The most striking result of the current study is the lack of significant subjective or objective health effects from exposure to mixtures of VOCs with and without ozone. Although numerous epidemiologic studies suggest that symptoms are significantly increased among workers in buildings with poor ventilation and mixtures of VOCs (e.g., Mendell et al., 2002; Mendell, 1993; Sieber et al., 1996), the current controlled acute exposure to similar chemical mixtures did not validate these epidemiologic findings. Relative to clean air masked with a pulse of VOCs, neither the VOCs nor the VOCs + O exposures caused significantly increased symptom reports, reduced neurobehavioral performance, elevations in markers of nasal inflammation or reductions in lung function. A marginal trend, toward increased severity of lower respiratory symptoms relative to baseline, was observed approximately one hour (minute 125) after exposure onset in the VOCs and VOCs + O conditions relative to MCA. Subjects were also more likely to report some VOC general symptoms in the VOCs and VOCs + O conditions relative to the MCA condition. However, these effects were of marginal significance, became non-significant with appropriate correction for multiple comparisons, and were not validated by spirometry results.

The current findings regarding neurobehavioral performance are consistent with Otto et al., 1992, who reported no changes in neurobehavioral performance among subjects exposed to a similar mixture of 23 VOCs relative to clean air. However, the research group from the Technical University of Denmark report several studies in which subjects are exposed to off-gassing from a 20 year old carpet and show reductions in productivity on tasks that simulate office work (e.g., typing, calculations) (Wargocki et al., 1999; Wargocki et al., 2000). While the total VOCs were of similar concentrations between the exposure conditions with and without the

carpet (~2.34 ppm), the composition of the chemical mixture differed for the condition with the carpet (e.g., acetone, acetic acid) compared to without the carpet. Consistent with ratings indicating poorer air quality associated with the VOCs and VOCs + O exposures in the present study, subjects were more dissatisfied with the air quality in the Wargocki studies. In contrast to the negative findings in the present study, Wargocki et al. also reported more symptoms (e.g., headache) and slower typing speed in response to the condition with the old carpet (polluted condition). Since the overall exposure concentration (2.35 ppm) was less than in the present study, symptom and performance differences may be due to differences in the chemical composition or to differences in task demands. Neither acetone nor acetic acid was present in the current VOCs mixture. Also, subjects in the Wargocki et al. (1999,2000) study performed the tasks over a longer period of time than in the present study (265 minutes) and the typing task was more sensitive than traditional measures such as the Stroop, for which no effects were observed in the Wargocki et al. (1999,2000) study. However, the POL has been shown to be sensitive to the effects of acute alcohol at relatively low blood alcohol concentrations (Mills).

Several factors in the present study were hypothesized to exacerbate exposure effects. For example, stress alone and in combination with negative affect significantly increased anxiety symptoms. The effect of stress was further validated by the significant difference in cortisol for those who received the stressor relative to subjects who did not. Although exposure alone did not have significant impact on symptoms, the interaction of negative affect, stress, and exposure was significant. Essentially after controlling for baseline, subjects low in negative affect reported more symptoms of anxiety after the stressor and during exposure to VOCs + O relative to the VOCs and MCA conditions. This result was not true for subjects high in negative affect

who reported more anxiety symptoms following the stressor regardless of exposure condition. Thus, the current study is similar to previous studies in finding that individuals who score relatively higher on a scale of negative affect report more anxiety and somatic symptoms across the board than those who do not possess this quality (e.g., Watson & Clark, 1984; Diefenbach et al., 1996). Furthermore, stress seemed to overwhelm any specific effect of exposure for those high in negative affect. Stegen et al. (2000) also found that high NA subjects were unable to discriminate 5.5% CO_2 with 21% O_2 and 73.5% N_2 from room air when subjects were given uncertain information about the air mixture. That is, subjects were told that it was unclear whether breathing the air mixtures would produce a negative or positive feeling. Low NA subjects were able to discern when the elevated CO_2 mixture was administered while high NA subjects could not. Subjects in the current study were not intentionally given any expectations about the exposures although informed consent required that subjects be told about common symptoms associated with poor indoor air quality and that these symptoms would be transitory. One could interpret this information as ambiguous. In a manner similar to Stegen et al.'s (2000) study, high NA subjects' anxiety was elevated regardless of exposure while low NA subjects' anxiety was not.

