

A Controlled Short-term Exposure Study to Investigate the Odor Differences Among Three Different Formulations of Gasoline

Richard E. Opiekun^{1,2}, Kathie Kelly-McNeil¹, Susan Knasko^{3,4}, Paul Lioy¹ and Nancy Fiedler¹

¹Environmental and Occupational Health Sciences Institute of Rutgers University and the University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School, 170 Frelinghuysen Road, Piscataway, NJ 08854, ²Joint PhD Program in Exposure Measurement and Assessment, Department of Environmental Sciences, Rutgers, The State University of New Jersey and The UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ and ³Monell Chemical Senses Center, 3500 Market Street, Philadelphia, PA 19104-3308, USA

⁴Present address: Unilever Research USA, 45 River Road, Edgewater, NJ 07020, USA

Correspondence to be sent to: Nancy Fiedler, Environmental and Occupational Health Sciences Institute, Room 210, 170 Frelinghuysen Road, Piscataway, NJ 08854, USA. e-mail: nfiedler@eohsi.rutgers.edu

Abstract

Control subjects (CON) and self-reported methyl tertiary butyl ether (MTBE)-sensitive subjects (SRS) were evaluated to distinguish between the following gasoline blends: gasoline versus gasoline + MTBE (15% MTBE v/v); and gasoline versus gasoline + MTBE + reodorant. The study also investigated the ability of a reodorant to conceal the odor of MTBE in a gasoline mixture. In each of two separate sessions, seven men (four CON, three SRS) and seven women (four CON, three SRS) were asked, in a forced-choice format, to sniff 28 randomized bottle pairs to determine if the odors in each pair were the same or different. Chi-square analyses revealed that, with the exception of one male CON, subjects were unable to distinguish between gasoline and gasoline with MTBE or gasoline with MTBE and the reodorant. Thus, a reodorant is of limited value as an additive which alters the ability of an individual to detect MTBE in a blended gasoline. The results suggest that at the level used in the experiment, no mask would be required to blind a participant from the odor of MTBE if that level is used in a controlled human health effects study of the additive.

Introduction

Methyl tertiary butyl ether (MTBE) has been used as an octane-enhancing fuel additive (between 3 and 11% by volume) in the super grade of gasoline since the 1970s. After passage of the Clean Air Act in 1990, 15% MTBE by volume was added to all grades of gasoline during the winter months in carbon monoxide non-attainment areas throughout the USA. The purpose of adding 15% MTBE (v/v) was to promote better combustion of gasoline and reduce carbon monoxide emissions. While refueling their vehicles, a subpopulation of individuals have reported that this higher level of MTBE causes them to have headaches, nausea, disorientation, elevated incidence of fatigue and burning in the upper respiratory tract (Fiedler *et al.*, 1994; Moolenaar *et al.*, 1994; White *et al.*, 1995; European Center for Ecotoxicology and Toxicology of Chemicals, 1997). One of the proposed explanations for this increase in symptom reporting is that the complaints are related to the unpleasant odor of oxygenated gasoline. The ability of ambient odors (Ludvigson and Rottman, 1989) and chemicals, such as

acetone (Dalton *et al.*, 1997), to affect an individual's mood and other health symptoms has been demonstrated.

Odor studies have shown that suggestion of a malodor in the absence of an actual odor can lead to an increase in reporting of health symptoms (Knasko *et al.*, 1990). Furthermore, the presence of an unfamiliar odor has been shown to increase symptom reporting (Stahl *et al.*, 1974). Individuals have also claimed retrospectively that the presence of a malodor adversely affected their mood, health and behavioral performance, but measures taken during actual exposure found that the malodors had no effect on these factors (Knasko, 1993).

Due to the reported malodor and purported identifiability of MTBE, a reodorant was developed to conceal the scent of the oxygenate. An oxygenated fuel mixture containing the reodorant should have the odor characteristics of unoxygenated gasoline. The purpose of this study was twofold: (i) to test the ability of the reodorant to successfully conceal the odor of MTBE and (ii) to compare the ability of

control (CON) and subjects self-reported as sensitive (SRS) to MTBE to accurately detect an odor difference between samples of gasoline alone versus gasoline containing 15% (v/v) MTBE and gasoline alone versus gasoline containing 15% MTBE and the reodorant. These results were used to determine if masking of MTBE odor is required to blind exposure in a controlled environment study of MTBE-related human health effects.

