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Toxicol Ind Health 1999; 15; 339
DOI: 10.1177/074823379901500308

Toxicology and Industrial Health **(1999) 15, 339–345**

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Sensory irritation and multiple chemical sensitivity

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Many of the symptoms described in Sick Building Syndrome (SBS) and multiple chemical sensitivity (MCS) resemble the symptoms known to be elicited by airborne irritant chemicals. Irritation of the eye, nose, and throat is common to SBS, MCS, and sensory irritation (SI). Difficulty of breathing is often seen with SBS, MCS, and pulmonary irritation (PI). We therefore asked the question: can indoor air pollutants cause SI and/or PI? In laboratory testing in which mice breathed the dilute volatile emissions of air fresheners, fabric softeners, colognes, and mattresses for 1 h, we measured various combinations of SI and PI as well as airflow decreases (analogous to asthma attacks). Air samples taken from sites associated with repeated human complaints of poor air quality also caused SI, PI, and airflow limitation (AFL) in the mice. In previous publications, we have documented numerous behavior changes in mice (which we formally studied with a functional observational battery) after exposure to product emissions or complaint site air; neurological complaints are a prominent part of SBS and MCS. All together, these data suggest that many symptoms of SBS and MCS can be described as SI, PI, AFL, and neurotoxicity. All these problems can be caused by airborne irritant chemicals such as those emitted by common commercial products and found in polluted indoor air. With some chemical mixtures (e.g., emissions of some fabric softeners, disposable diapers, and vinyl mattress covers) but not others (e.g., emissions of a solid air freshener), the SI response became larger (2- to 4-fold) when we administered a series of two or three 1-h exposures over a 24-h period. Since with each exposure the intensity of the stimulus was constant yet the magnitude of the response increased, we concluded that there was a change in the sensitivity of the mice to these chemicals. The response was not a generalized stress response because it occurred with only some mixtures of irritants and not others; it is a specific response to certain mixtures of airborne chemicals. This is one of the few times in MCS research that one can actually measure *both* the intensity of the stimulus *and* the magnitude of the response and thus be allowed to discuss sensitivity changes. The changing SI response of the mice might serve as a model of how people develop increasing sensitivity to environmental pollutants. Intensive study of this system should teach us much about how people respond to and change sensitivity to airborne irritant chemicals.

Keywords: *mice, sensitivity, sensory irritation, toxicity.*

Introduction

Our laboratory has been studying some of the acute biological effects of the emissions which offgas from various common consumer products such as air fresheners, colognes, fabric softeners, and mattresses. These items were chosen because they are frequently identified as noxious to individuals with multiple chemical sensitivity (MCS). We found that these mixtures of airborne chemicals can cause several acute toxic effects in normal laboratory mice: sensory irritation (SI), pulmonary irritation (PI), reduction of expiratory airflow velocity, and neurotoxicity (Anderson and Anderson, 1997a–c, 1998, 1999a–c). SI is caused by activation of the nerve endings of the trigeminal nerve in the face, eyes, and nasal passages. PI is caused by activation of the vagus nerve endings in the lower airway (Alarie, 1973). Decreased expiratory airflow velocity can be caused by several mechanisms, especially narrowing of the airway by muscular contraction, edema, or exudate. Neurotoxicity presumably reflects absorption of the airborne chemicals into the circulatory system and entry into some part of the nervous system. We believe these four types of effects seen in mice have counterparts in various aspects of Sick Building Syndrome (SBS) and MCS. In humans, SI manifests as a sensation of sore eyes, burning face, or sore throat; PI produces a feeling of tight chest or difficulty of breathing. Irritation of the eyes, nose, and face plus difficulty of breathing are frequently parts of the SBS and MCS syndrome (Ashford and Miller, 1998). Some people with chemical sensitivity have problems with airflow velocity (including asthma), and neurological problems are a prominent complaint in some individuals suffering from MCS (e.g., confusion, fatigue, difficulty concentrating, poor memory, etc.). Thus, there is considerable overlap between symptoms related to SI, PI, and AFL and many of the symptoms described in SBS and MCS. This overlap led us to study whether common air pollutant mixtures can cause SI, PI, AFL, or neurotoxicity in mice; this paper presents some of the data which demonstrate that common air pollutants can produce these toxic effects in mice.

