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Acute exposure to low-level methyl tertiary-butyl ether (MTBE): Human reactions and pharmacokinetic response

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Abstract

This two-part investigation assessed the effects of 1-h exposures to methyl tertiary-butyl ether (MTBE) at an ambient concentration of 1.7 ppm on healthy adults. In one part, four subjects participated in a pharmacokinetic study of blood levels of MTBE. Concentration in blood rose 20-fold from baseline to 17 μg/L by the end of exposure and declined to one half that level at 40 min after exposure. In another part, 43 subjects participated in a double-blind study of reactions to exposures to MTBE (1.7 ppm), to a mixture of 17 volatile organic compounds (VOCs) (7.1 ppm), and to air. Subjects rated symptoms (e.g., irritation, headache, mental fatigue), their mood, and various environmental attributes (e.g., odor, air quality, temperature), and also took computerized performance tests during exposures. Measures of eye irritation (e.g., tear-film breakup, eye redness) and nasal inflammation (i.e., measurement of polymorphonuclear neutrophilic leukocytes, PMNs) were taken before and after exposure as objective correlates of symptoms. Although subjects could perceive differences among the exposures via odor and air quality, they showed no increase in symptoms in response to MTBE and VOCs over those to air. The only significant objective effect comprised a time-by-agent interaction in number PMNs due principally to an increase in PMNs after exposure to VOCs. The level was higher 18-24 h after exposure than immediately after It. Because the subjects' ratings replicated effects found previously (e.g., time dependence in irritation judgments, effect of gender on rated odor intensity and pleasantness, and thermal comfort), it would appear that the absence of differential effects of MTBE on symptoms can be accepted at face value.

Introduction

The use of methyl tertiary-butyl ether (MTBE) as a fuel additive to reduce automotive emissions of carbon monoxide has received scientific and public attention on two accounts (U.S. Environmental Protection Agency, 1993): one for efficacy as an oxygenate, and the other for effects on the comfort and health of persons exposed to it in a mixture of gasoline vapors. Some persons exposed to MTBE in actual-use situations have reported acute adverse effects, including aversion to the odor of this ethereal-smelling compound, and various symptoms, such as headache, dizziness, obtundency, nasal and ocular irritation, nausea, and cough (e.g., Moolenaar et al., 1994). Symptoms of this sort may occur from exposure to various types of agents and have similarity to those reported by occupants of "complaint buildings" with poor apparent indoor air quality (Mendell, 1993).

We became involved in characterization of acute responses to MTBE after scientists at the Health Effects Research Laboratory (HERL) of the U.S. Environmental Protection Agency (EPA) had started to plan an investigation of such responses (Prah et al., 1994). The HERL researchers had noted the similarity of symptoms reported in connection with exposure to MTBE and those reported in complaint buildings (Otto et al., 1992; Otto, 1992). Research on that topic has begun to address matters of symptom verification; for example, if a person perceives eye irritation, will he or she show objective signs of changes in the surface of the eye? Such matters have also received attention in the workplace (Iregren et al., 1993). As a correlate of perceived eye irritation, for example, one might measure a decrease in the time it takes for the tear film over the eye to break up when people are exposed to vapors (Kjaergaard, 1992). Such a reduction could reflect a physiological response, such as secretion of fluid of lower surface tension, or could reflect a direct reduction of surface tension from dissolution of the vapors into the tear film (Kjaergaard et al., 1989).

Measurement of polymorphonuclear leukocytes (PMNs) in nasal lavage is an emerging tool in verification. Koren et al. (1992) found an increase in PMNs in subjects exposed to a mixture of volatile organic compounds (VOCs), and Graham et al. (1988) found an increase

following exposure to ozone. Since PMNs indicate inflammation, their presence might serve to verify symptoms of irritation.

A controlled study of acute health effects to an environmental agent generally entails creation of exposure-response functions for the effects of interest over concentrations that span and or exceed the range encountered environmentally. Where the response is a subjective report, it is advisable to have some control for overreporting. Our approach entailed exposure to an agent smelling similar, both in character and intensity, to the agent of interest to invite some positive reporting, but benign enough not to cause other acute effects of concern (e.g., headache, eye irritation) at concentrations with comparable odor.

In the ideal case, objective measures would correlate well enough with subjective reports to verify the presence of symptoms. Experience suggests, however, that for acute exposures objective measures have less sensitivity than subjective ratings; one can feel a stimulus before a response can be measured objectively (Cain & Cometto-Muñiz, 1995).

Because of urgency to gather data in time to permit decisions regarding the use of MTBE for the 1993-1994 fuel season, neither the present study nor the study of HERL gathered exposure-response functions, but explored only one level and for one duration of exposure. Under this constraint, the focus for generalization of our results became the commuter exposed to a concentration at the high end of that found in field sampling. The selected concentration (1.7 ppm) was based upon results from studies of commuters (Lioy et al., 1993). (HERL selected 1.4 ppm for its tests. A duration of 1 h was chosen since it lay at the high end of actual commuting durations.

In real-world circumstances, commuters receive exposure to MTBE only in conjunction with gasoline. It would seem advisable to simulate such exposures and to compare effects of MTBE in a gasoline-vapor background with that of the background alone. This, however, would have required technical knowledge unavailable at the time.

Pharmacokinetics

We also measured accumulation of MTBE and its elimination from blood during and after exposures to airborne MTBE. This part of the investigation served two ends: (1) It showed how incident concentration at the level used for the study of acute effects would be converted into blood-level, and (2) it provided a benchmark for comparison of effects obtained in a chamber to those associated with some symptoms. The Centers for Disease Control (CDC) obtained blood concentrations in circumstances generally associated with complaints in Alaska (viz., commuting and occupational exposure to gasoline). The CDC data provide a frame of reference for effects of exposures in the chamber (Moolenaar et al., 1994).

Methods

Subjects

The protocol for the study received approval from the Human Investigation Committee of the Yale School of Medicine. The sample of subjects comprised healthy young adults from the New Haven area: 4 persons for the pharmacokinetic study and 43 for the study of acute effects. These persons were recruited principally through ads in local newspapers. A total of 190 people were given phone interviews, which contained a description of the study, an outline of inclusion and exclusion criteria including the need for a physical exam, and specification of time commitment, risks and rate of pay.

Exclusion criteria were:

1. Chronic cough, history of chronic pulmonary disease, respiratory allergies, allergic rhinitis, or childhood asthma.
2. History of acute or chronic cardiovascular, hepatic, or renal disease.
3. Acute illness within the previous month.
4. Current use of medication, including vitamin supplements and aspirin (or similar analgesics), but excluding birth control pills.
5. Investigational exposures to pollutants within the previous 2 wk.
6. History or evidence of chemical sensitivity.

7. Pregnancy (checked daily during the investigation).
8. Abnormal eye exam, other than need for refractive correction, or ocular surgery.
9. Smoking within the previous year.

Subjects who remained interested after the interview were scheduled for a 1-h exam by a physician from the Yale Occupational Medicine Clinic. The exam included medical history, checking of vital signs, cardiac exam, chest exam, nasal exam, and spirometry. Of 94 persons who completed the physical, 4 were chosen for participation in the pharmacokinetic study (2 males and 2 females, aged 18-26 yr) and 52 for participation in the acute effects study. Forty-three of the 52 completed the series: 22 males between 18 and 32 yr of age and 21 females between 18 and 34. Two females who began the acute effects series were dropped when they tested positive for pregnancy. The other seven persons found themselves unable to follow the schedule of the study.

Facilities and Equipment

Environmental Chambers. Exposures took place in twin environmental chambers with volumes of 650 ft^3 (18.5 m^3). These tightly constructed aluminum chambers provided a temperature of 24 ± 0.2°C, relative humidity of 40 ± 3%, and fresh air rates of 60 ± 2 ft^3/min (28 L/s). Before it entered the chambers, the fresh air passed through an air cleaning and dehumidification station that contained fiber filters for particulate filtration, charcoal and Purafil filters for gas filtration, and a mechanical dehumidifier that dried the air to a dew point of 5°C. Humidity was raised as necessary by injection of clean steam from an electric boiler that used filtered water. Rate of airflow through the distribution system equaled 800 ft^3/min (377 L/s).

Injection and Monitoring of Vapors. Atmospheres with the desired concentrations of MTBE or VOCs were obtained by injecting vapors into the recirculation air stream of the chamber. A cylinder of compressed gas with a concentration of 0.20 mol% (2000 ppm) in nitrogen (Phillips 66 Co.) served as the source of MTBE. A cylinder also served as the source of a 16-component mixture of VOCs, supplied at 3.7 mol% (37,000 ppm) (Phillips 66 Co.). Composition of

the mixture, with accompanying concentrations (mol%), was as follows: butene-1 (0.06), isobutylene (0.06), *trans*-butene-2 (0.06), *cis*-butene-2 (0.05), isobutane (0.33), *n*-butane (1.36), *trans*-pentene-2 (0.06), pentene-1 (0.02), 2-methyl butene-1 (0.07), cyclopentane (0.02), *n*-pentane (0.32), isopentane (0.97), methyl cyclopentane (0.02), 2-methylpentane (0.14), 3-methylpentane (0.07), and 2,3-dimethylbutane (0.09). These components reflect the profile of air samples taken in surveys of service stations (American Petroleum Institute, 1991). These account for about 90% of the mass in such samples.