The same interaction effect was not observed when subjects high and low in chemical intolerance were compared. It appears that chemical intolerance, as defined in the current study, did not confer increased risk of symptoms in response to either increasing concentrations of acute chemical mixtures or potentially irritating secondary products produced by the addition of ozone. Although chemical intolerance increased the odds of reporting general VOC symptoms,

combining high negative affect and chemical intolerance did not increase the risk of symptom reporting with exposure to VOCs or VOCs + O.

In contrast to symptoms, ratings of odor intensity and unpleasantness of the odor were significantly worse in the VOCs and VOCs + O conditions than the MCA condition. Subjects also reported the odor as significantly more stressful in the VOCs and VOCs + O conditions. Subjects rated air quality as worse and indicated that they needed to ventilate the room in the VOCs and VOCs + O conditions relative to MCA. Although the "clean air" condition was "masked" with a pulse of VOCs in an effort to blind subjects to the exposure condition, analysis of the guess data and ratings of the strength of exposure revealed that subjects were able to differentiate the MCA condition from the VOCs and VOCs + O conditions. Subjects could not distinguish between the VOCs and VOCs + O conditions. Thus, ratings of odor and air quality appear to be consistent with subjects' awareness of relative exposure concentrations. This awareness and negative ratings of air quality, however, did not influence health symptom reports even among those high in chemical odor intolerance or negative affect.

Subjects were asked after each exposure session to think about the conditions they experienced as if this was their work environment. They were then asked to rate their annoyance level with these physical work conditions and with their symptoms. Similar to the odor and environmental quality ratings, subjects rated the physical work conditions and their symptoms during the VOCs and VOCs + O conditions significantly more annoying than following the MCA condition. This is particularly interesting since their symptoms were not significantly worse between these exposure conditions. Results of the symptom, environment, and odor ratings suggest that an

indoor environment contaminated with VOCs is unpleasant and annoying but does not necessarily induce health symptoms among healthy women.

While some increased symptoms were observed in previous controlled exposures using similar "indoor air" mixtures (e.g., Hudnell et al. (1992), Prah et al., 1998), actually only a few out of many symptoms assessed were significantly increased. Careful examination of those symptoms exacerbated by exposure reveals some consistency with the current findings. Prah et al. (1998) reported that relative to clean air, mixtures of indoor contaminants increased ratings of odor intensity and nasal irritation and reduced air quality and pleasantness, sensory qualities rather than health effects. Similarly comparing responses of a VOC mixture to clean air, Hudnell et al. (1992) reported significantly reduced air quality and increased odor level but they also reported increased symptoms of headache, eye irritation, drowsiness, and throat irritation. However, Hudnell et al. (1992) did not control for multiple statistical comparisons among the individual tests of 22 symptoms. Furthermore, no previous indoor air study has "masked" the clean air condition to control for the effects of odor on symptoms.

For all subjects combined, VOCs and VOCs + O exposures also did not cause a significant increase in PMNs, IL-6, IL-8 or total protein in NL fluid (Tables 28-30). These results contrast with the findings of Koren et al, (1992) who observed a significant two-fold increase in PMNs in NL immediately after a 4-hour exposure to a mixture of 22 VOCs at 25 mg/m^3 (Koren, Graham, & Devlin 1992). However, our results are consistent with the findings of Pappas et al, (2000) who found no significant increase in NL PMNs at 2 hours after 4 hour exposures to the same VOCs mixture at 25 mg/m^3, as well as at a concentration of 50 mg/m^3 (Pappas et al., 2000.

Differences in time-course and technique may explain some of the differences in results. The exposure period in the current study was shorter, with post-exposure NL taking place about 3 hours after the start of the 135-minute exposure. However, it seems unlikely that this would fully explain the lack of positive findings in a study that had a much larger sample size than the previously reported studies.