1. Abbreviations: AFL, airflow limitation; GC/MS, gas chromatography mass spectroscopy; MCS, multiple chemical sensitivity; PI, pulmonary irritation; SBS, Sick Building Syndrome; SI, sensory irritation; TB, time of break; TP, time of pause; VD, velocity of deflation.

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One of the unexplained aspects of the MCS syndrome is the apparent increased sensitivity of the victims to the presence of low concentrations of a variety of airborne chemicals (Ashford and Miller, 1998). We will describe data obtained in experiments involving repeated exposures of mice to airborne irritant chemicals, and illustrate how the SI system can change in sensitivity to the effects of these chemical mixtures.

Materials and methods

Samples

Samples of various commercial products were purchased in several retail stores in New England and stored in their original packages until used. These items were placed in an all-glass chamber (37 l, $0.25 \times 0.30 \times 0.50$ m^3), which was sealed, warmed to 27°C, and allowed to equilibrate for 1 h before use. Temperatures in the sample chamber and in the animal chamber were monitored by a Cole-Parmer thermistor model 8402-00.

Animals

Male Swiss–Webster mice were obtained from Taconic Farms in Germantown, New York, and housed in polypropylene cages according to published guidelines (National Research Council, 1996). The animal weights were between 25 and 28 g after a 1-week holding period with 12 h light/12 h dark cycles. Cage bedding was corncob chips. Purina lab chow and bottled water were available except during exposures. Extensive reports of source colony animal health were provided regularly by the animal supplier. Histological evaluation of lungs from our unexposed colony mice showed no signs of bacterial, viral, or parasitic infections.

Animal Exposure

For each test, four mice were positioned in the glass exposure chamber as described in ASTM-E-981 (ASTM, 1984) and later modified by Vijayaraghavan et al. (1993). The details of the exposure chamber, exposure techniques, and data collection have been extensively described previously (Vijayaraghavan et al., 1993). The head of each mouse extended into the central exposure area with the body in a side arm which served as a whole body plethysmograph. During the 15-min control (baseline) and the 15-min recovery periods, the animal exposure chamber was continuously ventilated with charcoal-filtered air. During the exposure (60 min), the animals breathed product emissions carried by charcoal-filtered air. Air flow was 6 l/min. The temperature in the animal exposure chamber was 22°–24°C. During the experiment, the system was open (dynamic): charcoal-filtered air was passed through the sample-holding chamber to the animal exposure chamber, and was then exhausted out of the building. The animals were returned to their cages with food and water for 4 h between the exposures.

Bioassay ASTM-E-981

ASTM-E-981 is a standardized toxicological test method for measuring biological effects of airborne irritant chemicals (ASTM, 1984). The original protocol uses plethysmographs to monitor respiratory changes in volume and frequency; we used this procedure for many of the air sample tests reported here and previously (Anderson and Coogan, 1994). More recently, (Vijayaraghavan et al. 1993, 1994 and Boylstein et al. 1995) automated the data collection portion of this procedure to yield quantitative data on the frequency and severity of several changes in the respiratory pattern. This newer technique involves the use of a pneumotachograph to continuously measure flow of air during each breath of each mouse. Digital computer programs (obtained from Dr. Y. Alarie, University of Pittsburgh School of Public Health) integrate flow rates to calculate volume changes on each portion of each respiratory cycle of each mouse.