Although intended as a positive control for reporting symptoms to a service station-type odor, the 16-component mixture at environmentally representative concentrations had a weaker odor than the concentration of MTBE chosen for the investigation. In order to amplify the odor, a 17th component of isopropyl mercaptan (IPM) was added from another cylinder (supplied at 0.08-0.10 mol%) at approximately 0.5 ppb. IPM is a component in some gasoline blends and natural gas warning agents. The perceived intensity of a sample of gasoline at a vapor concentration of 7-9 ppm, characteristic of concentrations within the perimeters of service stations, formed the criterion for the amount of IPM added.

The target concentration of MTBE to be achieved was 1.7 ppm (6 mg/m^3). The target concentration of VOCs was 7.1 ppm (19 mg/m^3). Concentrations of vapors inside and outside the chambers was monitored online by an HP 5890 gas chromatograph (GC) with a photoionization detector and with a Bendix 8201 hydrocarbon analyzer (HCA).

The GC was calibrated daily for MTBE with vapor standards of 0.3, 1.5, and 5.0 ppm. Calibration for VOCs was performed daily using serial dilutions in nitrogen of the 16-component mixture. Average level of MTBE taken across the days of the experiments equaled 1.74 ppm ± 3.5% with no individual reading falling outside the band 1.6-2.0 ppm. Average level of VOCs taken across the days of the experiments equaled 7.14 ppm ± 5.7% with no individual reading falling outside the band 6.4-9.0 ppm.

Blood Sampling and Analysis. Blood was collected into 7-ml or 10-ml

gray-capped vacutainer tubes. Studies with such tubes at CDC and Midwest Research Laboratories (MRI), Kansas City, KS, the analytical laboratory for this study, showed little interference in high-resolution analysis for VOCs in blood. A 3-day leachability test of saline stored in the vacutainers detected no MTBE or its metabolite, tertiary-butyl alcohol (TBA).

Measurements of MTBE and TBA were performed by gas chromatography/mass spectroscopy (GC/MS). The method involved purge and trap extraction of MTBE and TBA from blood with GC separation and detection via a Finnigan 5100 mass spectrometer (quadrupole detector and operated in the selective ion monitoring mode). An Incos data system served to acquire the data from the GC/MS analysis. The analytical outcome included total ion current chromatograms and mass chromatograms for assessment of ions characteristic of the target analytes and isotopically labeled internal standards. The chromatograms served to calibrate the instrument and quantitate concentration of samples.

Pharmacokinetic Study Procedure

Prior to a session, the chamber was brought to 1.7 ppm MTBE for at least 1 h. When a subject arrived at the laboratory, a venous catheter was placed into the antecubital vein. A baseline sample of blood was drawn into a vacutainer containing potassium oxalate/sodium fluoride anticoagulant 5 min before the subject entered the chamber. Six samples were taken during the 1-h exposure to MTBE (t_{exp} = 2, 5, 10, 20, 30, and 60 min) and 7 in the 1.5-h period after exposure (t_{post} = 2, 5, 10, 20, 40, 60, and 90 min).

Blood samples were uniquely coded to conceal the sequence of sampling to MRI. At two time points, preexposure and t_{exp} = 60 min, duplicate samples were obtained for quality assurance. Four preexposure samples were spiked with 7.6 μg/L MTBE after blood collection, in order to evaluate stability during shipping. Samples were packed with blue ice but kept from freezing. Other features built into the program for quality control included daily calibration of equipment (1-100 μg/L range for MTBE), analysis of blanks, duplicate analyses

of split samples, storage stability, and comparison of results against internal standards (deuterated butanone, toluene, and TBA).

Acute Effects Study Procedure

Each subject came to the laboratory on 7 days: the first for 3 h for familiarization with the techniques to be employed (nasal lavage, ocular examination, neurobehavioral testing, ratings of attributes and symptoms); the second, fourth, and sixth days for 4.5 h for tests before, during, and after 1-lr exposures in the chambers; and the third, fifth, and seventh days for 30 min for postexposure nasal lavage. The contingent of subjects was divided into four groups. Groups A and C had exposures in the order MTBE-air-VOCs, in chamber 1, and groups B and D had exposures in the order VOCs-air-MTBE in chamber 2. Groups A and D had sessions on the same day and Groups B and C had sessions on the same day, a day after groups A and D. Order of exposure for any given group was known to only one member of the research team who had no role in charting the reactions and who blinded both subjects and members of the team to the order.

Ocular Measurements. Ocular parameters included duration of tear-film breakup, scoring of the conjunctiva for epithelial damage, eye redness, and presence of inflammatory cells in tear fluid. Equipment employed for the first two parameters comprised a Haag-Streit slit-lamp. For assessment of redness, photographs of the eye were taken with a Nikon N90 camera equipped with a Tamron-F AF Tele-converter, an AF Micro Nikkor 105-mm lens, and a Nikon Speed light SB-25 flash. For evaluation of eye redness by a panel, two Kodak Ektagraphic III slide projectors were used. Measurement of cells in tear fluid required a 5-μl capillary tube and the equipment for the analysis of nasal lavage fluid.

Subjects were examined for eye redness (hyperemia), tear-film breakup time, and epithelial damage in the left eye only, before and after exposure to MTBE, VOCs, and air. For conjunctival hyperemia, two close-up pictures of the nasal conjunctiva and two of the temporal conjunctiva were taken with color slide film. The subject looked right

and then left to expose the temporal and nasal conjunctivae, respectively.

After the slides were developed, pairs that included a picture taken before exposure and one taken after were viewed side by side and scored in duplicate by five trained judges blinded regarding pre- versus postexposure order (Kjaergaard et al., 1990). Each pair of slides was scored for difference in redness on a scale of 1–5, where 1 indicated that the eye shown on the right was much less red than the eye shown on the left, 2 indicated that the eye on the right was slightly less red than the eye on the left, 3 indicated no difference, 4 indicated that the eye on the right was slightly more red than the eye on the left, and 5 indicated that the eye on the right was much more red than the eye on the left. Right-left positions of the before-after photos were counterbalanced.

Measurement of tear-film breakup time occurred second in the sequence of ocular measurements before and after exposure (Kjaergaard et al., 1991). Ten microliters of sterile 1% sodium fluorescein dye was transferred to the lower lid using a sterilized glass rod. The slit-lamp microscope was focused on the eye using cobalt blue illumination. The subject was told to blink once and then to keep both eyes open while staring directly into the microscope. As the subject blinked, a chronograph was started. The corneal surface was then scanned. The chronograph was stopped when spots or lines indicating breakup of the tear film were observed. Three consecutive measurements were recorded.

Measurement of epithelial cell turnover occurred third in the sequence of ocular measurements. Ten microliters of sterile 1% lissamine green B dye was transferred to the lower lid. The subject blinked normally for 1–4 min in order to distribute the dye. He then placed his head in the slit-lamp apparatus and was asked to look to the left and slightly upward so that the number of blue-green dots on the nasal bulbar conjunctiva could be counted and recorded. The subject was then asked to look to the right and slightly upward so that the dots on the temporal bulbar conjunctiva could be counted. Finally, the subject was asked to look upward, while pulling down the lower eyelid, so that the dots on the inferior bulbar conjunctiva could be counted. Epithelial score marks were categorized as follows: 1, 0

dots; 2, 1-10 dots; 3, 11-50 dots; 4, 51-100 dots; and 5, >100 dots.

For cytological assessment, 5-μl samples of tear fluid were taken via capillary pipette at the outer canthus just before and just after exposures, as the fourth ocular measurement. Sampling took anywhere from 30 s to several minutes. A dry eye required more time. Samples were placed on ice for up to 1 h and then spun down onto slides on a Cytospin3 and stained by a Leukostat stain kit (Fisher Scientific). Stained slides were read for the total number of cells and differentially for PMNs and other cells. The variable of principal interest was number of PMNs, as an index of inflammation.

Nasal Lavage. Equipment employed for measurement of cells in nasal lavage fluid and tear samples included a centrifuge, a hemacytometer (Hausser Scientific), and a light microscope. Nasal lavage entailed 3 washes per exposure: preexposure, immediate postexposure, and 18–24-h postexposure. Nasal spray bottles containing 0.9% NaCl solution warmed to 37°C were used to spray lavage fluid into the nostril. A total of 3 ml was sprayed into each nostril in 3 deliveries of 1 ml each. The subject then allowed the fluid to run out into a cup that was immediately put on ice.

Directly following collection of lavage fluid, the cells in the hemacytometer were counted under a light microscope. Viability was determined using a 1:1 dilution of lavage fluid with 0.4% trypan blue. Differential counts for PMNs, epithelial cells, monocytes, eosinophils, and lymphocytes were made using 0.3 ml of lavage fluid in Cytospin-made slides stained with a Leukostat stain kit. A total of 300 cells was counted under oil immersion. As with the ocular cell counts, principal interest focused on number of PMNs.

Neurobehavioral tests. The testing employed the Neurobehavioral Evaluation System (NES2), an automated battery of tests of motor performance, perception, and cognitive functioning, run on IBM 386 computers (Baker et al., 1985). The investigation employed three tests in the NES2 (Baker et al., 1985; Arcia & Otto, 1992) given in the hour before and then in the last 15 min of the exposure:

1. Symbol-Digit Substitution: Subjects coded nine figures displayed in a row on the lower portion of the computer screen according to a set of figure-digit pairs presented in an array in the upper portion. This was repeated eight times.

2. Switching Attention: In the first part, subjects responded as quickly as possible to the side of the screen on which a rectangle appeared (two series of eight stimuli). In the second part, subjects responded to the direction in which an arrowhead pointed (two series of eight stimuli). In the third part, subjects responded to side or direction, as cued by the screen (eight series of eight stimuli). The cue varied.

