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DETERMINATION OF URINARY CONCENTRATIONS OF ORGANIC OXYGENATES IN URBAN WORKERS

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ABSTRACT

Methyl *tert*-butyl ether, ethyl *tert*-butyl ether and *tert*-amyl methyl ether are oxygenated compounds added to gasoline to enhance octane rating in replacement of alkyl–lead compounds. In the present study the excretion of urinary methyl *tert*-butyl ether, ethyl *tert*-butyl ether and *tert*-amyl methyl ether were evaluated as biomarkers of exposure to gasoline. With this aim, 10 policemen engaged in traffic control, 10 gas station workers and ten occupationally non-exposed persons were investigated. Spot urine samples were obtained prior to and at the end of the work shift from each subject. The urinary levels of oxygenated ethers were determined by using head-space gas chromatography and mass spectrometry detection. There were significant differences among the mean urinary concentrations of methyl *tert*-butyl ether in pre-shift samples of gas station workers (1193 ng/L), policemen (734 ng/L) and occupationally non-exposed persons (49 ng/L). The mean urinary concentrations of methyl *tert*-butyl ether and *tert*-amyl methyl ether differed significantly among post-shift samples of gas station workers (16636 and 8655 ng/L), policemen (14458 and 1472 ng/L) and occupationally non-exposed persons (324 and 59 ng/L) (ANOVA: $p<0.05$ and Kruskal-Wallis test: $p<0.05$). There was a significant difference in methyl *tert*-butyl ether concentrations between job categories ($p<0.05$ by ANOVA and Kruskal-Wallis test), and gas station workers and policemen were found to be probably the most exposed groups in this study.

Key words: Methyl *tert*-butyl ether, Ethyl *tert*-butyl ether, *Tert*-amyl methyl ether**,** Urinary levels**,** Gasoline

INTRODUCTION

Methyl *tert*-butyl ether (MTBE), ethyl *tert*-butyl ether (ETBE) and *tert*-amyl methyl ether (TAME) are oxygenated compounds added to gasoline to enhance octane rating, in replacement of alkyl-lead compounds and to improve the combustion process, in order to reduce emissions of partially oxidated compounds as carbon monoxide from motor vehicles. Five or more carbon atoms in gasoline cannot be higher than 15%(v/v) (EFOA, 2006). Data from National Iranian Oil Products Refining & Distribution Company reports that in Iranian fuels an average amount of 10% (v/v) of ethers is present (NIORDC, 2008).

MTBE is currently the most common oxygenate of gasoline, used to reduce carbon monoxide and hydrocarbon levels in motor vehicle emissions. MTBE was originally introduced as an octane enhancer in unleaded gasolines in the 1970s. Recently, TAME and ETBE, two congeners of MTBE with one additional methyl group, have been introduced as a gasoline additive because of their favorable physical and chemical properties.

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The three substances are also used as mixtures in various proportions.

Increasing use of these oxygenates together with concerns about the effects of oxygenated fuels on human health has prompted research efforts in several fields (Nihle´n *et al.,*1998; Lin *et al.,* 2005; Alimohammadi *et al.,* 2005; Khavanin *et al.,* 2005; Tomei *et al.,* 2006; Nikpay *et al.,* 2006; Silva *et al.,* 2008;). Short-term effects such as nausea, headache, and sensory irritation have been studied in response to complaints by service station customers. Further investigations are also under way on the basis of the acute neurotoxic and carcinogenic effects obtained in animal studies at high exposure levels (Rudo, 1995; HEI, 1996).

Exposure to gasoline has been a long time concern in many occupational fields, including gasoline transport by tanker lorries, service stations, policemen and the petroleum industry. Traffic policemen and gas station workers are two of the groups most heavily exposed to vehicle exhausts during their work shifts (Egeghy *et al.,* 2000; Crebelli *et al.,* 2001; Scibetta *et al.,* 2005). According to literature, in estimation of workers' exposure with biological samples, analysis of the concentration of MTBE in urine detected after the work shift seems to give the most reliable estimation of exposure (Saarinen *et al.,* 1998; Hakkola *et al.,* 2001; Vainiotalo *et al.,* 2006), so in this study these ethers were selected as suitable biomarkers of gasoline exposure.

The aim of this study was to determine the exposure levels for traffic policemen and gas station workers during routine work shift, by biological monitoring.