Chemical analysis confirmed the formation of potentially irritating compounds such as aldehydes and fine particles in the VOCs + O exposure as reported earlier (Fan, et al., 2003), yet no significant differences in nasal irritation symptoms or PMNs were observed between the VOCs + O, VOCs and MCA exposures. Perhaps this result is not entirely unexpected, given that the concentrations of ozone (40 ppb) and terpenes (285 ppb) were several orders of magnitude lower than concentrations used in positive animal irritation studies (3 ppm and 50 ppm, respectively) (Wolkoff, et al., 2000).{2027} Yet the terpene concentration was higher than levels typically reported in indoor air, indicating that terpene oxidation products may not be important causes of acute nasal irritation and inflammation at ambient indoor concentrations.

A nasal spray lavage method that had been used successfully to study nasal markers of inflammation in previously several published studies was used in the present study (Alexis et al. 2002; Peden et al. 1999; Noah et al. 1995). The earlier VOCs exposure studies, including the positive study by Koren et al, (1992) used a "bolus lavage" method in which 5 ml of saline was instilled into the nasal cavity with a blunt-tipped syringe. Several observations suggest that the two methods may yield somewhat different results. The shape of the distribution of pre-exposure PMN concentrations was similar to that obtained in 200 unexposed subjects using the

bolus lavage method, although the counts in the present study were more highly skewed towards zero values (Koren, Hatch, & Graham 1990). Microtrauma, resulting from repeatedly sniffing the lavage fluid and gently blowing the nose, likely accounted for the blood observed in the specimens. Although the presence of blood in the specimens was not significantly associated with exposure or PMN concentration, it is possible, but seems unlikely, that a nondifferential effect of blood-derived PMNs may have obscured the effect of exposure. It has been suggested that the spray technique may preferentially wash the anterior nasal cavity mucosa, while the bolus technique, in which fluid is held in the nasopharygeal space, may preferentially wash the posterior nasal cavity and nasopharynx (Peden 1996). Finally, the intraclass correlation coefficient (ICC) of the pre-exposure PMN concentrations, R=0.52 (95% CI R>=0.42), suggests that week-to-week within-subject variability is greater than between-subject variability. Hauser et al. (1994) reported an ICC of R= .88 (95% CI R.>= 0.75) with NL 72 hours apart. The longer time period between the NL samples in the present study (~ one week), may have contributed to greater within-subject variability. The low ICC calls into question the reliability of the spray lavage technique for investigating nasal responses. Unfortunately, any differences due to these two NL techniques cannot be evaluated directly in the absence of controlled study comparing the two techniques.

Reports of NSBRI typically describe a subgroup of individuals among the total building population who are symptomatic (Norback, Ingegerd, & Widstrom1990). Although only healthy women were included and subjects with a history of multiple chemical sensitivity were excluded in the present study, the possibility that "sensitive" subgroups existed within our sample of healthy women was explored. It was hypothesized that symptomatic "responders" might have

had significantly increased nasal PMN influx after the VOCs and/or VOCs + O exposures. "Responders" (N=49) were defined as individuals who reported larger increases in the nasal irritation symptom from baseline to minute 125 during either the VOCs or the VOCs + O exposure as compared to during the MCA exposure. This analysis (as described above) yielded results similar to those in the analysis of all subjects, with no significant effect of exposure.

As reviewed by Shusterman, subjects with seasonal allergic rhinitis have had increased sensory and/or physiological responses to nasal irritants in some studies, although this has not been a consistent finding (Shusterman 2002). In the present study, subjects were not thoroughly evaluated for atopy, but RAST test results were used as a crude measure of atopy. Separate analysis of symptoms among the "atopic" subgroup (defined as having a Class 2 or greater response to at least one of the 5 RAST) showed no significant effect of exposure on nasal symptoms or concentrations of PMNs, total protein, IL-6, and IL-8 in nasal lavage fluid .