3. Profile of Mood States (POMS): Subjects rated their feelings (scale 1-5) with respect to 25 adjectives that represented various feelings.

Ratings of Attributes and Symptoms. Subjects filled out 2 paper-and -pencil questionnaires at 10-min intervals during exposures. One, termed the Air Quality Questionnaire (AQ), contained 11 environmental attributes rated on 10-cm visual analog scales by marking the lines (for 10 items) or filling in a box for a judgment of yes–no (for one item) as follows:

1. What is the intensity of the odor in the room now?
No odor……..Extremely strong

2. How pleasant is the odor in the room now?
Extremely acceptable……Extremely unacceptable

3. Do you feel any eye irritation now?
Not at all irritated……...Extremely irritated

4. Do you feel any nasal irritation now?
Not at all irritated……...Extremely irritated

5" Do you feel any throat irritation now?
Not at all irritated.....Extremely irritated

6. Do you have any headache now?
No headache.....Extremely strong

7. How is the air quality in the room now?
Very good.....Very poor

8. How is the temperature in the room now?
Too cool.....Too hot

9. How is the noise level in the room now?
Quiet.....Noisy

10. How is the amount of light in the room now?
Too dim.....Too bright

11. Is the air quality acceptable now?
Yes No

Its format and eight of the items on this questionnaire came from a questionnaire developed for self-report epidemiological studies by the Environmental and Occupational Health Sciences Institute (see Mohr et al., 1994; Fiedler et al., 1994). The other, termed the Symptom Questionnaire (SQ), contained 22 symptoms such as headache and irritation of the nose, rated on a scale of 0–4 as follows:

0 = None (Symptom is not present)
1 = Mild (Symptom is barely detectable)
2 = Moderate (Symptom is present, but not annoying)
3 = Strong (Symptom is present, but somewhat annoying)
4 = Severe (Symptom is present and very annoying or painful)

1. Headache
2. Irritation of the nose
3. Cough
4. Wheezing, chest tightness, or shortness of breath
5. Dry, itching, or irritated eyes
6. Tired or strained eyes
7. Burning eyes
8. Irritation of the throat
9. Difficulty in remembering things or concentrating
10. Dry throat

11. Sore throat
12. Feeling depressed
13. Unusual tiredness, fatigue, or drowsiness
14. Stuffy or runny nose, or sinus congestion
15. Tension, irritability, or nervousness
16. Pain or stiffness in back, shoulders, or neck
17. Skin rash
18. Sneezing
19. Dizziness or lightheadedness
20. Mental fatigue or "fuzziness"
21. Pain or numbness in the hand or wrists
22. Dry skin
23. Rate odor level in room (none, mild, moderate, strong, very strong)
24. Rate the air quality in room (very poor, poor, fair, good, very good)
25. Rate the odor pleasantness (very bad, bad, neutral, good, very good)

This instrument was developed at the U.S. EPA, based upon questions asked in a large study of building-related complaints of occupants (Prah et al., 1994). Except for questions on noise and lighting, added by the present experimenters, the AQ and the SQ were those used in the U.S. EPA study.

Data Analysis

Pharmacokinetic Study. Quality control studies at MRI revealed the analysis of MTBE to be reproducible with differences between duplicate samples ranging from 0.6% to 29.7%. Recovery of MTBE from blood spiked at MRI ranged from 84% to 139% (mean = 108%). Recovery of MTBE from 2 blood samples spiked at the Pierce Lab and sent overnight to MRI equaled approximately 60%, which suggested losses during shipment. However, extensive stability studies conducted at MRI showed no systematic losses during refrigeration lasting up to 6 wk.

Acute Effects Study. The raw data from the sheets filled out for each subject for the objective ocular and nasal measurements, and by each subject for the subjective measurements, and the NES2 data files were entered into spreadsheets (Excel). Some initial data reduction was performed at this stage. The three tear-film breakup

time (BUT) measures made each time a subject was seen were averaged. Eye redness ratings were averaged across judges.

Initial reduction of the NES2 data used the program SUMM, provided along with the battery that calculated means and standard deviations over the trials of a test. NES2 variables included mean reaction time (in seconds) for the symbol-digit task; mean reaction time (in milliseconds) and number of errors for the four conditions of the attention task; and mean score (on a 1–5 scale) for each of five mood states (tension, depression, anger, fatigue, and confusion).

For the AQ, which required the subject to place a mark on a 10-cm line, the marks were converted to a 0–10 scale, where a mark made from 0 to 0.5 cm was treated as noise and called "0", a mark made above 0.5 cm and up to 1.5 cm was called "1", etc.

Multivariate analysis of variance (MANOVA), repeated measures design, was used to make decisions about the significance of effects (SPSS for Windows). Within-subjects factors were exposure (three conditions: air, MTBE, VOCs) and time [two levels (pre, post), three levels (pre, post, 24 h), or six levels (10-min intervals during exposure)].

Results

Pharmacokinetic Study

Concentrations of MTBE in blood before, during, and after exposure appear in Figure 1. The amount detected rose steeply from 0.83 ± 0.50 μg/L (SD) preexposure to 17.1 ± 2.01 μg/L after 60 min. Concentrations fell immediately upon cessation of exposure, reaching about one-half peak at 40 min postexposure. Concentration continued to decline through 60 min postexposure. At 90 min, only subject 2 showed a continuing decline (Table 1). In subject 3, the declining trend ended at 40 min. For the other 2 subjects, measured concentration was virtually unchanged (S1) or increased (S4) between 60 and 90 min. The rapid elimination phase appears therefore to last approximately 1 h.

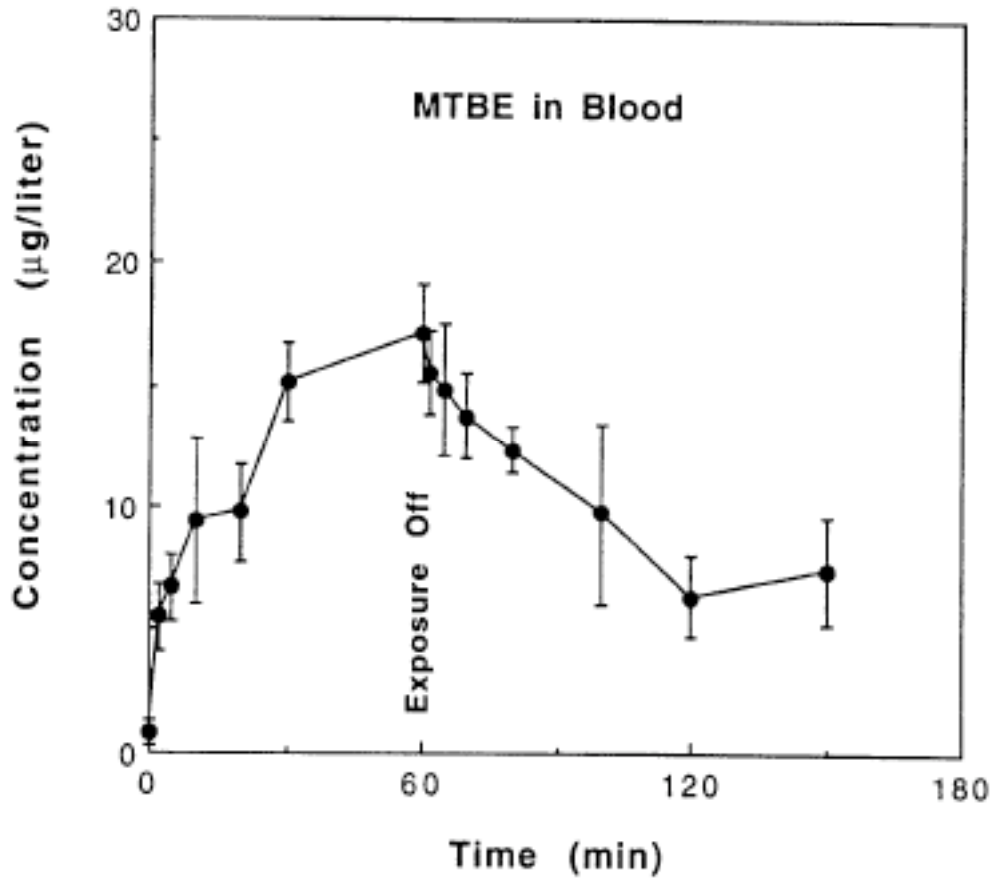


Figure 1. Concentration (average ± standard deviation) of MTBE in the blood of 4 subjects exposed to an ambient concentration 1.7 ppm for 1 h and followed for 1.5 h after exposure.

Table 1. Concentration of MTBE (μg/L) in blood of 4 subjects before, during, and after exposure to 1.7 ppm MTBE in the vapor phase.

Time (min)	S1	S2	S3	S4	Mean	SD
Preexposure	1.1	0.5	0.4	1.4	0.9	0.5
2	4.5	6.3	6.8	4.1	5.4	1.3
5	6.8	7.1	7.9	4.8	6.7	1.3
10	13.9	8.3	9.8	5.7	9.4	3.4
20	10.3	10.4	11.5	6.8	9.8	2.0
30	13.6	15.8	17.1	14.2	15.2	1.6
60	16.7	14.9	17.4	19.7	17.2	2.0
62	16.3	15.0	13.4	17.4	15.5	1.7
65	13.2	13.6	13.7	18.9	14.9	2.7
70	13.6	14.7	11.4	15.4	13.8	1.8
80	12.8	11.9	11.4	13.5	12.4	0.9
100	8.6	10.5	5.4	14.4	9.7	3.8
120	6.2	7.6	7.4	4.1	6.3	1.6
150	6.1	6.3	6.5	10.7	7.4	2.2

An attempt to quantify TBA in blood had poor reproducibility, apparently due to problems of storage and recovery. The results generally indicated concentrations similar in magnitude to those seen for MTBE, but with a slower rate of decline. Such results were consistent with those found elsewhere (U.S. EPA, 1993; Laboratoires Bio-Researches, 1990.