In these assays, abnormalities caused by airborne irritant chemicals are recognized only if they cause changes in respiratory parameters which are statistically significant as compared to the average values determined for each mouse each day during the 15-min baseline period. Specifically SI, PI, and AFL are diagnosed by this rule-based computer program by statistical comparison of each breath during the exposure period to the 3500 breaths measured during the baseline period for each mouse at the beginning of each experiment. The respiratory cycle of the normal mouse contains very brief pauses after inspiration and expiration. The pause after inspiration is called 'time of break', it is abbreviated TB and is typically about 0.02 to 0.03 s duration. The pause after expiration is called 'time of pause', it is abbreviated TP and is typically about 0.025 to 0.035 s duration. Diagnoses of SI or PI require increases in TB or TP greater than 2.0 times the standard deviation of the mean value for that mouse during the baseline period for that experiment. Airflow limitations (AFLs) were diagnosed when the expiratory flow rate at 50% expiration (VD, velocity of deflation) fell 1.5 standard deviations below the mean value for that animal's baseline VD. In each experiment, approximately 90,000 breaths were analyzed.

Most of the data concerning effects of consumer product emissions come from experiments using this computerized version of ASTM-E-981. The computer program splits the data into 15-s intervals and for each interval calculates the percentage of the breaths which fits the criteria for each of the possible diagnoses. The programs calculate the mean for each of the four mice for each 15-s period; these data are plotted against time to determine the peak effect for each

mouse (or for the group as in Figure 2). We have found it useful (especially when we have more than four mice exposed and their peak responses occur at different times) to use the peak effect for each diagnosis for each mouse to summarize the results of an entire experiment. Many of the tables and graphs show the average of these peak values.

Chemical Analysis of the Test Atmosphere

To monitor the intensity of the exposures, the total volatile organic chemicals were determined from the test mixture using flame ionization detection (FID, Beckman Industrial, Model 400 A) with methane (100 ppm) as calibration gas. To determine the major constituents of the test mixtures, samples were adsorbed onto Carbotrap 300 or charcoal tubes. These samples were subjected to gas chromatography mass spectroscopy (GC/MS) analysis by Citizens' Environmental Laboratory, Cambridge, MA.

Results

Figure 1 illustrates several computer-based analyses of the respiratory patterns of mice exposed to fabric-softener emissions. Figure 1A shows tracings diagnosed as normal; the intervals considered to represent TB and TP are indicated by the vertical lines. Tracings in Figure 1B have prolonged TB intervals and are diagnosed as S (for SI); in tracing Figure 1C, the second breath is followed by a long pause (TP), diagnosed as P (for PI). The second and third breaths in Figure 1D have prolonged expiration with low velocity airflow at the mid-expiratory point and are diagnosed as A (for AFL).

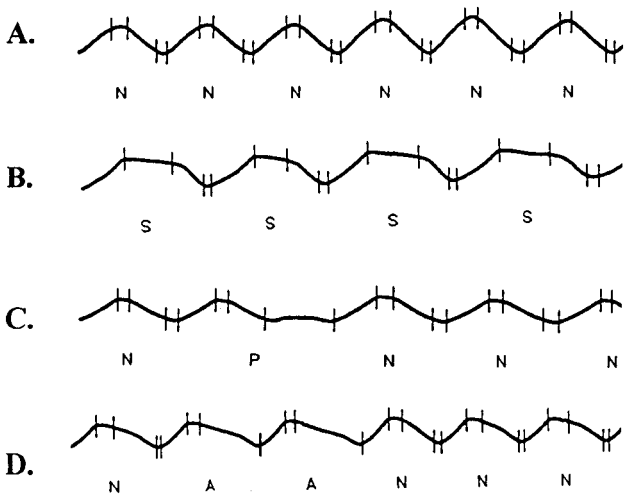

Figure 1. Respiratory cycles in exposed mice. The ordinant represents volume and the abscissa shows time (approximately 1.5 s). Inspiration results in upward movement of the line; expiration causes downward movement of the line. The tracings are the result of computer integration of the airflow velocity data. Letters below tracings show computer-assigned diagnoses of the individual breaths.