Materials and methods

Subjects

Seven non-smoking men between 38 and 77 years of age ($\bar{x} = 66$, $\sigma = 14.3$; four CON, three SRS) and seven non-smoking women between 36 and 62 years of age ($\bar{x} = 51.9$, $\sigma = 8.4$; four CON, three SRS) participated in this study. All subjects were Caucasian and had at least completed high school. The SRS were age and gender matched with CON. Self-reported sensitives were recruited with a mass mailing to ~1000 members of an activist organization (eg. Oxy-busters), and through advertisements on a local morning radio program and in a local newspaper. Responders to the advertisements were sent and asked to complete a symptom screening questionnaire pertaining to how they felt around gasoline, listing symptoms associated with colds, flu, allergies (27 questions) and MTBE exposure (seven questions). Questions not usually associated with MTBE (e.g. fever, diarrhea, muscle ache) were used as an overall assessment of health status and also controlled for response bias for symptom reporting, but were not used as a study exclusion criteria. Subjects were asked to rate all cold, flu and allergy symptoms and their MTBE-related symptoms (headache, cough, nausea, sleepiness, burning within respiratory tract, dizziness, difficulty concentrating) from 0 to 3 as follows: 0 = do not have this symptom; 1 = symptom is slight; 2 = symptom is moderate; 3 = symptom is severe. Summing the scores, the highest attainable MTBE symptom score was 21. SRSs were categorized as those individuals who reported experiencing adverse symptoms associated with MTBE exposure while driving or refueling and who scored in the upper quartile of all responders (score ≥ 10). CON were members of the university community who did not claim to experience any adverse symptoms when exposed to oxygenated gasoline and who scored < 9 on the symptom survey. CON and SRS with any of the following medical conditions were excluded from the study: neurologic disease or brain injury, significant exposure to other neurotoxicants, chronic fatigue syndrome or multiple chemical sensitivity, stroke or cardiovascular disease, serious pulmonary disease, liver or kidney disease, serious gastrointestinal disorders (e.g. colitis), nasal polyps, nasal surgery, sinus disease and major psychiatric conditions including psychoses, manic depression, alcoholism and drug abuse. No pregnant or lactating women were included in the study. From among the remaining pool of subjects, eight CON and six SRS were selected

randomly and asked to participate. One female SRS left the study after one visit due to complaints of nausea.

Preparation of subjects

The subjects were given a preparatory session to inform them of the experimental design and possible health risks involved with the study. The explanation of risks included those associated with exposure to gasoline (arrhythmia, dizziness, headache, euphoria, vertigo, blurred vision, nausea, fatigue and sleep problems), MTBE (nausea, vomiting, hypotension, headaches, sleepiness, cough, disorientation and angina) and the reodorant (allergic dermatitis, eye and skin irritation). All subjects were instructed not to wear scented body products or eat pungent foods, such as onion or garlic, on the day of testing. Participants were also asked not to eat or drink anything at least half an hour before arriving for their appointment. Subjects were tested at the same time of day for each visit in order to control for circadian variations. If subjects had a cold, flu or active allergy on the day of testing they were rescheduled for another day. Immediately prior to each testing session, participants were trained in the task they were to perform, shown the proper sniffing technique and, in accordance with University Institutional Review Board guidelines, given adequate opportunity to have questions and concerns addressed prior to signing the consent document. Each subject was shown how to properly hold a sample bottle and insert the bottle's nose-piece securely into one nostril while occluding the opposite nostril prior to taking a sniff. This demonstration was then presented for the second nostril and each participant was allowed to practice with an empty bottle until they were proficient with the technique. Subjects were also informed that they would be remunerated with \$15 per visit for their time.

Sniff bottle materials and assembly

Glass bottles (255 ml) were purchased through Fisher Scientific (Pittsburgh, PA; cat. no. 02883CC) and fitted with plastic flip-top caps (J. Rice Plastic Containers, Brooklyn, NY; cat. no. 24-410). Each cap had two holes (6 mm each) drilled beneath the flip top for insertion of a nose-piece adapter and return air tubing. Prior to fitting the caps, all bottle threads were triple-wrapped with half-inch Teflon™ thread tape to ensure a tight seal between cap and bottle. The posterior hole of each cap was fitted with a Teflon™ tube (133 mm) which extended toward the bottom of the bottle, allowing return air to enter each bottle as the subject sniffed the vapors of the fuel mixture from the bottle. The anterior hole was fitted with a nose-piece adapter comprising a Teflon™ tubing connector (Bel-Art Products, Pequannock, NJ; cat. no. 19730-0002) into which a 43 mm piece of Teflon™ tubing was inserted into the wider end. The narrower top portion of the adapter, with the barbed end, contained an extra-virgin Teflon™ nose-piece (50 mm \times 14 mm OD; 8 mm ID) with a tapered end to fit