Acute Effects: Objective Measures

Ocular Measurements. Ocular indices yielded a nonsignificant tendency for the eyes to become more irritated as subjects sat in the chamber. Dots on the conjunctiva increased, tear-film breakup time decreased (i.e., the film showed less stability), and rated redness increased. These effects occurred irrespective of exposure group.

The average category for dots on the conjunctiva was 3, that is, 11–50 dots. Although the number increased slightly from before to after exposure, this change did not reached significance, nor did the MANOVA performed on the post-pre difference scores for nasal dots [0.20 ± 0.09 (SE), 0.10 ± 0.10, and -0.02 ± 0.11 for air, MTBE, and VOCs, respectively], temporal dots (0.10 ± 0.11, 0.10 ± 0.11, and 0.20 ± 0.13), and inferior bulbar dots (0.00 ± 0.12, 0.20 ± 0.10, and 0.00 ± 0.10) revealed any differential effect of exposure.

From an average of 9 s preexposure, breakup time (BUT) of the tear film decreased with exposure, with the largest change seen for exposure to MTBE [-4.00 s ± 1.12 (SE)]. Nevertheless, as with dots on the conjunctiva, there was no significant differential effect of exposure (-1.5 ± 1.04 s for air, -0.77 ± 0.81 s for VOCs). Redness increased one-half a category step from before to after exposure, but with no differential effect of exposure (on nasal conjunctiva, 0.67 ± 0.64, 0.42 ± 0.41, and 0.46 ± 0.40 for air, MTBE, and VOCs, respectively, and on temporal conjunctiva, 0.56 ± 0.70, 0.44 ± 0.50, and 0.46 ± 0.51, respectively). MANOVA run on the difference in number of PMNs revealed an increase from pre- to postexposure, but no significant effect of exposure (-4580 ± 3151, -3988 ± 2081, and -9595 ± 4510 for air, MTBE, and VOCs, respectively).

Nasal Lavage. The variable of principal interest for the nasal lavage was the number of PMNs. Since lavage was performed both immediately after exposure and then again 18-24 h later, time entered as a variable. MANOVA run on the post-pre differences uncovered no significant main effects of exposure, time, or gender for either live cells or PMNs. It did, however, reveal a significant effect of exposure x time for number of PMNs [$F(2,82) = 6.02$, $p < .004$], which derived from changes from the immediate to the delayed lavage (Figure 2). The effect was driven principally by a rise (t-test, $p < .01$) from immediate to delayed lavage after exposure to VOCs; PMNs in the delayed lavage also increased significantly from the number in preexposure lavage ($p < .031$). Exposure to neither air nor MTBE caused a notable change.

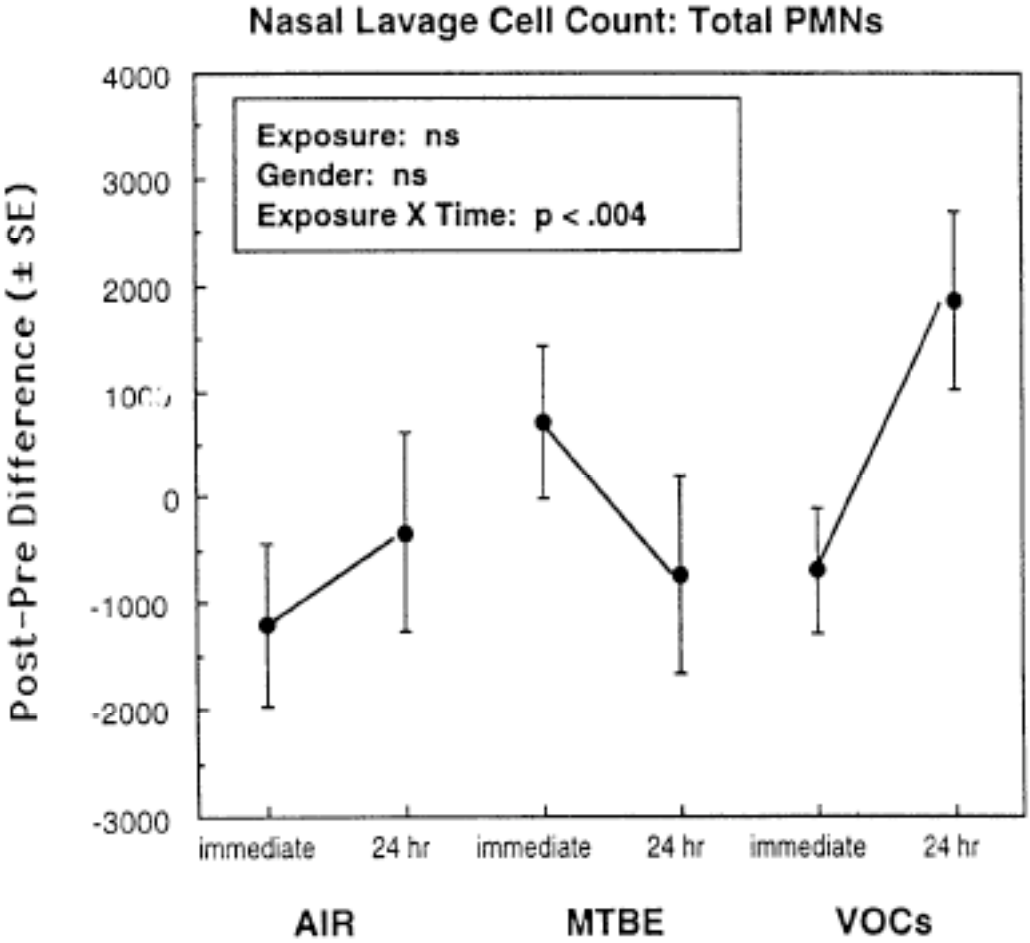


Figure 2. Difference (average ± standard error) between both immediate and delayed postexposure and preexposure in PMNs in nasal lavage fluid.

CNS Function. For digit-symbol substitution on the NES2, latency decreased from preexposure to the last 15 min of exposure, but without a differential effect of exposure (-25 ± 30, -3 ± 40, and -17 ± 20 ms for air, MTBE, and VOCs, respectively). For switching attention, performance changed slightly though not differentially from preexposure to the last 15 min of exposure (for switching direction, the subtask that showed the largest net effect: -10 ± 12, -12 ± 9, and -9 ± 13 ms for air, MTBE, and VOCs, respectively).

Acute Effects: Subjective Measures

Overview. The Air Quality Questionnaire (AQ) and Symptom Questionnaire (SQ), both listed earlier, were administered six times per session. The percent of responses, by item, above minimal across all administrations provides a quick overview of the outcome. Figure 3 shows such results for the AQ. A majority of responses exceeded 1 (technically, exceeded 15% of total line length on the visual analog scale) on 6 of the 10 attributes, including odor intensity, odor pleasantness, and air quality. Exposure to air, however, led to about as high a percentage of such responses as did exposure to MTBE. Exposure to VOCs led to more responses greater than 1 for odor intensity and pleasantness, implying higher impact. For attributes pertaining to irritation and headache, fewer than half the responses rose above 1.

The 22 symptoms in the SQ overlapped with attributes in the AQ, offering in some instances finer resolution, such as differentiation of dry, itching, irritated eyes from burning eyes. Nevertheless, the results showed great compatibility with those on the AQ. On the SQ scale, where 0 equaled absence of symptom and 1 through 4 equaled mild through severe, the majority of responses to questions pertaining to eye irritation were 0. Questions about nasal irritation generally led to even fewer positive responses. With the exception of headache, no other symptoms showed much tendency to rise above even 20% positive responding, and few gave any indication of a differential effect of exposure. In general, exposure to VOCs led to

the highest frequency of positive responses.