In conclusion, relatively brief, one-time exposures to mixtures of low-level VOCs or VOCs and their oxidation products do not appear to cause significant acute changes in symptoms, nasal inflammation, or neurobehavioral performance in healthy women. Subjects found the air quality to be worse and the intensity of the odor to be greater in these conditions and projected that this would be annoying if these conditions were present in their every day working environment. In contrast, the psychological stressor was effective in producing increased autonomic arousal as indicated by salivary cortisol and in causing increased symptoms of anxiety, particularly among individuals high in negative affect. This "stress effect" seemed to overwhelm any exposure effect on anxiety among those high in negative affect while anxiety was exacerbated by exposure to VOCs + O for those low in negative affect. The effect of stress and exposure, however, was

isolated to symptoms of anxiety and did not generalize to other more typical symptoms associated with poor indoor air such as nasal irritation or headache. Although the irritation potency of complex and variable mixtures of VOCs and their oxidation products is difficult to predict, reported air concentrations of VOCs in buildings with poor indoor air quality are typically lower than the VOC concentrations used in this study. Therefore, the present results support the conclusion that VOCs at concentrations typically found in non-industrial buildings are unlikely to be a significant cause of acute upper respiratory irritation for most occupants.

Several caveats need to be considered for the present study. Although this study included the largest number of subjects, to date, and intentionally selected only women for study due to their hypothesized vulnerability to report indoor air quality symptoms, the ability to recruit subjects at the extremes of either negative affect or chemical intolerance was not possible. Subjects with higher levels of these characteristics simply did not volunteer for the study. This will likely be a problem in any chemical exposure study since individuals who regard themselves as intolerant of chemicals or who are relatively more distressed by daily life circumstances are probably less likely to want to subject themselves to an environment that they are told could result in some discomfort or even health effects that are transitory. The extent to which an acute model of indoor air exposure applies to indoor air problems experienced in a work environment with many other demands and chronic exposure to chemical mixtures is problematic. Thus, the lack of health effects observed may simply be a function of the necessarily acute exposure paradigm. On the other hand, the exposure concentrations were relatively higher than those documented in most buildings with indoor air complaints. Furthermore, work demands were modeled in the present study through the use of a known stressor as well as requirements for computerized

neurobehavioral tasks and the former was successful in causing autonomic arousal and symptoms of anxiety. Overall, this study suggests that psychological stress and annoyance with the unpleasant odor of the air may be more potent factors than chemical mixtures in the complaints that coincide with poor indoor environments.

Reference List

1. Agresti A, Lang JB: A proportional odds model with subject-specific effects for repeated ordered categorical responses. *Biometrika* 80:527-534, 1993

2. al'Absi M, Bongard S, Buchanan T, Pincomb GW, Licinio J, Lovallo WR: Cardiovascular and neuroendocrine adjustment to public speaking and mental arithmetic stressors. *Psychophysiology* 34:266-275, 1997

3. Alexis N, Griffith K, Almond M, Peden DB: IL-4 induces IL-6 and signs of allergic-type inflammation in the nasal airways of nonallergic individuals. *Clin.Immunol.* 104:217-220, 2002

4. Apter A, Bracker A, Hodgson M, Sidman J, Leung W-Y: Epidemiology of the sick building syndrome. *J Allergy Clin Immunol* 94:277-288, 1994

5. Bachmann MO, Myers JE: Influences on sick building syndrome symptoms in three buildings. *Soc.Sci.Med.* 40:245-251, 1995

6. Baldwin CM, Bell IR, O'Rourke MK, Lebowitz MD: The association of respiratory problems in a community sample with self-reported chemical intolerance. *Eur.J.Epidemiol.* 13:547-552, 1997

7. Bauer RM, Greve KW, Besch EL, Schramke CJ, Crouch J, Hicks A, Ware MR, Lyles WB: The role of psychological factors in the report of building-related symptoms in sick building syndrome. *J Consulting and Clinical Psychology* 60:213-219, 1992