MATERIALS AND METHODS

Study population

Thirty healthy men from the city of Tehran, Iran, were enrolled in the study. The study population consisted of 10 traffic policemen engaged in traffic control and 10 gas station workers in five districts with medium to high traffic level, selected on the basis of traffic flow data recorded by the Regional Agency for Environmental Protection. For the measurement of any background levels originating from other activities such as car refueling, urine samples were collected from 10 occupationally non-exposed persons in the same districts acted as referents. All of the subjects were men between 27 and 57 (mean 41) years of age and none of them were smokers. Since there may be a significant risk of dermal exposure, it was requested from gas station workers to wear plastic gloves during the work shift.

Sampling

The exposure measurements were carried out in August 2008. Urine samples were collected at the beginning and at the end of the shift. Urine samples were stored in a cooling box until they were received by the laboratory, where they were divided into several fractions and frozen at -20°C until analysis.

Analysis of urine samples

Solid-phase microextraction (SPME) appears to be a solvent- free extraction technique that presents some of the characteristics outlined before as an attractive alternative to most of the conventional sampling techniques (Pawliszyn, 1997). This technique is based on distribution of the analytes to a fused-silica fiber coated with a stationary phase.

For the SPME determinations, a manual SPME holder and fibers were used. The fibers were conditioned at 20°C higher than desorption temperature (230°C). Two blank injections were performed before the actual analysis. Between uses, fibers were kept sealed from ambient air by piercing the tip of the SPME needle into a small piece of septum to prevent accidental contamination. The HS-SPME parameters were determined by experiments in which some parameters kept constant and the remaining one was modified to find optimum condition.

The sampling procedure involved placing 10mL of sample into a 20 mL vial containing 0.88 g NaCl (1.5 M) and sealing with a screw-top septum containing cap. The sample was then spiked with a 50 µL of methanol containing 1µg/L of dioxane as internal standard. The vial was placed in a water bath maintained at 55±0.1 °C for 15 min to establish phase equilibrium. The vial and SPME holder were clamped into a stand that allowed the vial to be immersed in the water bath only up to the level of the liquid in the vial. The SPME needle was then inserted through the septum into the headspace so as to locate the tip of the exposed fiber approximately 0.5 cm from the top of the liquid; the fiber was allowed to equilibrate for 30 min. The fiber was then retracted, removed from the vial, and placed immediately into the injector of the GC system. The SPME holder was adjusted so that the exposed fiber tip was positioned about halfway (1.5 in.; 1 in.=2.54 cm) into the gas chromatography (GC) injector port liner when extended from the protective needle. Injection was accomplished by extending the fiber in the heated inlet for 4.5 min, and the splitter was opened after 3 min. The additional 1.5 min of exposure time in the injector port allowed the fiber to be cleaned of any compounds that were not desorbed in the initial 3 min.

Preliminary studies indicated that the above procedure allowed for reproducible, quantitative transfer of MTBE into the injector port of the GC system. Blank samples containing internal standards were run at the beginning and at the end of the sample queue. Triplicates of each sample were extracted by the SPME technique (Rastkari *et al.,* 2009).

The instrument used for GC-MS analysis was provided from Agilent Technologies, Palo Alto, CA, USA- 6890, plus gas chromatograph equipped with a 5973 mass selective detector quadrupole mass spectrometer.

The gas chromatograph was fitted with a DB-1 capillary column (60 m, 0.25 mm id, 0.25 μm film thickness). The instrumental temperature program was as follows: injector temperature=230°C; initial oven temperature=40°C (held for 6 min), increased to 200°C at a rate of 30°C/min and held for 1 min. The total time required for one GC-Mass spectrometry (GC-MS) run was approximately 12 min. The inlet was operated in splitless mode. The mass selective detector was operated in electron impact (EI) mode using selected ion monitoring (SIM). The dwell time of each ion was set at 100 ms. The GC conditions were selected to minimize the time of analysis while allowing all analytes to elute in acquisition groups containing suitable number of ions for monitoring (Table 1). The limits of quantification (LOQ) values for all compounds were 20 ng/L.

Statistics

Mean urinary concentration of MTBE, TAME and ETBE among three groups (gas station workers, policemen and occupationally non-exposed persons) were analyzed and because the distribution of data was not normal, the analysis was carried out by means of two statistical procedures: analysis of variance (one- way ANOVA) followed by Scheff's post hoc test and Kruskal-Wallis test. Results were expressed as mean ± S.D. and 95% confidence intervals. The level of significance was set to 0.05 and p values >0.05 were assumed to be nonsignificant.