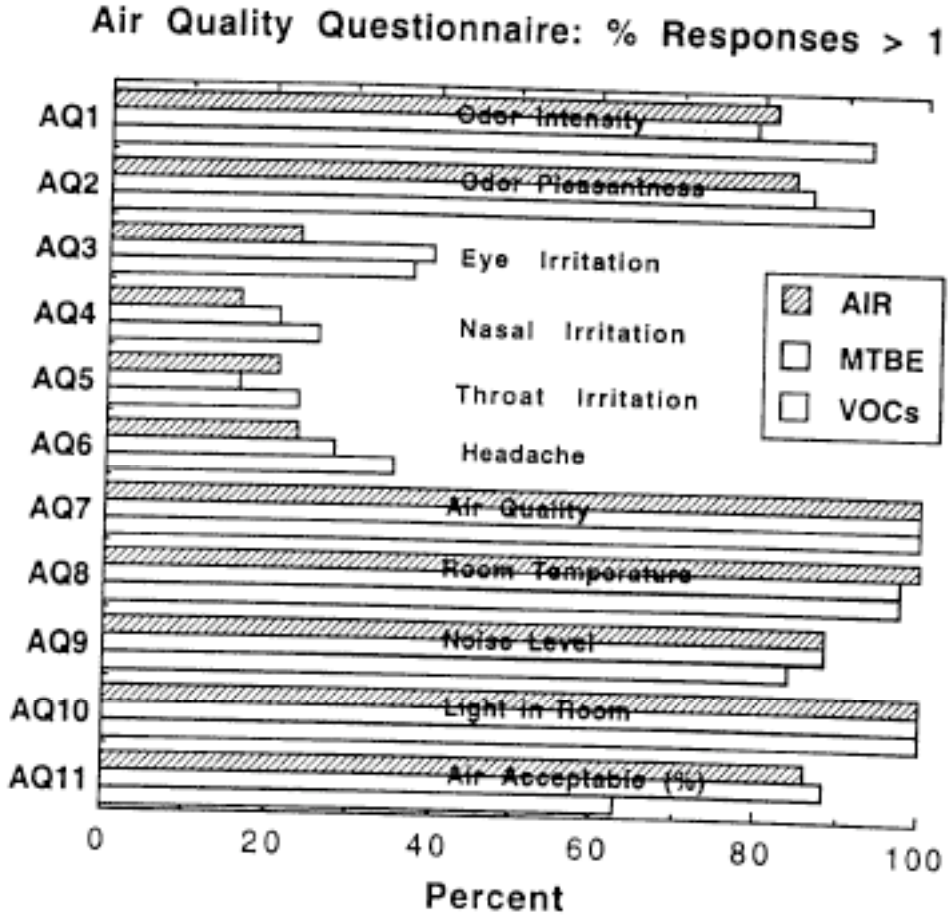


Figure 3. The percentage of responses that exceeded 15% of the range of the visual analog scale for attributes 1-10 of the AQ, and the percentage "yes" on attribute 11.

Scaled Responses of Adverse Effects on AQ and SQ. As we shift from minimal responding to averages of the scaled responses, the attributes of eye and nasal irritation, headache, tiredness, difficulty remembering, and tension deserve attention. These are symptoms where, based on nonminimal responding, some differential effect seemed possible. Calculations indicated that the power to pick up differences of at least one scale point on the AQ and of one-half point on the SQ approached the maximum value of 1.0.

As the MANOVAs in Tables 2 and 3 indicate, significant differential

effects of exposure never occurred among the scaled responses. The most common significant effect occurred for time—that is, some responses tended to drift upward during exposures.

Table 2. Subjective Measures: Eye, Nose, Throat Irritation, MANOVA Summary

Measure	Parameter	Multivariate *F* [Univariate *F*]	*p*	df
Eye irritation				
AQ3	Gender		ns	
	Exposure		ns	
	Time	2.336	<.011	(10,318)
SQ5, SQ6, SQ7	Exposure		ns	
	Gender		ns	
	Time	3.883	<.0001	(15,436)
	[SQ5	*F* = 7.252	<.0001	(5,160)]
	[SQ6	*F* = 6.355	<.0001	(5,160)]
	[SQ7	*F* = 5.724	<.0001	(5,160)]
Nasal irritation				
AQ4	Exposure		ns	
	Gender		ns	
	Time		ns	
SQ2, SQ14	Exposure		ns	
	Gender		ns	
	Time		ns	
Irritation of the throat				
AQ5	Exposure		ns	
	Gender		ns	
	Time	3.005	<.001	(10,308)
SQ8, SQ10, SQ11	Exposure		ns	
	Gender		ns	
	Time	2.729	<.0001	(15,464)
	[SQ8	*F* = 5.018	<.0001	(5,170)]
	[SQ10	*F* = 7.805	<.0001	(5,170)]
	[SQ11	*F* = 4.111	<.0001	(5,170)]

Profile of Mood Scales (POMS). Ratings on the NES2 test battery indicated no significant effects of exposure in the post- minus preexposure difference scores for the five mood scales (tension, depression, anger, fatigue, and confusion).

Table 3. Subjective Measures: Miscellaneous Symptoms, MANOVA Summary

Measure	Parameter	Multivariate *F* [Univariate *F*]	*p*	df
Other respiratory symptoms				
SQ3, SQ4	Exposure		ns	
	Gender		ns	
	Time	3.473	<.0001	(15,464)
	[SQ3		ns]	
	[SQ4	*F* = 4.261	<.001	(5,170)]
Headache				
AQ6	Exposure		ns	
	Gender		ns	
	Time	3.005	<.001	(10,308)
SQ1	Exposure		ns	
	Gender		ns	
	Time	4.595	<.0001	(10,338)
Other symptoms				
SQ13, SQ19, SQ20	Exposure		ns	
	Gender		ns	
	Time	3.253	<.0001	(15,450)
	[SQ13	*F* = 3.875	<.002	(5,165)]
	[SQ19	*F* = 7.083	<.0001	(5,165)]
	[SQ20	*F* = 2.625	<.026	(5,165)]
SQ9, SQ12, SQ15, SQ16, SQ21, SQ22	Exposure		ns	
	Gender		ns	
	Time		ns	
Profile of mood scales	Exposure			
Tension, depression, anger, fatigue, confusion	Gender		ns	
			ns	

Air Quality Assessed on the AQ and SQ. Questions about air quality, odor intensity, and odor pleasantness relate more to a person's assessment of the environment than to symptoms and are treated separately. Assessments of air quality were addressed via questions AQ7 ("How is the air quality in the room now?" rated from very poor to very good), AQ11 ("Is the air quality acceptable now?" rated as yes or no), and SQ24 ("Rate the air quality in room" from 0 = very poor to 4 = very good). No subjects ever responded to AQ7 with 0 or 1 (very poor) during any of the exposure conditions; that is, all subjects rated air quality as better than very poor. In response to the question about

acceptability (AQ11), 86% of judgments were “yes” during exposure to air, 88% during exposure to MTBE, but only 63% during exposure to VOCs. Except for one subject during exposure to VOCs, all responded with ratings above zero (better than very poor) to SQ24.

As Figure 4a illustrates, there were significant differential effects for exposure for acceptability of the air (see Table 4, items AQ7, AQ11, and SQ24). Contrasts revealed significant differences between VOCs versus air and MTBE and between VOCs and MTBE. Air and MTBE did not differ significantly. Gender proved significant on AQ7, where women gave lower ratings. Gender did not reach significance, however, on item SQ24.

Assessment of odor intensity included responses to AQ1 (What is the intensity of the odor in the room now?" from no odor to extremely strong) and SQ23 ("Rate the odor level in room" from none to very strong). The types of exposure differed significantly one from another in the order VOCs > MTBE > air (Table 4). On the SQ, rated intensity of the VOCs equaled about moderate whereas that of air and MTBE equaled about mild (Figure 4b).

Assessment of odor pleasantness included responses to AQ2 (“How pleasant is the odor in the room now?" from extremely acceptable to extremely unacceptable) and SQ25 ("Rate the odor pleasantness” from 0 = very bad to 4 = very good). Exposure showed a differential influence on these (Table 4). VOCs differed significantly from both air and MTBE on both items. MTBE differed significantly from air on AQ2 (Figure 5), but not on SQ25. Females gave significantly lower ratings on SQ25; in this and other cases where females differed from males, the internal pattern was the same (see Discussion).

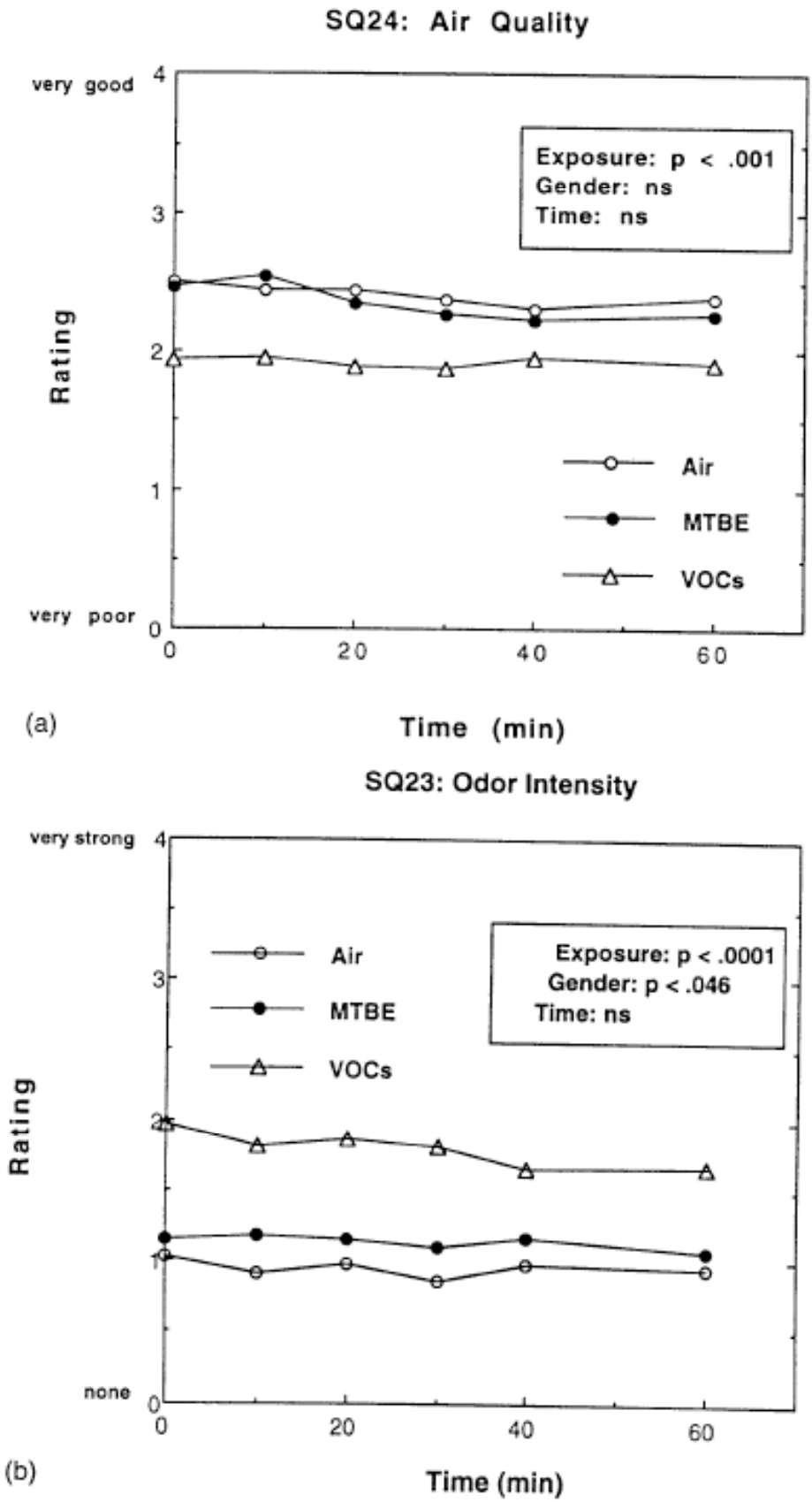


Figure 4. (a) Average ratings of air quality on the SQ. (b) Average ratings of odor intensity on the SQ.