8. Bell IR, Peterson JM, Schwartz GE: Medical histories and psychological profiles of middle-aged women with and without self-reported illness from environmental chemicals. *J Clin Psychiatry* 56:151-160, 1995

9. Bell IR, Schwartz GE, Peterson JM, Amend D: Self-reported illness from chemical odors in young adults without clinical syndromes or occupational exposures. *Arch.Environ.Health* 48:6-13, 1993

10. Bell IR, Wyatt JK, Bootzin RR, Schwartz GE: Slowed reaction time performance on a divided attention task in elderly with environmental chemical odor intolerance. *Intern J.Neuroscience* 84:127-135, 1996

11. Clark LA, Watson D, Mineka S: Temperament, personality, and the mood and anxiety disorders. *J Abn Psychology* 103:103-116, 1994

12. Clark LA, Watson D: Tripartite model of anxiety and depression: psychometric evidence and taxonomic implications. *J.Abnorm.Psychol.* 100:316-336, 1991

13. Cowart BJ: Relationships between taste and smell across the adult life span. *Annals New York Academy of Sciences* 561:39-55, 1989

14. Dalton P, Wysocki CJ, Brody MJ, Lawley HJ: Perceived odor, irritation, and health symptoms following short-term exposure to acetone. *Am.J.Ind.Med.* 31:558-569, 1997

15. Davidian M, Giltinan DM: *Nonlinear Models for Repeated Measurement Data*. New York, NY, Chapman & Hall, 1995

16. Deshields TL, Tait RC, Gfeller JD, Chinbnall JT: Relationship between social desirability and self-report in chronic pain patients. *The Clinical Journal of Pain* 11:189-193, 1995

17. Diefenbach MA, Leventhal EA, Leventhal H, Patrick-Miller L: Negative affect relates to cross-sectional but not longitudinal symptom reporting: data from elderly adults. *Health Psychol.* 15:282-288, 1996

18. Dirks H, Colic D, Haeckel R, et al: Die Bestimmung von cortisol im speichel und ihre klinische Anwendung. In Speicheldiagnostik. Haeckel R, Ed. Darmstadt, Germany, GIT-Verlag, 1988, p. 86-93

19. Dohrenwend BS, Krasnoff L, Askenasy AR, Dohrenwend BP: Exemplification of a method for scaling life events: the peri life events scale. *J.Health Soc.Behav.* 19:205-229, 1978

20. Fan Z, Lioy P, Weschler C, Fiedler N, Kipen H, Zhang J: Ozone-initiated reactions with mixtures of volatile organic compounds under simulated indoor conditions. *Environ Sci Technol* 37:1811-1821, 2003

21. Fiedler N, Kelly-McNeil K, Mohr S, Lehrer P, Opiekun R, Lee CW, Wainman T, Hamer R, Weisel C, Edelberg R, Lioy PJ: Controlled human exposure to methyl tertiary butyl ether in gasoline: symptoms, psychophysiologic and neurobehavioral responses of self-reported sensitives. *Environ.Health Perspect.* 108:753-763, 2000

22. Green HG, Dalton P, Cowart B, Shaffer G, Rankin K, Higgins J: Evaluating the labeled magnitude scale for measuring sensations of taste and smell. *Chem.Senses* 21:323-334, 1996

23. Hauser R, Garcia-Closas M, Kelsey KT, Christiani DC: Variability of nasal lavage polymorphonuclear leukocyte counts in unexposed subjects: its potential utility for epidemiology. *Arch.Environ.Health* 49:267-272, 1994

24. Hodgson MJ, Frohliger J, Permar E, Tidwell C, Traven ND, Olenchock SA, Karpf M: Symptoms and microenvironmental measures in nonproblem buildings. *J Occupational Medicine* 33:527-533, 1991

25. Hoppe P, Praml G, Rabe G, Linder J, Fruhmann G, Kessel R: Environmental ozone field study on pulmonary and subjective responses of assumed risk groups. *Environmental Research* 71:109-121, 1995