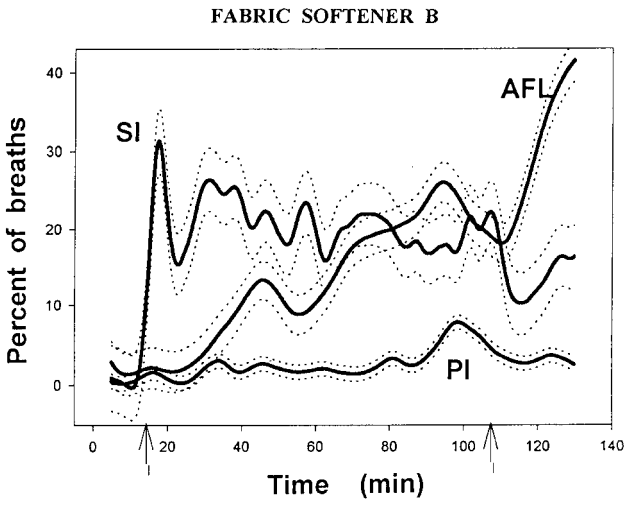

Figure 2. Time course of representative experiment. Emissions of 3.1 g of fabric softener B were present from 15 to 75 min. The graph shows the average of four mice calculated for each 15-s period. The dotted lines show the 95% confidence intervals for the curves.

Figure 2 shows a representative experiment in which four mice were exposed to the emissions of one of the fabric softeners (Brand B). The graph shows the average of the four mice calculated at each 15-s period. For the first 15 min, the mice breathe charcoal-filtered air, and data are collected to calculate the mean baseline values for TB, TP, and mid-expiratory air flow velocity for each mouse. After introduction of the fabric-softener emissions at 15 min, the mice rapidly developed prolonged TB; the prolongation was sufficiently large that approximately 32% of the breaths were diagnosed as having SI at the peak of the response. In this experiment, there were few times when the TP interval was prolonged, so very few breaths were diagnosed as showing PI. In contrast, there was progressive onset of expiratory AFL, and towards the end of the exposure, over 20% of the breaths showed significant airflow decrease (greater than 1.5 SDs) and were therefore diagnosed as having AFL.

Sixteen mice were exposed to the fabric softener emissions at the same concentration for a total of three exposures over a 24-h period. As the mice were exposed to this mixture of chemicals (Figure 3), they developed increasing TB values. The first encounter produced on the average 22% SI, the second encounter produced 52% SI, while the third encounter produced 47% SI at the peaks. The concentration of the emission mixture was determined by FID and was fairly constant (250 ppm ±10%) during the three exposures. Since the exposure concentration was essentially constant but the biological response increased, this is an example of increasing sensitivity of the mice; it developed over a total period of less than 24 h.

The change in sensitivity was due to the chemicals encountered; it was not an artifact of exposure to the

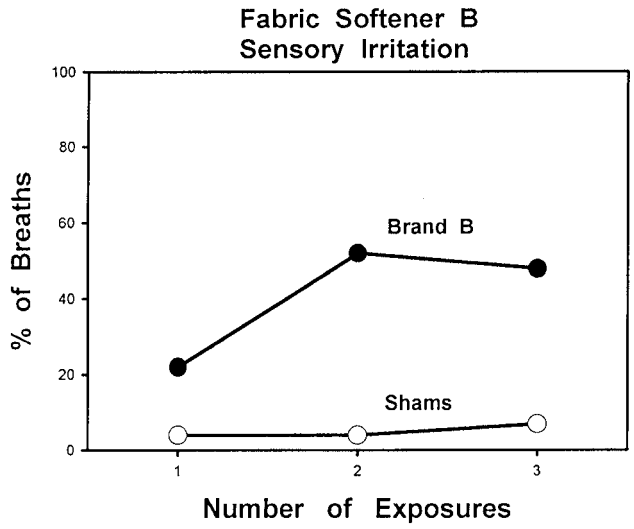

Figure 3. SI with repeat exposures to fabric softener B emissions. Sixteen mice were exposed to the emissions of 3.1 g of fabric softener B three times over a 24-h period. The upper line plots the means of the percentage of the breaths showing the SI effect at the peak response of each animal during the three 1-h encounters with this chemical mixture. The lower curve shows the results of sham exposures of 34 mice to charcoal-filtered air.