Table 4. Subjective Measures: Air Quality MANOVA Summary.

Measure	Paramter	Multivariate *F* [Univariate *F*]	*p*	df
Air quality				
AQ7	Exposure	8.059	<.001	(2,33)
[Air vs. MTBE)			ns]	
[Air and MTBE vs. VOC		*F* = 15.198	<.0001	(1,34)]
	Gender	4.150	<.049	(1,34)
	Time		ns	
AQ11	Exposure	6.387	<.005	(2,33)
[Air vs. MTBE			ns]	
[Air and MTBE vs. VOC		*F* = 12.743	<.001	(1,34)]
	Gender		ns	
	Time		ns	
SQ24	Exposure	9.310	<.001	(2,34)
[Air vs. MTBE			ns]	
[Air and MTBE vs. VOC		*F* = 18.09	<.0001	(1,35)]
	Gender		ns	
	Time		ns	
Odor intensity and pleasantness				
AQ1, AQ2	Exposure	6.996	<.0001	(4,32)
[A1: Air vs. MTBE		*F* = 5.489	<.025	(1,35)]
[A1: Air vs. VOC		*F* = 33.616	<.0001	(1,38)]
[A1: MTBE vs. VOC		*F* = 10.474	<.003	(1,34)]
[A2: Air vs. MTBE		*F* = 7.475	<.01	(1,35)]
[A2: Air vs. VOC		*F* = 30.267	<.0001	(1,38)]
[A2: MTBE vs. VOC		*F* = 12.014	<.001	(1,34)]
	Gender		ns	
	Time	*F* = 2.231	<.049	(10,26)]
	[A1:	*F* = 3.219	<.008	(5,175)]
	[A2:		ns]	
SQ23, SQ25	Exposure	9.557	<.0001	(4,32)
[SQ23: Air vs. MTBE		*F* = 4.849	<.034	(1,35)]
[SQ23: Air vs. VOC		*F* = 34.358	<.0001	(1,39)]
[SQ23: MTBE vs. VOC		*F* = 16.988	<.0001	(1,35)]
[SQ25: Air vs. MTBE			ns]	
[SQ25: Air vs. VOC		*F* = 27.167	<.0001	(1,39)]
[SQ25: MTBE vs. VOC		*F* = 25.315	<.0001	(1,35)]
	Gender	3.384	<.046	(2,34)
	[SQ23	*F* = 5.398	<.026	(1,35)]
	[SQ25	*F* = 4.416	<.043	(1,35)]
	Time		ns	
Rating of other room attributes				
AQ8, AQ9, AQ10	Exposure		ns	
	Gender	4.820	<.008	(3,29)
	[AQ8:	*F* = 13.482	<.001	(1,31)]
	[AQ9:		ns]	
	[AQ10:		ns]	
	Time		ns	
	Gender × time	2.208	<.006	(15,423)
	[AQ8:	*F* = 3.079	<.011	(5,155)]
	[AQ9:		ns]	
	[AQ10:	*F* = 2.583	<.028	(5,155)]

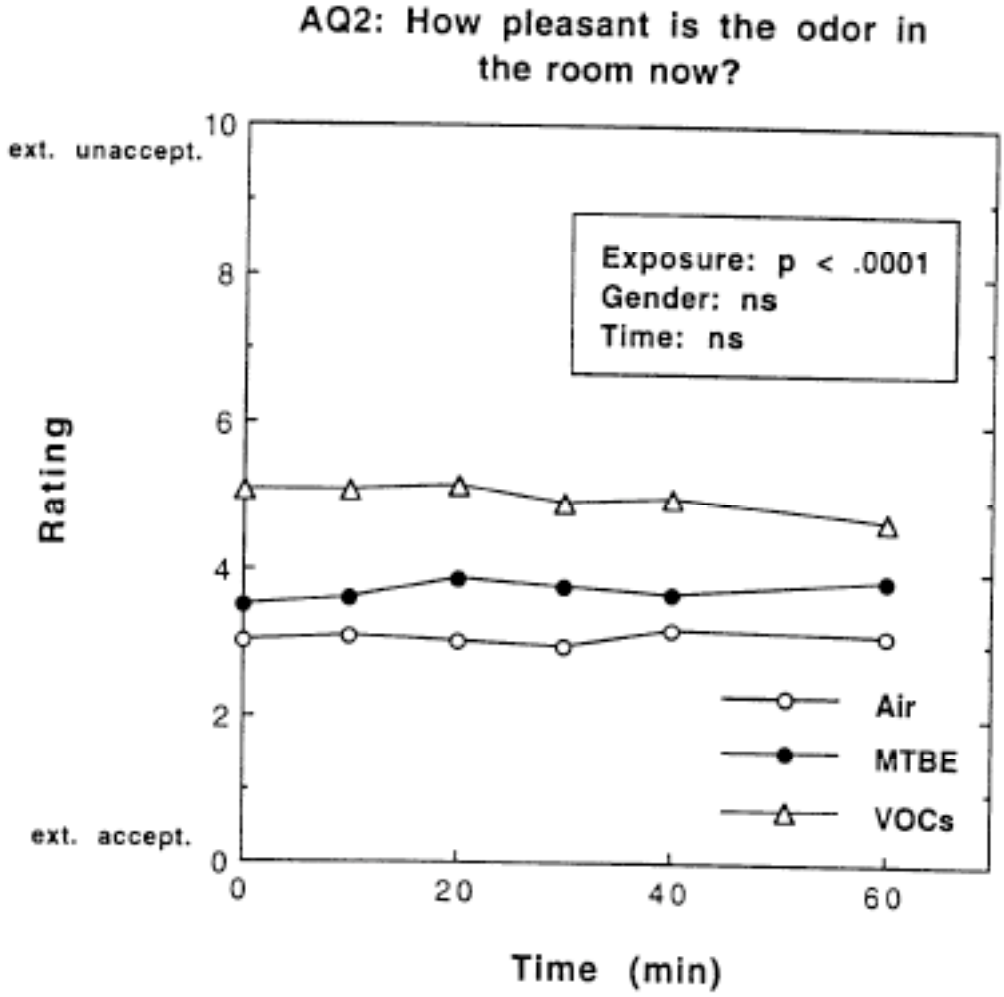


Figure 5. Average ratings of odor pleasantness on the AQ.

Ratings of Other Attributes. Subjects also rated temperature (AQ8: "How is the temperature in the room now?" from too cool to too hot), noise level (AQ9: "How is the noise level in the room now?" from quiet to noisy), and amount of light (AQ10: "How is the amount of light in the room now?" from too dim to too bright). Neither exposure nor time led to significant effects (Table 4). Gender led to a significant multivariate effect with a corresponding significant univariate test for AQ8, the thermal attribute. Gender also interacted significantly with time in univariate tests for AQ8 and AQ10.

Discussion

Pharmacokinetic Study

The pharmacokinetic study was designed to measure the level of internal exposure associated with the constant ambient exposures and, within that context, to establish blood concentrations associated with acute effects, if any. The results were also expected to provide a starting point to predict rate of clearance of MTBE. The relationship between blood concentration and acute effects could be relevant to understanding symptoms reported in Fairbanks, AK, during the oxygenated fuel season of 1992-1993 (Moolenaar et al., 1994). In that study, blood concentrations were the principal measure of exposure.

Moolenaar et al. (1994) reported a mean blood concentration of 9.44 μg/L in 18 workers exposed to vehicle exhaust or gasoline fumes over an 8 to 10-h workshift. Maximum blood levels ranged up to 37 μg/L Mean preshift to postshift differential equaled 6.71 μg/L from a measured ambient level of 0.21 ppm. The mean blood concentration of 7 commuters analyzed after their commute (length unspecified) equaled 1.19 μg/L. Despite large intersubject variability in their small sample, the data do provide a useful first approximation to levels that might be seen in occupational and environmental exposures.

Our exposures produced a mean peak blood level of 17 μg/L, which exceeded that for all commuters and exceeded the mean for the workers in Fairbanks, AK. None of our subjects reached levels as high as the highest among the workers. Our results indicated that blood concentration would have risen above 17 μg/L had exposure continued. Our exposure, though, could have been of sufficient intensity to generate symptoms, if MTBE alone and an exposure as short as 1 h were sufficient to generate them.