26. Horstman DH, Folinsbee LJ, Ives PJ, Abdul-Salaam S, McDonnell WF: Ozone concentration and pulmonary response relationships for 6.6-hour exposures with five hours of moderate exercise to 0.08, 0.10, and 0.12 ppm. *Am Rev Resp Dis* 142:1158-1163, 1990

27. Hudnell HK, Otto DA, House DE, Molhave L: Exposure of humans to a volatile organic mixture. II. Sensory. *Arch.Environ.Health* 47:31-38, 1992

28. Kahn J-P, Rubinow DR, Davis CL, et al: Salivary cortisol: a practical method for evaluation of adrenal function. *Biol Psychiatry* 23:335-349, 1988

29. Kirschbaum C, Hellhammer DH: Salivary cortisol in psychobiological research: an overview. *Neuropsychobiology* 22:150-169, 1989

30. Kirschbaum C, Hellhammer DH: Salivary cortisol in psychoneuroendocrine research: recent developments and applications. *Psychoneuroendocrinology* 19:313-333, 1994

31. Kjaergaard SK, Molhave L, Pedersen OF: Human reactions to a mixture of indoor air volatile organic compounds. *Atmospheric Environ* 25:1417-1426, 1991

32. Knasko SC, Gilbert AN, Sabini J: Emotional state, physical well-being and performance in the presence of feigned ambient odor. *J Appl Soc Psychol* 20:1345-1357, 1990

33. Knasko SC: Performance, mood, and health during exposure to intermittent odors. *Arch.Environ.Health* 48:305-308, 1993

34. Kobal G, Hummel T, Van Toller S: Differences in human chemosensory evoked potentials to olfactory and somatosensory chemical stimuli presented to left and right nostrils. *Chem.Senses* 17:223-244, 1992

35. Koren HS, Hatch GE, Graham DE: Nasal lavage as a tool in assessing acute inflammation in response to inhaled pollutants. *Toxicology* 60:15-25, 1990

36. Koren HS, Graham DE, Devlin RB: Exposure of humans to a volatile organic mixture. III. Inflammatory response. *Archives of Envirionmental Health* 47:39-44, 1992

37. Kreiss K: The sick building syndrome: where is the epidemiologic basis. *Am J Publ Health* 80:1172-1173, 1990

38. Landis JR, Miller ME, Davis CS, Koch GG: Some general methods for the analysis of categorical data in longitudinal studies. *Stat.Med.* 7:109-137, 1998

39. Liang KY, Zeger LL: Longitudinal Data Analysis Using Generalized Linear Models. *Biometrika* 73:13-22, 1986

40. Lumley MA, Mader C, Gramzow J, Papineau K: Family factors related to alexithymia characteristics. *Psychosom.Med.* 58:211-216, 1996

41. Lumley MA, Asselin LA, Norman S: Alexithymia in chronic pain patients. *Compr.Psychiatry* 38:160-165, 1997

42. McCulloch CE, Searle SR: *Generalized, Linear, and Mixed Models.* New York, NY, John Wiley & sons, Inc., 2000

43. Mendell M, Smith A: Consistent pattern of elevated symptom rates in air-conditioned office buildings: a re-analysis of epidemiologic studies. *Am J Public Health* 80:1193-1199, 1990

44. Mendell MJ: Non-specific symptoms in office workers: a review and summary of the epidemiologic literature. *Indoor Air* 3:227-236, 1993

45. Mendell MJ, Fisk WJ, Kreiss K, Levin H, Alexander D, Cain WS, Girman JR, Hines CJ, Jensen PA, Milton DK, Rexroat LP, Wallingford KM: Improving the health of workers in indoor environments: priority research needs for a national occupational research agenda. *Am.J.Public Health* 92:1430-1440, 2002

46. Menzies D, Tamblyn RM, Nunes F, Hanley J, Tamblyn RT: Exposure to varying levels of contaminants and symptoms among workers in two office buildings. *Am J Publ Health* 86:1629-1633, 1996