Figure 1 Sniff bottle assembly.

comfortably into a subject's nostril (see Figure 1; design specifications provided by Monell Chemical Senses Center). Each nose-piece was washed in detergent and sonicated in a mixture of distilled water and Fisherbrand ultrasonic cleaning solution (Fisher Scientific; cat. no. 15-336-26) for half an hour after manufacturing to remove loose particles and machine oils. After use, nose-pieces were soaked in a 10% chlorine bleach solution, rinsed 10 times with distilled water to ensure removal of any residual chlorine odor and autoclaved (240°F and 16 psi) for 20 min.

Prior to use, each bottle was flushed 12 times with dehumidified sterile air and the outside was wiped with a lint-free cloth moistened with deionized water. Powderless latex gloves were worn during handling of the bottles to prevent oils and other residues from contaminating the glassware. To provide the best representation of concentrations inhaled by the subjects, the bottles were then directly filled with the vapors of the gasoline mixture which was previously blended in 20.3 l Tedlar® bags (Cole-Parmer Instrument Company, Vernon Hills, IL). The air mixture, containing ~1.7 ppm MTBE (range 1.4–1.8 ppm) and gasoline at 33 ppm total hydrocarbons, was humidified to 50% relative humidity for subject comfort (see Gas mixture preparation). Transfer of the gasoline mixture from bags to bottles was accomplished with a Teflon-lined pump set to a flow rate of 1.2 l/min. This flow rate completely flushed each bottle four times with the fuel mixture in order to fill the headspace of the bottle with the desired MTBE concentration. After filling, each bottle cap was immediately closed and triple-wrapped with Parafilm® to limit diffusion of the mixture from the bottle. Bottles were filled ~1 h prior to testing of a subject. Gas chromatography (DB-VRX column; J&W Scientific, Folsom, CA) had shown ~10% deterioration in the vapor concentrations from the initial vapor concentration present within the bottles. This leakage

was not seen as problematic inasmuch as this experiment was conducted with concentrations of MTBE well above the odor detection threshold in air of 0.33 ppm (Neste and Viljanen, 1989).

Gas mixture preparation

Gasoline without oxygenates (Sunoco Custom Blends, Philadelphia, PA) was obtained for use in this study. In addition, two blends were also created in the laboratory to ensure that the formulations remained consistent for all participants throughout the study. The first blend contained the gasoline with 15% (v/v) MTBE (1.7 ppm in air) (Mallinckrodt Baker Inc., Paris, KY) added to simulate the winter blend of gasoline used throughout much of the nation. The second blend contained the same amount of MTBE and a reodorant compound (Firmenich Inc., Plainsboro, NJ; proprietary formulation) added at a concentration of 25 ppm by weight. The reodorant compound had a fruity fragrance and was added to mask the odor of MTBE in the blend.

Each gasoline blend was flash evaporated into a heated (68°C) 2 l gastight mixing flask containing only dehumidified air. After introduction, the vapors were thoroughly mixed using a Teflon-coated mixing bar and 20 glass beads. A gastight syringe was used to remove the vapors from the flask and inject them into a Tedlar® bag filled with humidified air. A gas chromatograph, with a DB-VRX column (J&W Scientific) initially heated to 35°C, was used to determine the MTBE concentration in each bag. The initial temperature of the column was held for 10 min and increased at a rate of 5°C per min to a final temperature of 150°C, where it was again held for 10 min. The total evaporated gasoline hydrocarbon concentration within a bag averaged 33 ppm [reported as methane using a total hydrocarbon analyzer (GOW-MAC, model 23-500)].

Exposure protocol

To determine if an individual could detect a difference in odor between gasoline alone versus gasoline with MTBE and gasoline alone versus gasoline containing both MTBE and a reodorant, subjects were given a total of 56 bottles to sniff during each of two visits separated by 1 week. Each bottle contained one of the three formulations of gasoline.