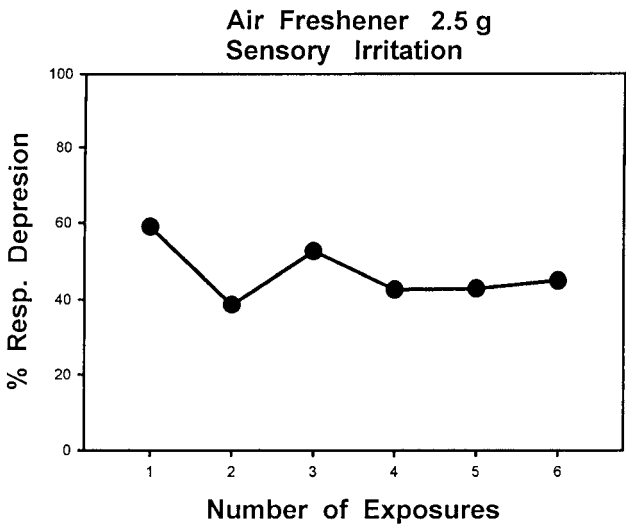

Figure 4. SI with repeat exposure to air-freshener emissions. Similar to Figure 3 but using 12 mice for six 1-h exposures to emissions of 2.5 g air freshener over a 3-day period.

apparatus itself. In sham exposures (mice breathing charcoal-filtered air only), less than 10% of the breaths was diagnosed as abnormal and this percentage did not increase with repeat exposures to charcoal-filtered air (lower line, Figure 3).

By contrast, similar experiments with a different brand of fabric softener (Brand A) did not lead to significant increases in SI; the percentage of breaths showing SI was 58 on the first exposure, 65 on the second, and 68 on the third. Likewise, six repeat exposures to air-freshener emissions did not result in increasing sensitivity (Figure 4); the percentage of respiratory depression (another measure of SI) ranged between 60% and 40% with no trend over the course of these experiments. In both these experiments (i.e., fabric softener A and air freshener), the emissions were quite noxious (i.e., produced significant SI), but no particular change in sensitivity was seen over the time course of these experiments. Thus, certain mixtures of chemicals (such as fabric softener B) elicit SI and change the sensitivity, while other mixtures which cause SI (such as air freshener and fabric softener A) do not elicit a change in sensitivity.

At the present time, we cannot relate these findings to specific chemical components of these mixtures. We know the names of many of the principal components, but we do not know their relative contributions to the total irritant effects of the mixtures. We have not yet tested the effects of the individual components. Fuller understanding will come only after extensive testing of numerous combinations of pure airborne chemicals.

We have seen increasing sensitivity of the SI phenomenon on repeat exposure of mice to several other mixtures. Figure 5 shows the effects of repeated exposure to the emissions of several brands of vinyl mattress covers; with Brand B, SI was 22% on the first exposure, 46% on the second, and 96% on the third (Figure 5). The other brands of mattress covers (A, D, F) emitted mixtures of chemicals with less dramatic effects. Emissions of certain disposable diapers produced analogous results. Figure 6 shows repeated exposures to emissions of Brand B of disposable

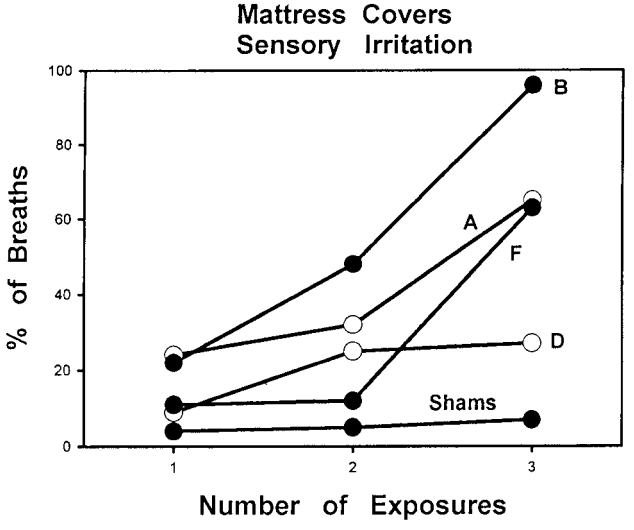

Figure 5. SI with repeated exposure to mattress-cover emissions. Similar to Figure 3 but using 12 mice for three 1-h exposures to each brand of vinyl mattress cover (A, B, D, F) over a 2-day period and 55 mice for sham exposures to charcoal-filtered air.

