

**Archives of Environmental Health**  
**January 2000**  
**Respiratory Toxicity of Mattress Emissions in Mice.**

Author/s: Rosalind C. Anderson

THE RECENT INCREASED prevalence of childhood asthma[1] has prompted further investigation of environmental factors that might cause or exacerbate asthmatic symptoms. The roles of mold, cockroach, and dust-mite antigens have been further delineated.[1] The results of epidemiological studies have shown that use of latex paint,[2] limonene-scented products,[3] and wall-to-wall carpeting[3] are also associated with asthma-related symptoms. Our laboratory has reported airflow decreases in mice exposed to air fresheners,[4] colognes,[5] vinyl mattress covers,[6] and fabric softeners.[7] In the current study, we evaluated respiratory-tract irritation and airflow decreases caused by volatile chemicals emitted by four types of children's mattresses.

#### Materials and Method

**Samples.** Three brands of crib-sized mattresses (i.e., 0.7 m x 1.3 m) and a diaper-changing pad were purchased in local stores. Brand A was polyurethane foam covered with vinyl. Brand B was 20% polyurethane foam enclosed in a vinyl cover, and it also contained springs and fibrous material. Brand C was a 100% polyurethane foam pad with a vinyl cover designed for use as a diaper-changing pad, but otherwise it was similar to brand A in appearance. Brand D was a traditional mattress with metal springs, fibers, and cotton padding. Brand E was a sample of organic cotton used in mattress construction. Portions of brands A, B, C, or E (i.e., .15 m x .15 m) were placed in an all-glass, .25-m x .30-m x .50-m 40-l chamber, which was sealed, warmed to 37 [degrees] C, and allowed to equilibrate for 1 hr before use. For brand D, we used a .25-m x .50-m portion of the mattress. 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 were housed in polypropylene cages in accordance with published guidelines.[8] The mice were exposed during a 1-wk holding period to a 12-hr light-dark cycle, and their weights following this protocol ranged between 25 g and 28 g. 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. No signs of infection were detected in mice in our quarantine room. Histological evaluation of lungs from our colony mice showed no evidence of infection with bacteria, viruses, or parasites.

**Animal exposures.** Each experiment involved two exposures of the mice to mattress emissions during a 24-hr period. During each test, we positioned 4 mice in the glass exposure chamber, as described in ASTM-E-981[9] and later modified by Vijayaraghavan et al.[10] The head of each mouse extended into the central exposure area, with the body in a side arm that served as a whole-body plethysmograph. During the 15-min baseline and 15-min recovery periods, the animal exposure chamber was ventilated continuously with charcoal-filtered air. During the 60-min exposure, animals breathed mattress emissions carried by charcoal-filtered air, which flowed at 6 l/min. The temperature in the animal-exposure chamber was between 22 [degrees] C and 24 [degrees] C. The system was open (i.e., dynamic); charcoal-filtered air was passed at a measured rate through the sample chamber to the animal-exposure chamber and was subsequently exhausted from the building. Between the two exposures, we returned the mice to their cages, which housed both food and water.

**Bioassay ASTM-E-981.** ASTM-E-981 is a standardized method for measuring biological effects of airborne irritant chemicals.[9] Alarie et al.[10-12] described a modification of this method, which allowed for more quantitative measurements and computerized diagnosis of the frequency and severity of sensory irritation (SI), pulmonary irritation (PI), and airflow limitation (AFL). This newer technique involves use of a pneumotachograph, which measures flow of air continuously on each breath. Digital computer programs, which integrate flow rates, calculate volume changes on each portion of each respiratory cycle of each mouse. We diagnosed SI, PI, and AFL by comparing statistically each breath to the 3,500 breaths measured during the baseline period for each mouse before each experiment occurred. Diagnoses of SI or PI required respiratory pattern changes (i.e., a break after inspiration [TB] or a pause after expiration [TP], respectively) that exceeded 2.0 times the standard deviation (SD) of the mean for that mouse during the baseline period for that experiment. We diagnosed AFL when the expiratory airflow rate at 50% expiration (VD)

fell 1.5 standard deviations below the mean for that animal's baseline expiratory flow rates. During each experimental hour, approximately 60,000 breaths were analyzed and assigned diagnoses. The computer programs allowed us to filter and/or smooth the data, using a maximum likelihood method.[12]

Statistics and graphics. The program described by Boylstein et al.[12] performs the basic analysis of respiratory patterns and assigns diagnoses when statistical criteria are achieved. We used SigmaPlot, version 3 (Jandel Corporation [San Raphael, California]) to graph the data and to perform additional statistical tests (unpaired t tests).