47. Menzies D, Bourbeau J: Building-related illnesses. *The New England Journal of Medicine* 337:1524-1531, 1997

48. Mills KC, Parkman KM, Spruill SE: A pc based software test for measuring alcohol and drug effects in human subjects. *Alcoholism Clinical and Experimental Research* 20:1582-1591, 1996

49. Molhave L, Back B, Pedersen OF: Human reactions to low concentrations of volatile organic compounds. *Environ Internatl* 12:167-175, 1986

50. Noah TL, Henderson FW, Henry MM, Peden DB, Devlin RB: Nasal lavage cytokines in normal, allergic, and asthmatic school-age children. *Am.J.Respir.Crit Care Med.* 152:1290-1296, 1995

51. Norback D, Ingegerd M, Widstrom J: Indoor air quality and personal factors related to the sick building syndrome. *Scan J Work Environ & Health* 16:121-128, 1990

52. Nordstrom K, Norback D, Akselsson R: Effect of air humidification on the sick building syndrome and perceived indoor air quality in hospitals: a four month longitudinal study. *Occup Environ Med* 51:683-688, 1994

53. Ohman Strickland PA, Lu S-E: Estimates, power and sample size calculations for two-sample ordinal outcomes under before-after study designs. *Stat.Med.* 22:1807-1818, 2003

54. Otto D, Hudnell HK, House D, Molhave L, Counts W: Exposure of humans to a volatile organic mixture, behavioral assessment. *Arch Environ Health* 47:23-30, 1992

55. Pappas GP, Herbert RJ, Henderson W, Koenig J, Stover B, Barnhart S: The respiratory effects of volatile organic compounds. *International Journal of Occupational & Environmental Health* 6:1-8, 2000

56. Peden DB, Tucker K, Murphy P, Newlin-Clapp L, Boehlecke B, Hazucha M, Bromberg P, Reed W: Eosinophil influx to the nasal airway after local, low-level LPS challenge in humans. *J.Allergy Clin.Immunol.* 104:388-394, 1999

57. Prah JD, Case MW, Goldstein GM: 1998 Equivalence of sensory responses to single and mixed volatile organic compounds at equimolar concentrations. *Environ.Health Perspect.* 106:739-744, 1998

58. Rohr AC, Wilkins CK, Clausen PA, Hammer M, Nielsen GD, Wolkoff P, Spengler JD: Upper airway and pulmonary effects of oxidation products of (+)-alpha-pinene, d-limonene, and isoprene in BALB/c mice. *Inhal.Toxicol.* 14:663-684, 2002

59. Ryan CM, Morrow KA: Dysfunctional buildings or dysfunctional people: an examination of the sick building syndrome and allied disorders. *J Consulting and Clinical Psychology* 60:220-224, 1992

60. SAS Institute, Inc. SAS/STAT User's Guide. Version 8. 1999. NC, SAS Institute, Inc.

61. Shusterman D: Review of the upper airway, including olfaction, as mediator of symptoms. *Environ.Health Perspect.* 110 Suppl 4:649-653, 2002

62. Sieber WK, Petersen MR, Stayner L: The NIOSH indoor environmental evaluation experience: part three - associations between environmental factors and self-reported health outcomes. *Appl Occup Environ Hyg* 11:1387-1392, 1996

63. Skov P, Valbjorn O, Pedersen BV, Danish Indoor Climate Study Group: Influence of personal characteristics, job-related factors and psychosocial factors on the sick building syndrome. *Scan J Work Environ & Health* 15:286-295, 1989

64. Stegen K, Van Diest I, Van de Woestijne KP, Van den Bergh O: Negative affectivity and bodily sensations induced by 5.5% CO2 enriched air inhalation: is there a bias to interpret bodily sensations negatively in persons with negative affect? *Psychology & Health* 15:513-525, 2000

65. Stenberg B, Eriksson N, Hoog J, Sundell J, Wall S: The sick building syndrome (SBS) in office workers. A case-referent study of personal, psychosocial and building-related risk indicators. *Int.J.Epidemiol.* 23:1190-1197, 1994