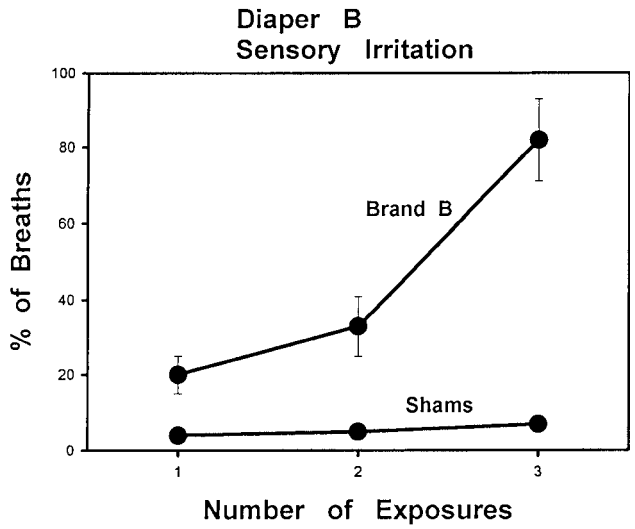

Figure 6. SI with repeated exposure to disposable diaper B emissions. Similar to Figure 3 but using 12 mice for 1-h exposures to emissions of the disposable diaper over a 2-day period and 34 mice for sham exposures. Brackets indicate standard error of the means.

diaper; SI increased from 20% on the first exposure, to 32% on the second exposure, to 84% on the third. Another brand of disposable diaper produced a chemical mixture which produced SI of 45% on the first exposure and 90% on the second exposure. Emissions of a foam mattress produced similar but less dramatic effects; SI increased from 29% on the first exposure to 38% on the second exposure.

Sham-exposed mice generally showed very little fluctuation in the value of TB (TB normally is approximately 0.025 s with standard deviation of mean being 0.003 to 0.006 s), and fewer than 5% of the breaths had TB values which were 2 standard deviations above the mean (and were therefore diagnosed as 'apparent SI') during each of three successive exposures (see lowest line on Figures 3, 5, 6).

Pulmonary irritation effect showed less change between exposures, while the AFL response increased with some of the mixtures but not others. Behavioral changes (suggestive of neurotoxicity) increased dramatically with repeat exposures to the emissions of an air freshener (Anderson and Anderson, 1997a), volatilized colognes (Anderson and Anderson, 1998), and emissions of some carpets (Anderson, 1993, 1995). Different chemical mixtures caused various combinations of abnormal posture, abnormal gait, loss of righting reflexes, loss of balance, abnormal stereotyped behavior, paralysis of one or more limbs, twitching, convulsions, and coma (Anderson, 1993, 1995; Anderson and Anderson, 1997a, 1998). Most of these effects slowly dissipated after the mice breathed clean air. Dose–response curves were obtained for several of these effects, and some of these behavioral changes occurred at concentrations actually encountered in simulated consumer use of at least one of these products (Anderson and Anderson, 1997a).

Scaling Up

Small chamber studies are always subject to potential criticism that, despite dilution of the emissions with charcoal-filtered air before presentation to the mice, we were using higher concentrations than would be found in real rooms. Therefore, we conducted a series of experiments with real rooms. First, one solid air freshener (5 g) was hung in a small room (760 ft^3 and temperature 65°F) with no forced ventilation and left to offgas for 24 h. The air from this room was then used as test atmosphere for the mice to breathe. The mice demonstrated SI, PI, and neurotoxicity, just as they did in the small chamber tests (see Anderson and Anderson, 1997a for further details). Second, we did analogous studies with another small test room (192 ft^3 and temperature 75°F, no forced ventilation), using either carpet, disposable diapers, air freshener, or fabric softener pads as the sources of air pollutants. In each case, we were able to demonstrate that the air had acquired irritant chemicals in sufficient strength to elicit changes in the respiratory parameters (TB, TP, and VD) in the mice breathing this air (Anderson and Anderson, unpublished observations). As we placed additional sources into the room, the SI, PI, and AFL effects increased.