Chemical analysis of the test atmosphere. We determined total volatile organic chemicals (TVOCs) from the gas mixture in the sample chamber at the end of each experiment, using flame-ionization detection (Beckman Industrial 400A) with 100-ppm methane as the calibration gas. The emission mixture from one of the mattresses was absorbed into a Carbotrap 300 tube and was submitted for chemical analysis for VOCs by gas chromatography/mass spectrometry (GC/MS) techniques at Citizens Environmental Laboratory (Cambridge, Massachusetts). The chemical moieties were identified by computer matching of the GC/MS peak characteristics against those for a library of known compounds. The results were qualitative (recovery percentages unknown). Carbon dioxide ([CO.sub.2]) concentrations in the test atmospheres were measured with a Horiba (Mexa 311GE) carbon monoxide-carbon dioxide (CO-[CO.sub.2]) analyzer.

### Results

Test atmospheres. The TVOCs in the charcoal-filtered air were 10 or less. Brands A through D generated TVOC values between 270 and 830 ppm methane equivalents (Table 1); brand E, however, generated only 130 ppm. These values were fairly stable; for example, the TVOC values for brand A decreased slowly, from 380 to 300 ppm, during a 100-min ventilation of the sample-holding chamber with charcoal-filtered air at 6 l/min.

Table 1.--Overall Responses of Mice Exposed to Various Mattress Emissions  
Percentage of breaths

Exposure	n	TVOC (ppm)	SI	SEM	PI
Sham	24	< 10	6	1	5
Brand A	28	320	39 (*)	5	23 (*)
Brand B	32	830	26 (*)	9	19 (*)
Brand C	20	270	13 (*)	4	17 (*)
Brand D	12	720	57 (*)	12	23 (*)
Brand E	11	130	14	7	3

Percentage of breaths

Exposure	SEM	AFL	SEM
Sham	1	5	1
Brand A	5	26 (*)	5
Brand B	9	14 (*)	7
Brand C	5	14	5
Brand D	8	11 (*)	7
Brand E	1	1	1

Notes: Brands A through E refer to the five types of mattresses to which the mice were exposed. Brand A was polyurethane foam with a vinyl cover; brand B was 20% polyurethane foam enclosed in a vinyl cover, the rest being springs and fibrous material; brand C was a 100% polyurethane foam pad with a vinyl cover; brand D was a traditional mattress with metal springs, fibers, and cotton padding; and brand E was a sample organic cotton used in mattress construction.

TVOC = total volatile organic chemicals,

SI = sensory irritation,

PI = pulmonary irritation,

AFL = airflow limitation, and

SEM = standard error of the mean.

(\*) Statistically significant from sham at p ([is less than or equal to]) .05.

Sham experiments. In 6 sham experiments, 24 mice received exposures to charcoal-filtered air. In a typical sham experiment the average values for TB, TP, VD, tidal volume (TV), and respiratory rate (RR) remained within 10% of their baseline values over a 1-hr period (Fig. 1). In these sham experiments, a small percentage (i.e., generally less than 5%) of the breaths were diagnosed as abnormal because spontaneous variations in the measured parameters existed.

[Figure 1 ILLUSTRATION OMITTED]

Overview of mattress experiments. In 26 experiments, 104 mice were exposed twice to the emissions of one of the five brands of crib mattresses. In each of these 26 experiments, we observed SI, PI, and/or AFL in differing proportions. Effects were generally more profound during the second exposure; therefore, all data refer to the second-exposure results.

The peak effects observed are listed in Table 1. At its peak effect, brand A caused SI in 39% of the breaths of the 28 mice exposed to these emissions. Brand A also caused PI in 23% of the breaths and AFL in 26% of the breaths. Brands B and C were somewhat less potent for all endpoints, whereas brand D was more potent as a sensory irritant (SI peak effect = 57% of breaths) but was less potent (11%) in the causation of AFL. The organic cotton padding (brand E) produced only mild SI (Table 1).

Illustrative experiment with brand A mattress. One of the 7 experiments, with emissions from the brand A mattress, is shown in Figure 2. Immediately after introduction of the emissions at 15 min, TB exceeded 150% of the baseline value, and VD fell to 82% of the baseline average. The TP gradually rose to 120% of baseline average. The RR dropped 18% and accompanied the rise in TB. The TB effect showed an adaptation phenomenon and returned partially back toward baseline.