Bottles were presented in pairs to a subject. One bottle was placed into the subject's right hand first and he/she was asked to sniff the contents through the right nostril while occluding the left nostril. The other bottle of the pair was presented to the subject's left hand and he/she was asked to sniff the contents through the left nostril while occluding the right nostril. They were permitted only one sniff per bottle since it has been shown that a single, natural sniff provides as much information about odor presence as multiple sniffs (Laing, 1983). After sniffing each bottle of a given pair, the subject was asked if the odor of the first bottle was the same or different from the odor in the second bottle (Meligaard *et*

al., 1987). After responding, subjects were given a 2 min rest period before being asked to sniff another pair of bottles. This rest period was chosen to insure that a subject's nose did not become fatigued during the testing session and that any residual gasoline odor would be removed from the nostrils before they were presented with another pair of bottles.

Exposure to the mixtures within each bottle lasted for the duration of one sniff, or between 2 and 3 s. Sniff volumes for men and women were observed to differ slightly. However, since the volume of a normal adult sniff is ~200 ml, a larger volume bottle was used to minimize the dilution of the MTBE with make-up air entering the bottle during a sniff (Doty et al., 1986).

On the first day of testing, seven subjects were asked to sniff gasoline (A) versus gasoline containing MTBE (B). The other seven subjects sniffed gasoline (A) versus gasoline containing MTBE and a reodorant (C) on their first testing visit. On the second day of testing, the subjects tested the opposite pairs. There were four possible combinations for each testing period: AA, BB, AB, BA or AA, CC, AC, CA. Each subject sniffed 28 pairs of bottles, with the four possible combinations presented seven times each in random order. Thus, on their first day of testing, a subject who was assigned to sniff bottles containing gasoline (A) and gasoline with MTBE (B) was presented AA bottles seven times, BB bottles seven times, AB bottles seven times and BA bottles seven times. On that subject's second day of testing, bottles containing gasoline with MTBE and the reodorant (C) replaced the B bottles. Subjects were told that they were sniffing three different gasoline formulations in order to determine if they could differentiate between the various blends. This two-choice simple difference discrimination method (AB pairing versus AC pairing) was chosen over a three-choice discrimination method (AB pairing versus AC pairing versus BC pairing) because it was deemed less fatiguing for the subjects (personal communication from Monell Chemical Senses Center). This test presentation method minimized the participants' exposure to gasoline.

Statistical analysis

A $2 \times 2 \times 3 \times 2$ repeated measures analysis of variance (ANOVA), combining group (CON versus SRS), gender (male versus female), mixture (AB, AC, AA, BB, CC) and time (first 14 bottle pairs versus second 14 bottle pairs), was used to test the effect of the between (group and subject) and within (mixture and time) subject factors on the proportion of times a subject reported the bottle pairs as 'different'. The data were fit to a mixed model using subjects as the random factor and limiting the model to include no higher than two-way interactions due to the limited degrees of freedom (see Table 1). This ANOVA was followed by Chi-square analysis, at 95% confidence, to determine a subject's accuracy in distinguishing a difference in odor between either AB or AC pairing combinations (see Table

Table 1 Odor detection mixed model ANOVA for the effects of group, gender, mixture and time

	df	F	P
Main effects			
Group ^a	1, 10.1	4.07	0.0710
Gender ^b	1, 10.1	2.67	0.1327
Mixture ^c	2, 55.6	1.27	0.2897
Time ^d	1, 55.1	0.16	0.6914
Interaction effects			
Group × gender	1, 10.2	0.28	0.6107
Group × time	1, 55.1	0.15	0.7001
Gender × time	1, 55.1	0.09	0.7679
Group × mixture	2, 55.6	0.41	0.6633
Gender × mixture	2, 55.6	1.40	0.2548
Mixture × time	2, 55.1	0.04	0.9567

^aGroup = SRS versus CON.

^bGender = males versus females.

^cMixture = gasoline alone, gasoline with 15% MTBE, gasoline with 15% MTBE and reodorant compound.

^dTime = first half of testing session (i.e. first 14 bottle pairings) versus second half of testing session (i.e. second 14 bottle pairings).

2). A Chi-square value of ≥ 3.84 indicated that a subject was able to determine accurately the difference between gasoline versus either gasoline with MTBE alone or gasoline with MTBE and the reodorant.