The blood concentrations associated with the chamber exposure were also in the range associated with increased symptom reporting in Stamford, CT (U.S. EPA, 1993). In that epidemiological investigation carried out by CDC, individuals with blood

concentrations in the upper quartile (>2.4 μg/L) were more likely to report one or more key symptoms. Median blood concentrations ranged from 0.12 μg/L (commuters) to 15.2 μg/L (gas station attendants). Our investigation produced concentrations in that range. Nevertheless, gas station-commuter exposures differ in other ways from the present exposure. Factors possibly relevant to any comparison include presence of other ambient chemicals, temperature, humidity, length of exposure, and type of activity. The blood analyses in the epidemiological studies had insufficient time resolution to determine the shape of the concentration versus time curve. Hence, in terms of dose of MTBE integrated over time, the relationship between our study and the studies in Fairbanks, AK, or Stamford, CT, is uncertain.

The ratio of ambient to blood concentration in the Alaskan workers offers potentially useful information to extrapolate 1-h exposures to steady state blood concentration. The extrapolation assumes the workers to be at steady state by the end of their shift and to have a responsiveness not different from that of volunteers without a known history of exposure to MTBE or gasoline vapors. As indicated, the workers showed an incremental concentration of 6.71 μg/L to an exposure of 0.21 ppm over 8-10 h. The present study found an incremental concentration of 16.4 μg/L to an exposure of 1.7 ppm over 1 h. According to the Alaskan data, an exposure of 1.7 ppm would have led to an incremental blood level of 54 μg/L at steady state. Although speculative, this analysis suggests that peak levels measured here may have equaled one-third steady state.

A pharmacokinetic study of rats exposed to 400 or 8000 ppm indicated that MTBE rapidly equilibrated in blood, with steady state reached within 2 h (Laboratoires Bio-Researches, 1990). Plasma concentration after 1 h equaled 56-58% of steady state. Such data suggest about a 2 to 1, rather than a 3 to 1, ratio of steady-state concentration to concentration after 1 h. The comparison between the data of rats and that of humans has the customary limitations of any interspecies comparison with a large disparity in levels of exposure. Nevertheless, both human and animal data imply rapid uptake and elimination of MTBE. The rat data on elimination fit a one-

compartment model with a calculated half-life of approximately 30 min. This outcome comports in part with the present data where decay to one-half peak concentration took about 40 min. The decay phase in human blood suggested a slower component also, not seen in the rats. This became evident in the slow decay from 60 to 90 min postexposure. A longer period of followup would have been required to determine the time constant of this component. In a recent study, however, Nihlén et al. (1994) reported a decay with 3 half-lives of 1 min, 47 min, and 6.5 h in human subjects exposed to 5, 25, and 50 ppm for 2 h during light exercise (50 W).

Results of Leuschner et al. (1991) on MTBE in blood, urine, and breast milk after use of MTBE for dissolution of gallstones suggested somewhat slower, though still relatively rapid, clearance of MTBE. The amount of MTBE used for treatment was unspecified, but varied among patients (n = 26), as did duration of treatment, which averaged 5.1 h. Concentration in blood averaged 400 μg/L just after treatment and fell by half in 5 h. Since some absorption of MTBE may have continued after therapy, these data may underestimate the true rate of elimination. The results could be compatible with a multicompartment model relevant to high concentrations attained systemically.

A second point of similarity between the present results and those in the rat revealed itself in the ratio of exposure concentration to blood concentration. With standard assumptions regarding inhalation rate and body weight, the exposure to 400 ppm in the Laboratoires Bio-Researches (1990) study corresponded to a dosage of 48 mg/kg/h, whereas the 1.7 ppm exposure to humans corresponded to 0.074 mg/kg/h. For an exposure rate approximately 650 times greater, maximum blood concentration in rats was 880 times greater, indicating good correspondence.

In a study of subchronic inhalation of MTBE in rats, with exposures to 50, 100, or 300 ppm for 6 h/day, 5 days/wk for 2-15 wk (Savolainen et al., 1985), concentration of MTBE in blood varied, approximately linearly with level of exposure with no indication of accumulation in blood, brain, or fat, an outcome consistent with rapid clearance. The

ratio of blood concentration to exposure concentration was like that seen in the Laboratoires Bio-Researches (1990) study of rats, where a vapor phase concentration of 300 ppm resulted in about 6 mg/L in blood. TBA did accumulate over time; blood concentrations after 6 wk of exposure exceeded that after 2 wk by four- to sevenfold.

In summary, the pharmacokinetic results indicate that (1) 1-h exposures to 1.7 ppm MTBE achieve blood concentrations comparable to those measured by CDC in Alaska; (2) maximal blood concentrations of MTBE seen at 1 h may equal one-half to one-third of steady state; and (3) MTBE shows rapid uptake and relatively rapid clearance.

Acute Effects

As indicated earlier, sensory indices usually show greater sensitivity than objective indices. Subjects typically feel irritation, fatigue, headache, and the like before objective indices can confirm any corresponding signs. When objective indices verify the reality of subjective effects, they ordinarily do so with higher thresholds. In the study of Prah et al. (1994) of 1-h exposures to 1.39 ppm MTBE, the subjects showed neither consistent MTBE-associated symptoms nor MTBE-associated functional or somatic changes, such as cognitive changes or increases in biomarkers of ocular or nasal inflammation. Female subjects, however, did indicate poorer air quality during exposures to MTBE.

In the present case, the power of the items to pick up differences on the order of 10% of the range, that is, one scale point on the AQ and one-half point on the SQ, approached 1.0. The indices proved differentially sensitive across agents to the presence of odor, to odor intensity and pleasantness, and to deterioration of air quality. Moreover, the ratings proved sensitive to factors of time and gender known or suspected to cause systematic variation in odor intensity, irritation, pleasantness, and even thermal comfort. For example, ratings of odor intensity declined, whereas ratings of irritation, particularly of the eyes, throat, and lower airways increased. Hudnell

et al. (1993) reported similar findings for a mixture of VOCs. Cain and colleagues found such effects on various occasions, both in response to the actual presence of an odorant/irritant and, to a smaller degree, in response to air alone (Cain et al., 1986, 1987a, 1987b; Stevens et al., 1989). We have no ready explanation for why subjects show an increase in rated irritation even without any injected contaminant. It may derive from demand characteristics, that is, a kind of response bias, or from a tendency to notice low-level irritation that does indeed increase even in uncontaminated air. The second possibility suggests that subjects would be keenly aware of true irritation when it actually occurs.

Various of the items in the AQ and SQ proved sensitive to a differential effect of gender in more or less expectable ways (see Wysocki & Gilbert, 1989). For example, women found odor intensity greater, pleasantness worse, and air quality worse (Figures 6 and 7a). This outcome shows convergence with the work of Prah et al. (1994). Women also found the thermal environment cooler (see Nevins et al., 1966; Rohles, 1973) (Figure 7b). Such findings imply that any failure to find significant effects of exposure must have stemmed more from a true absence of differential influence of chemical exposure than from poor ability to resolve differences.

Just as ratings of eye irritation increased during exposure irrespective of condition, the ocular parameters of dots on the conjunctiva, redness, and tear-film breakup indicated a slight increase in irritation. Perhaps this was just a fortuitous association. If not, though, it would bode well for objective verification of more serious ocular effects than found here.