[Figure 2 ILLUSTRATION OMITTED]

In the 7 experiments with brand A, the peak TB elevations averaged 217% of baseline (SD = 17%), with a maximum value at 570% of baseline. The peak TP elevations averaged 170% (SD = 6%) of baseline values, with a maximum of 280% of baseline value. The average VD decreased by 30% (SD = 3%); the maximum VD decrease achieved in any mouse was 60%, which resulted in an expiratory airflow velocity at 40% of its baseline value. A statistical analysis of this experiment is shown in Figure 3. The SI and AFL diagnoses were frequent (i.e., involving approximately one-third of breaths) between 20 min and 60 min; the PI diagnoses waxed and waned and became most frequent after removal of the test atmosphere.

[Figure 3 ILLUSTRATION OMITTED]

Illustrative experiment with brand B mattress. The statistical analysis of an analogous experiment with the brand B mattress is shown in Figure 4. The SI diagnoses increased during the duration of exposure, and PI and AFL developed relatively late in the experiment. In the series of experiments with brand B, the maximum TB elevation observed was 500% of baseline, whereas the average peak elevation of TB was 240% (SD = 20%). The largest TP elevation was 260% of baseline, and the maximum VD decrease was 83%, thus resulting in an expiratory airflow velocity at 17% of baseline value.

[Figure 4 ILLUSTRATION OMITTED]

Illustrative experiment with brand C mattress. In response to emissions from brand C, SI, PI, and AFL developed over the course of the exposure and peaked late in the experiment (Fig. 5). In this series, the largest TB and TP seen were 360% and 240%, respectively, of baseline; the maximum VD decrease was 61%, which corresponded to a mid-expiratory airflow velocity at 39% of baseline rate.

[Figure 5 ILLUSTRATION OMITTED]

Illustrative experiment with brand E mattress. The emissions of organic cotton padding caused an immediate 10% increase in respiratory frequency and TV, and was accompanied by an increase in the mid-expiratory airflow velocity to 165% of baseline (Fig. 6). In this experiment, TB and TP decreased slightly, and very little SI, PI, or AFL was recorded. The concentration of [CO.sub.2] in the test atmosphere was between 300 and 400 ppm (i.e., not different from ambient air).

[Figure 6 ILLUSTRATION OMITTED]

Chemical composition of emissions. Approximately one-half of the volatile chemicals emitted by brand A were identified with at least 85% certainty (Table 2). Inasmuch as isomers are frequently difficult to differentiate with GC/MS, some of these emissions may have been isomers of the chemicals indicated.

Table 2.--Chemical Composition(\*) of Emissions of Brand A Mattress

Chemical	Chemical
Styrene	Dipentene
Isopropylbenzene	1,2,4-trimethylbenzene
Limonene	Nitrobenzene
Ethylbenzene	[Beta]-Ocimene
1,3-p-menthadiene	1-methyl-2-ethylbenzene
1,3-dichlorobenzene	

(\*) Only compounds identified with 85% or better confidence are listed.

## Discussion

The results of this study demonstrated that some crib mattresses emitted mixtures of chemicals capable of causing respiratory-tract irritation and generating combinations of SI, PI, and AFL.

Mechanism of effects. Our working hypothesis was that the results were caused by the combined action of several volatile chemicals in the emission mixtures. Despite 30 y of experience with ASTM-E-981 testing, there is no evidence that SI is caused by anything other than stimulation of the trigeminal nerve endings via airborne chemicals that encounter the eyes, face, and nasal passages (Y. Alarie, personal communication, 1998). Similarly, there is no evidence that PI results from anything other than stimulation of the vagus nerve endings by airborne chemicals that encounter the lower airways. We do not know the precise mechanism of the AFL response; perhaps it results from nasal passage edema, tracheal narrowing, or lower airways bronchospasm or inflammation. Whatever the mechanism, the AFL responses we observed were most likely also caused by airborne chemicals.

These results were obtained with two brief exposures over a 24-hr period; therefore, we believe the results were direct effects, rather than allergic phenomena. We have not investigated why the results were generally more profound in the second exposure. Perhaps there was an accumulation of the active chemicals at active sites, but a change in the sensitivity of the animals cannot be ruled out with the current data.