In a separate set of experiments, we washed and dried some laundry with one of the fabric softeners, then used the dry laundry as the source of air pollutants in the small chamber. Tee-shirts dried with fabric softener emitted sufficient chemicals to cause SI and AFL in several of the mice (Anderson and Anderson, 1997b).

We have also conducted the same bioassay using samples of actual air from real buildings as the test atmosphere breathed by the mice. The air was brought from these buildings to the lab in Tedlar bags and used as test

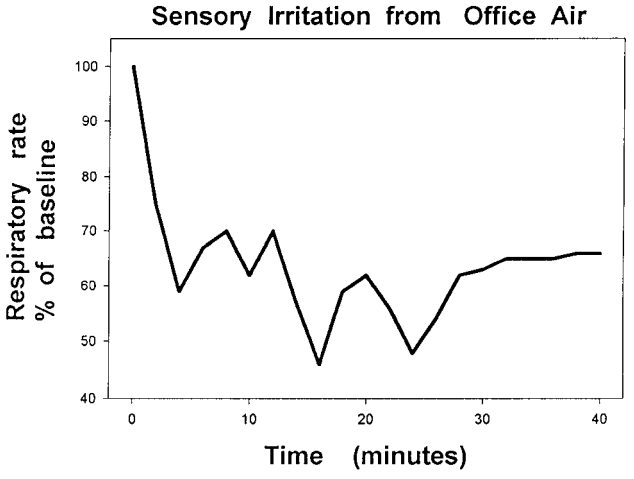

Figure 7. SI with exposure to air from a federal office building. The graph presents the average respiratory rate of four mice while breathing the test atmosphere. The results are expressed as percentage of the baseline respiratory rate (approximately 250 breaths/min).

Table 1. Chemicals in the test atmospheres.

Air freshener	Fabric softener B
toluene	toluene
alpha-pinene	styrene
beta-pinene	phenol
limonene	trimethylpentane
sabinene	isopropylbenzene
alpha-phellandrene	trimethylbenzene
meta-methoxybenzaldehyde	*meta*-xylene
terpinolene	*ortho*-xylene
alpha-terpinene	thymol

GC/MS was used to identify the major peaks in the emissions of the air freshener and the fabric softener (Brand B). The major peaks accounted for about half the peaks in each chromatogram. Listed are those peaks identified with a high degree of certainty by comparison to mass spectra in the computer library.

atmosphere for the monitored mice. Figure 7 shows the rapid onset of respiratory depression (a measure of SI) in mice breathing air from a Federal office building which was under litigation because of complaints of poor air quality. The mice corroborated the complaints and added a clearly objective dimension to these complaints. Air from a series of sites which people considered acceptable did not cause SI in the mice (Anderson and Coogan, 1994).

These complementary approaches demonstrated general consistency between our small-chamber studies, our room-size test studies, and our Tedlar bag studies of actual complaint site air.

Chemicals in these Test Atmospheres

Table 1 lists some of the major peaks in the GC/MS spectra of the air-freshener emissions and the emissions of fabric softener B. The chemicals are listed in an arbitrary order. Among the major peaks, the only overlap identified was toluene. Unfortunately in both the chromatograms, there were numerous minor peaks which were not identified.

Discussion

The complaints described in SBS and MCS (Ashford and Miller, 1998) overlap considerably with the symptoms associated with SI and PI. Our data show that the chemical mixtures which we know as polluting indoor air can cause toxic reactions (SI, PI, AFL) in mice. These data suggest that we can account for some to the SBS and MCS symptoms as SI, PI, and AFL. In addition, the mice undergo behavioral changes (consistent with neurotoxicity) while breathing some of the chemical mixtures. We believe that many of the neurological complaints in SBS and MCS can also be explained as direct toxic effects of common indoor air pollutants.