66. Stenberg B, Wall S: Why do women report "sick building symptoms" more often than men. *Soc.Sci.Med.* 40:491-502, 1995

67. Sundell J, Lindvall T, Stenberg B: Influence of type of ventilation and outdoor airflow rate on the prevalence of SBS symptoms. Indoor air quality 91: healthy buildings. *American Society of Heating, Refrigerating, and Air-Conditioning Engineers*85-90, 1991

68. Szarek MJ, Bell IR, Schwartz GE: Validation of a brief screening measure of environmental chemical sensitivity: the chemical odor intolerance index. *Journal of Environmental Psychology* 17:345-351, 1997

69. Wainman T, Zhang J, Weschler CJ, Lioy PJ: Ozone and limonene in indoor air: A source of submicron particle exposure. *Environ.Health Perspect.* 108:1139-1145, 2000

70. Walker RF, Joyce BG, Dyas J, et al: Immunoassays of steroids in saliva. In Salivary cortisol: 1. Monitoring changes in normal adrenal activity. Read GF, Riad-Fahmy D, Walker RF, Eds. Cardiff, Wales, Alpha Omega, 1984, p. 308-316

71. Wargocki P, Wyon DP, Baik YK, Clausen G, Fanger PO: Perceived air quality, sick building syndrome (SBS) symptoms, and productivity in an office with two different pollution loads. *Indoor Air* 9:165-179, 1999

72. Wargocki P, Wyon DP, Sundell J, Clausen G, Fanger P: The effects of outdoor supply rate in an office on perceived air quality, sick building syndrome (SBS) symptoms, and productivity. *Indoor Air* 10:222-236, 2000

73. Wargocki P, Wyon DP, Fanger P: Productivity is affected by the air quality in offices. In Healthy Buildings 2000. Sappanen O, Sateri J, Eds. Helsinki, Finland, Espo, Finland: SIY Indoor Air Information, 2000, p. 635-640

74. Watson D, Clark LA: Negative affectivity: the disposition to experience aversive emotional states. *Psychol.Bull.* 96:465-498, 1984

75. Watson, D and Clark, L. A. Manual for the Positive and Negative Affect Schedule (expanded form). 1994. Iowa City, Iowa, University of Iowa.

76. Watson D, Pennebaker JW: Health complaints, stress, and distress: exploring the central role of negative affectivity. *Psychol.Rev.* 96:234-254, 1989

77. Weschler CJ, Shields HC: The influence of ventilation on reactions among indoor pollutants: modeling and experimental observations. *Indoor Air* 10:92-100, 2000

78. Wilkins CK, Clausen PA, Wolkoff P, Larsen ST, Hammer M, Larsen K, Hansen V, Nielsen GD: Formation of strong airway irritants in mixtures of isoprene/ozone and isoprene/ozone/nitrogen dioxide. *Environ.Health Perspect.* 109:937-941, 2001

79. Wise TN, Mann LS: The relationship between somatosensory amplification, alexithymia, and neuroticism. *J.Psychosom.Res.* 38:515-521, 1994

80. Wolkoff P, Clausen PA, Jensen B, Nielsen GD: Are we measuring the relevant indoor pollutants? *Indoor Air* 7:92-106, 1997

81. Wolkoff P, Clausen PA, Wilkins CK, Nielsen GD: Formation of strong airway irritants in terpene/ozone mixtures. *Indoor Air* 10:82-91, 2000

82. Woods JE: Cost avoidance and productivity in owning and operating buildings. *Occup Med State Art Rev* 4:753-770, 1989

83. Zweers T, Preller L, Brunekreef B, Boleij JSM: Health and indoor climate complaints of 7043 office workers in 61 buildings in the Netherlands. *Indoor Air* 2:127-136, 1992

APPENDIX A

LIST OF PUBLICATIONS:

Zhihua, F., Lioy, P., Weschler, C., Fiedler, N., Kipen H., Zhang, J. Ozone-initiated reactions with mixtures of volatile organic compounds under simulated indoor conditions. Environ Sci Technol, 2003;37:1811-1821.