Chemicals. The chemicals in the test atmospheres were primarily solvents and other chemicals involved in the manufacture of the mattress and cover. Several of these chemicals have toxic properties. Styrene is toxic to lung, liver, and brain.[13,14] Isopropylbenzene[15] and limonene[16] are respiratory-tract irritants. Trimethylbenzene is carcinogenic and neurotoxic.[17] Nitrobenzene causes testicular degeneration and methemoglobinemia.[18] Ethylbenzene is toxic to liver, kidney, and brain.[19] Dichlorobenzene is carcinogenic.[20] About one-half of the peaks in the GC/MS analysis were not identified and might have contained other respiratory-tract irritants. We did not identify toluene di-isocyanate[21] or flame retardants among the volatile emissions. We investigated only new mattresses and did not determine the time course of dissipation of these emissions.

The emissions of brand E consistently elevated the RR, TV, and VD; we do not know what chemical caused these effects. Respiratory stimulation can occur with extremely high concentrations of benzene and toluene,[22] but in our study, these high concentrations were not approached. Carbon dioxide can increase RR and TV in mice, but [CO.sub.2] concentration in the test atmosphere was not elevated. Very few airborne chemicals stimulate RR and TV simultaneously in mice (Y. Alarie, personal communication, 1998).

Extrapolation to humans. Investigators can directly extrapolate data concerning SI in mice to predict human experience for more than 80 pure gases.[23] When humans develop SI, they feel burning or soreness in their eyes or faces; the human equivalent of PI is a feeling of difficult breathing.[24] It is not yet known to what extent one can extrapolate data concerning AFL from mice to humans, because this technique for measuring AFL in mice is relatively new.[10] Mice and humans share much anatomy, biochemistry, physiology, and pharmacology, but there are some major differences. Mice breathe four times per second, have stronger expression of their SI and PI reflexes than humans,[24,25] and have few respiratory bronchioles.[26] Nonetheless, both mice and humans can develop AFL after exposure to cholinergic agents, such as methacholine and carbamyl choline.[12]

Toxicology testing is based on the assumption that if a chemical has a definite effect in a laboratory animal, it will likely have a related (not necessarily identical) effect in man. Investigators can use the mouse test, which is an efficient way to screen consumer items, to determine which ones emit mixtures with potential human toxicity.[27] There should be few false-positive results because mice are less sensitive than humans to many chemicals tested.[28]

Relation of experimental concentrations to human exposures. In our study, we identified TVOC values that ranged from 130 to 720 ppm methane equivalents. We do not know the TVOC of the air immediately above a crib mattress. It is very difficult to compare two 1-hr exposures of normal mice with repeated 8-hr breathing of these mixtures by young children. Some children sleep in small rooms with multiple sources of air pollutants and minimal fresh air intake. Asthmatic children have airway hyperresponsiveness to airborne irritants[29]; therefore, extrapolations or risk assessments must not be based on assumptions of "average sensitivity." Laboratory results demonstrate a potential for toxicity, which may or may not exist in the "real world."

## Conclusions

Some commercial mattresses emit mixtures of chemicals that can cause acute toxic effects on the mammalian respiratory system, including upper airways irritation, lower airways irritation, and decreases in mid-expiratory airflow velocity. Several of the chemicals identified are known respiratory tract irritants. Epidemiological studies are needed for the evaluation of whether chemical emissions of mattresses are important in causing or exacerbating childhood asthma.

The provision of digital computer programs by Dr. Y. Alarie at the University of Pittsburgh School of Public Health is gratefully acknowledged.

Submitted for publication May 18, 1998; revised; accepted for publication February 2, 1999.

Requests for reprints should be sent to Rosalind C. Anderson, Ph.D., P.O. Box 323, West Hartford, VT 05084-0323.

## References

- [1.] Sears MR. Epidemiology of childhood asthma. *Lancet* 1997; 350:1015-20.
- [2.] Wieslander S, Norback D, Bjornson E, et al. Asthma and the indoor environment: the significance of emission of formaldehyde and volatile organic compounds from newly painted indoor surfaces. *Int Arch Occup Environ Health* 1997; 69:115-24.
- [3.] Norback D, Bjornsson E, Janson C, et al. Asthmatic symptoms and volatile organic compounds, formaldehyde, and carbon dioxide in dwellings. *Occup Environ Med* 1995; 52:388-95.
- [4.] Anderson RC, Anderson JH. Toxic effects of air freshener emissions. *Arch Environ Health* 1997; 52:433-41.
- [5.] Anderson RC, Anderson JH. Acute toxic effects of fragrance products. *Arch Environ Health* 1998; 53:138-46.
- [6.] Anderson RC, Anderson JH. Respiratory toxicity in mice exposed to mattress covers. *Arch Environ Health*, 1999; 54(3): 202-09.
- [7.] Anderson RC, Anderson JH. Airflow limitation in mice caused by emissions from fabric softeners. Abstracts of the Lancet Conference on Asthma and the Environment, Tours, France, October 9-10, 1997 (Abstract no 97).
- [8.] Guide to the Care and Use of Laboratory Animals. Washington, D.C.: National Academy Press, 1996.