In the data presented here concerning acute toxic effects of product emissions, we noted that repeat exposure of mice to certain mixtures of toxic chemicals yielded the same degree of SI, while repeat exposure to other mixtures resulted in increasing SI responses. The SI phenomenon is one of the few cases in toxicology in which we know the concentration of the active chemicals at the active site, namely the air bathing the trigeminal nerve endings on the eyes, face, and nasal passages (Neilsen, 1991). With a constant concentration at the active site yet increasing responses, we must be observing a change in the sensitivity of the mice to these airborne irritants.

The results presented here show that certain mixtures of airborne chemicals elicit large changes in the sensitivity of the SI system, while other mixtures do not. It would appear to be worthwhile to further characterize these mixtures and to study the effects of individual components. Perhaps then we could identify the 'sensitizing' chemical or chemicals. Previous reports describe the ability of acrolein and formaldehyde to cause increases in SI after repeat exposures of mice (Kane and Alarie, 1977).

The SI system may serve as a model of part of the MCS problem, and understanding how certain chemical mixtures produce enhanced sensitivity in the SI system may help us understand how certain people develop increased sensitivity to certain airborne chemicals (i.e., MCS). The data also demonstrate clearly that what we are seeing is related to certain specific mixtures of chemicals and not the result of a nonspecific stress such as being confined to the exposure manifold in the presence of noxious chemicals (i.e., the air-freshener emissions were quite noxious to the mice but did not change their sensitivity to those sensory irritants).

The SI system consists of poorly differentiated nerve endings (Cauna, 1957; Alarie, 1973; Neilsen, 1991) of the fifth cranial nerve (the trigeminal nerve) in the conjunctivae of the eyes, the skin of the face, and mucosa of the nasal passages. Stereospecific receptors in these nerve endings have been partially characterized by study of homologous series of chemicals and comparison of potency of various classes of volatile organic chemicals (Alarie et al., 1995; Neilsen et al., 1996; Kasanen et al., 1998). One can predict the potency of airborne chemicals to interact with the SI receptor from the physical properties of the chemical (Alarie et al., 1995). The trigeminal nerve takes the signal to the brainstem and alters the phrenic nerve signals (and other nerve firing patterns) to produce a delay after the end of inspiration and before active expiration (Alarie, 1973). Any part of this system—from the number of receptors to the processing in the brainstem and the firing of the nerves controlling the larynx, the diaphragm, and the intercostal muscles—could be changing as the mechanism bringing about a changing sensitivity of the mouse to the SI triggers.

Many experiments can now be done to characterize this phenomenon. What is the time course of development of

these changes? How long do they last in the absence of further stimuli? Does the increased sensitivity to one set of chemicals also involve increased sensitivity to other sets of chemicals? These and other questions need to be answered before we can determine how much this phenomenon resembles the development of MCS.

Compared to the ease of study of the SI system (the receptors are practically exposed to ambient air and the responses are generally crisp), the changes in the PI system and AFL response are much harder to study and the data much harder to interpret. Changes in neurotoxicity with repeat exposures (Anderson, 1995; Anderson and Anderson, 1998) might be due to the cumulative dose of neurotoxins rather than any changes in sensitivity to the toxic chemicals.

Which Chemicals Cause These Changes?

Comparison of the major peaks in the GC/MS chromatograms did not help us understand why fabric softener B produced changes which the air freshener did not. Instead of identifying minor peaks, it would probably be more useful to study how the SI system responds to repeated exposures to low concentrations of purified toluene, styrene, xylene, phenol, thymol, etc., alone and in combinations. We have not yet tested the effects of the various individual chemicals identified in the GC/MS analyses.

Conclusions

There is enough similarity of the SI system and portions of the SBS and MCS to warrant intensive study of the SI system and how it changes sensitivity after exposure to certain chemical mixtures. Understanding changes in the sensitivity of the SI system in mice may tell us how and why certain people develop MCS.

Acknowledgments

Scott Hopkinson, Bernice Archambault, and Carl Johnson provided excellent technical assistance. Dr. Yves Alarie made many helpful suggestions in the course of this work.

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