Results

The repeated measures ANOVA revealed a significant effect of group, but no other significant main effects or interactions. The SRS gave the 'different' response significantly more than the CON, while gender did not significantly affect the rate of 'different' response. Neither the mixture (i.e. different pairs—AB, AC—or same pairs—AA, BB, CC) nor the time (first half of testing session versus second half of testing session) affected the rate of 'different' responses. Hence, subjects did not appear to adapt or become sensitized to odors throughout their visit. With regard to accuracy of response for each subject, the Chi-square results revealed that only one CON (subject 13) reached significance, indicating that he accurately distinguished the odor difference between gasoline and gasoline containing MTBE. Two other CON, however, approached significance in their ability to detect a difference when the reodorant was present (A and C pairing) (see Table 2). The Chi-square analysis by group comparing accuracy for SRS (AB pairing Chi-square mean \pm SD = 0.79 ± 1.1 ; AC pairing Chi-square = 0.63 ± 0.79) and CON (AB pairing Chi-square = 1.23 ± 1.4 ; AC pairing Chi-square = 1.61 ± 1.4) revealed that neither group was able to accurately distinguish between mixtures for either the AB or AC pairing combinations. Thus, neither CON nor SRS subjects could accurately differentiate be-

Table 2 Individual results in response to gasoline and gasoline with MTBE (AB) and gasoline with MTBE and reodorant (AC) pairs—Chi-square values^a

Subject	Status	AB combination	AC combination
1	CON	0.14	3.74
2	CON	0.15	1.47
3	CON	0.62	0.58
4	CON	1.04	2.15
5	CON	0.57	3.59
6	CON	2.49	0.58
7	SRS	0.24	2.19
8	SRS	0.37	0.70
9	SRS	2.80	0.16
10	SRS	0.58	0.37
11	SRS	NR ^b	0.14
12	SRS	0.00	0.19
13	CON	4.09 ^c	0.57
14	CON	0.70	0.16

^aData based on results of Chi-square calculated values.

^bNR = not reported. Subject did not return for second exposure visit.

^cChi-square ≥ 3.84 = significantly different.

tween gasoline alone versus gasoline containing MTBE or gasoline alone versus gasoline with MTBE and the reodorant.

Discussion

The results indicate that neither the SRS nor the majority of CON could distinguish odor differences between gasoline and gasoline containing MTBE with or without a reodorant at a concentration likely to be experienced by a commuter in a gasoline station. This study also revealed that SRS, while no more accurate, were more likely than CON to report differences, regardless of whether bottles contained the same or different fuel mixtures. This tendency for sensitives to frequently give the response of 'different' was also observed during testing of gasoline versus gasoline with MTBE and a reodorant. These results suggest that SRS had a bias toward reporting a difference between bottles.

In summary, since most subjects could not distinguish gasoline with MTBE from gasoline alone, the present study suggests that a reodorant may not be of any value to mask MTBE at the level desired in future human health effects studies within a controlled environment.

Several caveats need to be mentioned when assessing the results of this study. First, even though the concentration of MTBE in each sniff bottle was well above the detection limit for this compound and was chosen to be similar to environmental concentrations found at a gas station while refueling (Lioy *et al.*, 1994), olfactory thresholds can vary between days and for individuals (Stevens *et al.*, 1988). Thus, it is possible that some subjects had a diminished

sensory capacity for odor detection on the day of testing and may not have been able to detect a difference between a gasoline only and a gasoline–MTBE mixture during a testing session. However, it has been demonstrated that addition of MTBE to certain gasolines can lower the detection threshold of those gasolines by up to 66%, allowing for an individual with diminished sensory capacity to detect an odor (TRC Environmental Corporation, 1995). Research has shown that the greater the difference in odor thresholds between MTBE and the respective gasoline to which it is added, the greater the decrease in the odor threshold of the MTBE–gasoline mixture (TRC Environmental Corporation, 1995). Thus, it is unlikely that day-to-day variation in the odor detection threshold could account for the current results.

It must also be recognized that the controlled conditions under which this experiment was conducted do not reflect conditions of the outdoor environment. Other atmospheric and climatic factors, such as temperature, humidity, wind direction and wind speed, may contribute to the detectability and perceived odor intensity of MTBE. Also, in the natural environment, individuals often take multiple sniffs, which has been shown to contribute to improved odor component identification (Laing, 1983). However, subjects in this study were not asked to accurately identify specific odors. Rather, their task was simply to determine whether the odor mixture was the same or different. Additional studies using a larger study population are required to examine these parameters since these data may not be representative of all individuals who are exposed to oxygenated gasoline.

Conclusion

This study has demonstrated that the majority of the individuals ($n = 13$) tested could not distinguish among the odors of different gasoline blends under controlled conditions, which included direct inhalation of a vapor mixture at the concentration found while refueling a vehicle. Moreover, there was no apparent difference in the ability of the SRS to more accurately detect the presence of MTBE in a fuel mixture.

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