- [9.] American Society for Testing and Materials (ASTM). Standard test method for estimating sensory irritancy of airborne chemicals (Designation: E 981-84). Philadelphia, PA: ASTM, 1984.
- [10.] Vijayaraghavan R, Schaper M, Thompson R, et al. Characteristic modification of the breathing pattern of mice to evaluate the effects of airborne chemicals on the respiratory tract. *Arch Toxicol* 1993; 67:478-90.
- [11.] Vijayaraghavan R, Schaper M, Thompson R, et al. Computer-assisted recognition and quantification of the effects of airborne chemicals acting at different areas of the respiratory tract in mice. *Arch Toxicol* 1994; 68:490-99.
- [12.] Boylstein LA, Anderson SJ, Thompson R, et al. Characterization of the effects of an airborne mixture of chemicals on the respiratory tract and smoothing polynomial spline analysis of the data. *Arch Toxicol* 1995; 69:579-89.
- [13.] Gadberry MG, De Nicola DB, Carlson GP. Pneumotoxicity and hepatotoxicity of styrene and styrene oxide. *J Toxicol Environ Health* 1996; 48:273-94.
- [14.] Coccini T, Fenoglio C, Nano R, et al. Styrene-induced alterations in the respiratory tract of rats treated by inhalation or intraperitoneally. *J Toxicol Environ Health* 1997; 52:62-77.
- [15.] Nielsen GD, Kristiansen U, Hansen L, et al. Irritation of the upper airway from mixtures of cumene and n-propranolol. *Arch Toxicol* 1988; 62:209-15.
- [16.] Anderson RC, Anderson JH. Respiratory toxicity of (+)-limonene in mice. Manuscript in preparation.
- [17.] Register of Toxic Effects of Chemical Substances. Cincinnati, OH: National Institute for Occupational Safety and Health, 1996.
- [18.] Levin AA, Bosakowski T, Earle LL, et al. The reversibility of nitrobenzene-induced testicular toxicity: continuous monitoring of sperm output from vasocystotomized rats. *Toxicol* 1988; 53:219-30.
- [19.] Agency for Toxic Substances and Disease Registry (ATSDR). Toxicological Profile for Ethylbenzene. Atlanta, GA: U.S. Department of Health and Human Services, Feb 17, 1998.
- [20.] Rall DP. Carcinogenicity of p-dichlorobenzene. *Science* 1987; 236:897-98.
- [21.] Kinber I, Bernstein IL, Karol MH, et al. Identification of respiratory allergens. *Fund Appl Toxicol* 1996; 33:1-10.
- [22.] Nielsen GD, Alarie Y. Sensory irritation, pulmonary irritation, and respiratory stimulation by airborne benzene and alkylbenzenes: prediction of safe industrial exposure levels and correlation with their thermodynamic properties. *Toxicol Appl Pharmacol* 1982; 65:459-77.
- [23.] Schaper M. Development of a database for sensory irritants and its use in establishing occupational exposure limits. *Am Ind Hygiene Assoc J* 1993; 54:488-544.
- [24.] Alarie Y. Sensory irritation by airborne chemicals. *CRC Crit Rev Toxicol* 1973; 2:299-363.
- [25.] Nielsen GD. Mechanism of activation of the sensory irritant receptor by airborne chemicals. *CRC Crit Rev Toxicol* 1991; 21:183-208.
- [26.] Mercer RR, Crapo JD. Architecture of the acinus. In: Parent RA (Ed). *Comparative Biology of the Normal Lung*. I. Boca Raton, FL: CRC Press, 1992; p 110.
- [27.] Nielsen GD, Alarie Y. Animal assays for upper airway irritation. *Annals NY Acad Sci* 1992; 641:164-75.
- [28.] Tepper JS, Costa DL. Will the mouse bioassay for estimating sensory irritancy of airborne chemicals (ASTM E981-84) be useful for evaluation of indoor air contaminants? *Indoor Environ* 1992; 1:367-72.
- [29.] American Thoracic Society. Guidelines for the evaluation of impairment/disability in patients with asthma. *Am Rev Respir Dis* 1993; 147:1056-61.

ROSALIND C. ANDERSON JULIUS H. ANDERSON Anderson Laboratories, Inc. West Hartford, Vermont