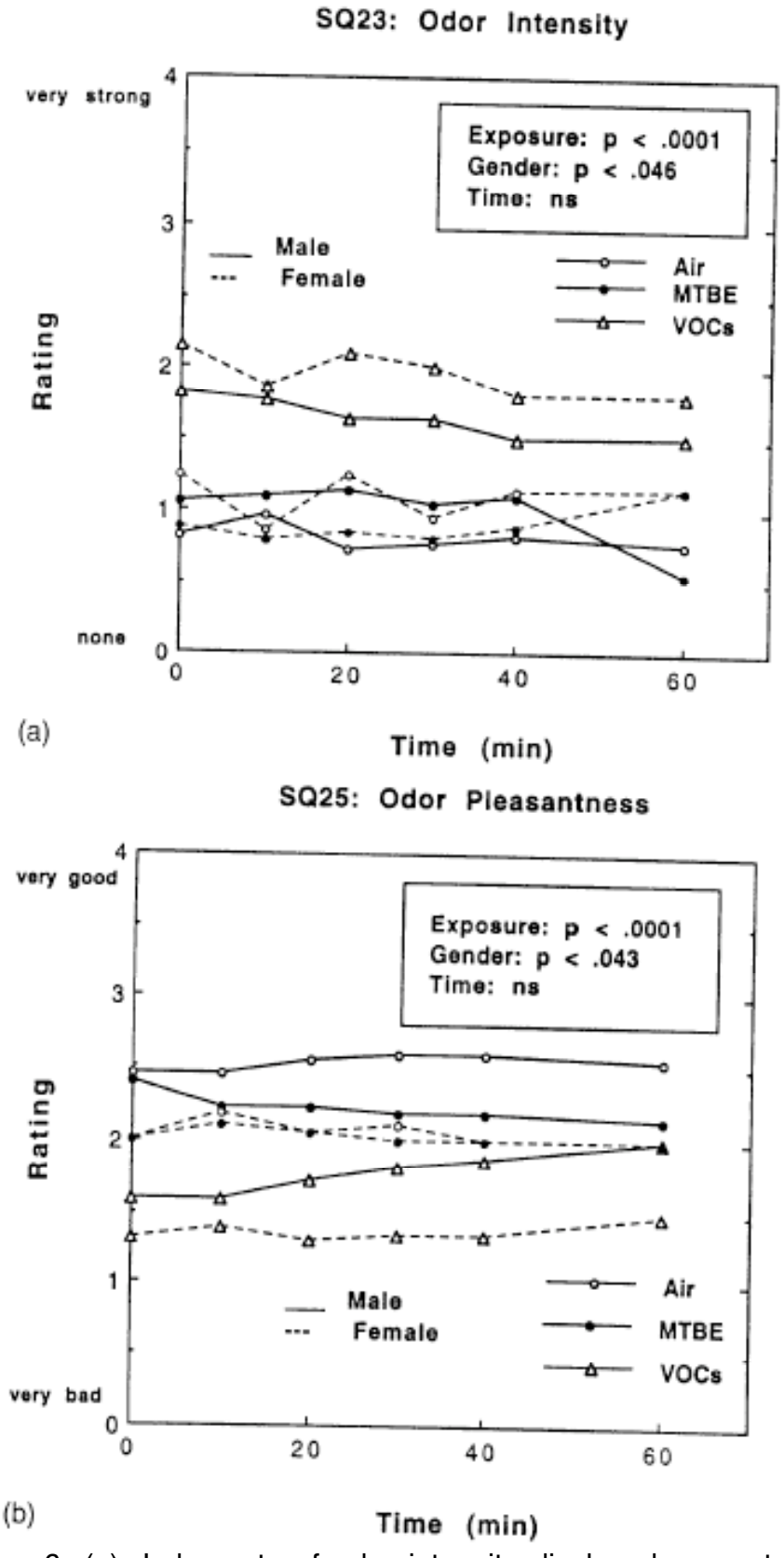
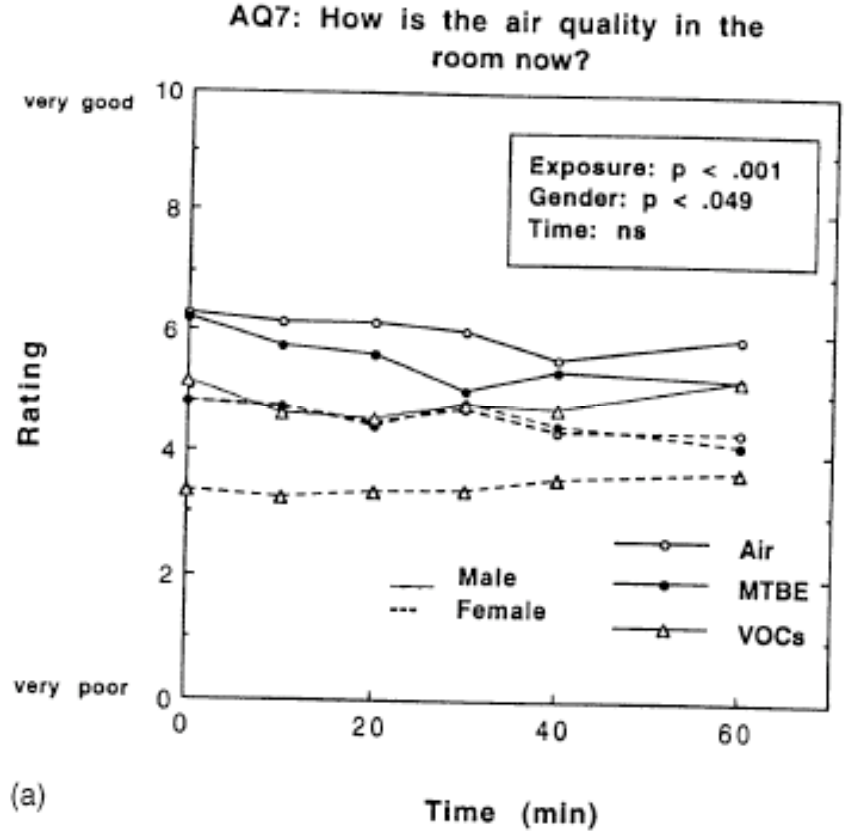


Figure 6. (a) Judgments of odor intensity displayed separately for males and females. (b) Judgments of odor pleasantness displayed separately for males and females.



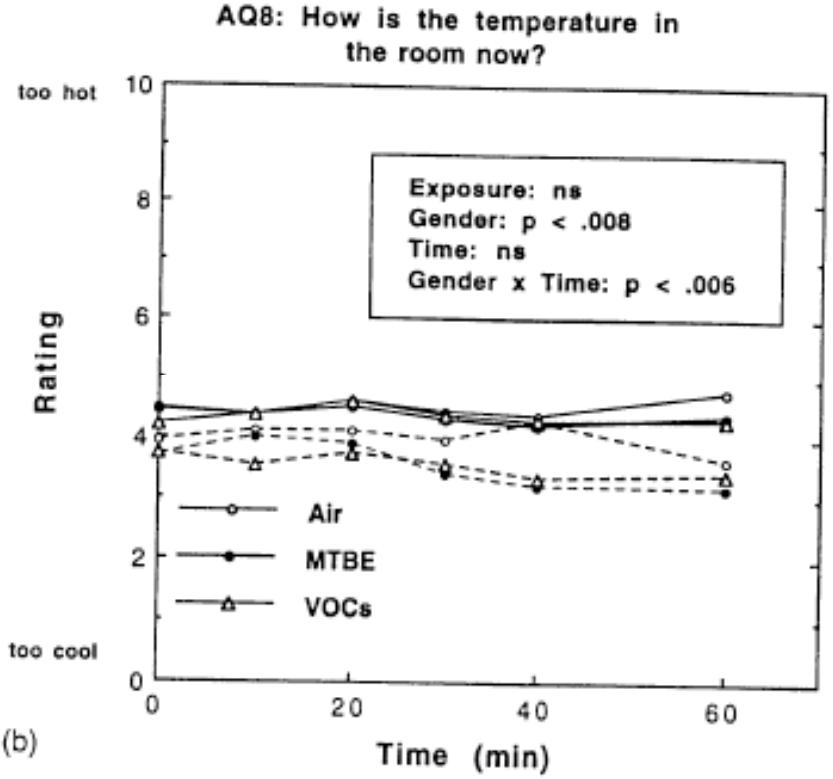


Figure 7. (a) Judgments of air quality displayed separately for males and females. (b) Judgments of temperature displayed separately for males and females.

For the nasal lavage, the significant interaction of exposure by time in PMNs derived principally from an increase from just post-exposure to 18-24 h postexposure to VOCs. Koren et al. (1992) similarly found a delayed increase in PMNs from exposure to their 22-component mixture of VOCs (4-hr exposure to 25 mg/m^3). For subjects with low baseline values, number of PMNs increased 50-fold. Our increase, obtained from a 1-h exposure to 19 mg/m^3, equaled just 65%. (None of our subjects showed the high baselines found among some subjects studied at HERL.) These two positive outcomes give incentive to continue ratings of nasal irritation beyond the period of exposure, since such ratings might capture delayed irritation where inflammation develops.

In summary, our young healthy adults did not react adversely to concentrations of MTBE at the high end of those found during commuting and over a duration realistic for a commute. Our subjects could detect the presence of MTBE via smell, but showed little objection to it. When subjects expressed symptoms, these were mild and no greater than those expressed to just air. The subjective results had an internal structure consistent with that seen in previous studies. For example, the results showed time dependent potentiation for ratings of irritation, time-dependent attenuation for ratings of odor, and higher ratings for odor impact and thermal discomfort among women.

Our various objective indices gave positive effects only where expected from either subjective reactions or from prior positive findings. Encouragingly, the indices of surface irritation of the eye (dots, tear-film breakup time, and redness) seem to have potential to reflect differential effects across stimuli. Furthermore, based on both previous and present positive outcomes, an increase in percent PMNs in nasal lavage fluid seems likely to reflect an impact of even mildly irritating vapors on the nasal mucosa. The interpretation of this immunological response requires caution, pending understanding of the time course of variation of PMNs with repeated lavage.

The present results have obvious constraints for generalization to the

population at large. Even aside from constraints imposed by situational differences, such as environmental temperature and humidity (cold climates vs. neutral conditions in a chamber), activity level of the persons exposed (the activity in real-world situations vs. passive conditions in a chamber), and the presence or absence of other vapors along with MTBE, there is the additional constraint that the general population contains people who would neither have passed our physical exam nor, perhaps because of self-decided sensitivity, would have presented themselves for such a study. Even if systematic exclusion of possible "responders" were not itself an overriding limitation, studies with samples of a few dozen people can hardly predict the reactions of the statistically rare individual.

Within constraints imposed by the ethics of exposing humans, it would be valuable to focus on one particular health effect per experiment and to seek the minimum conditions necessary to elicit that effect. If the effect occurred within environmentally realistic limits, then a dose-response function over an appropriate range would seem merited.

In the subjective realm, odors can become associated with virtually any symptom, such that a person can claim the odor caused the symptom (Shusterman, 1992). Not uncommonly, people exposed to an ambient odor can become bothered by and eventually symptomatic in multiple ways to its presence, even when no objective indices confirm adverse effects. Similar situations occur in office buildings. Whether the symptoms are “caused” by the experience of the odor or by the chemical acting on some target organ is often irrelevant to the complainant. Only by application of objective techniques of high sensitivity can we hope to resolve such issues. The present study, despite its limitations in terms of exposure, has progressed, we can hope, along lines of refinement of techniques as well as along lines of understanding the effects of MTBE.

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