Table 1. Selected ions and time windows used for detection of the analytes in SIM mode.

Ion groups	Analyte	Time window (min)	Selected Ions(m/z)
1	MTBE	5.9-7	57,73
2	ETBE	7-8.4	59,87
3	TAME	8.4-11	73,87,58
3	Dioxane	8.4-11	73,87,58

RESULTS

Tables 2 and 3 show the results of the mean urinary concentration of MTBE, ETBE and TAME determinations (ng/L) in the three groups of workers in two different samples collected: before starting work and at the end of the shift. The urinary MTBE concentration of subjects with jobs associated with exposure to gasoline (gas station workers and policemen) before starting the work were compared with the no or low exposure group.

The mean MTBE concentration in gas station workers was significantly greater than that the other groups. Although the mean levels of TAME and ETBE before the work shift in gas station workers and policemen were higher than the occupationally non-exposed group, a statistically significant difference could not be observed (p value equal to 0.243 and 0.082, respectively). The total MTBE uptake during the work shift in gas stations workers, policemen and occupationally non-exposed group were calculated to be on average 15442, 13724 and 247 ng/L, respectively.

The concentration of MTBE increased during the day for all three groups. The increase is more marked among gas station workers and policemen. As can be seen in Table 2, excretion of TAME and ETBE in gas station workers after the work shift were significantly higher than the policemen and occupationally non-exposed group ($p<0.001$). The concentration of TAME and ETBE in gas station workers increased during the day and reached 8655 and 4584 ng/L, respectively.

Table 2. Mean urinary concentration (S.D.) of MTBE, TAME and ETBE in three groups (n = 10) before starting work.

Groups	MTBE (ng/L)	ETBE (ng/L)	TAME (ng/L)
Gas station workers	1194 (835)	271 (473)	478 (748)
Policemen	735 (811)	35 (111)	324 (689)
Occupationally non-exposed persons	50 (105)	0 (0)	60 (105)
p value*	0.003	0.082	0.243
p value**	0.000	0.000	0.000

* one-way ANOVA
** Kruskal-Wallis test

Table 3. Mean urinary concentration (S.D.) of MTBE, TAME and ETBE in three groups (n = 10) at the end of work shift.

Groups	MTBE (ng/L)	ETBE (ng/L)	TAME (ng/L)
Gas station workers	16636 (8051)	4584 (3226)	8655 (4989)
Policemen	14458 (4946)	345 (384)	1472 (2551)
Occupationally non-exposed persons	324 (540)	11 (18)	59 (7)
p value*	0.000	0.000	0.000
p value**	0.000	0.000	0.000

* one-way ANOVA
** Kruskal-Wallis test

DISCUSSION

The aim of this study was to monitor the degree of chronic and acute exposure to oxygenated ethers (MTBE, ETBE and TAME) of three groups of workers (gas station workers, policemen and occupationally non-exposed persons) through the evaluation of urinary concentrations.

The gas stations workers and policemen were chosen for the study because their exposure was expected to be relatively high and there were few reports in literature on the biological monitoring of coincident gasoline components in urine samples from these groups. With regard to the choice of biomarkers (the hydrocarbon compounds, the ethers and tertiary alcohols) some pervious gasoline studies (Saarinen *et al.,* 1998; Hakkola *et al.,* 2001; Vainiotalo *et al.,* 2006) showed a good correlation between their airborne exposure concentrations and the biological results.

In general, the advantage of using unmetabolized compounds as biomarkers is that the urinary concentration of the unmetabolized substance is less influenced by inter individual metabolic differences than the urinary disposition of corresponding metabolites (Ghittori *et al.,* 2005; Chang lee *et al.,* 2002).

For the assessment of exposure to gasoline blends in which oxygenates constitute a certain percentage, the urinary concentrations of ethers

(MTBE, TAME and ETBE) can be used as an indicator of a day's (acute) exposure, where as the urinary concentration of ethers before the one day shift allows the monitoring of repeated (chronic) exposure. A significant difference was observed in MTBE concentrations in both pre and post-shift samples among job categories ($p<0.05$ by ANOVA and Kruskal-Wallis test). The mean MTBE concentrations in exposed workers (gas station workers and policemen) was significantly greater than that of no or low exposure group. The total oxygenate (MTBE, TAME and ETBE) content in gasoline was 11-13% (EFOA, 2006). Since MTBE is more volatile than TAME and ETBE, airborne MTBE usually occurs at higher concentrations than TAME and ETBE. In accordance, the concentrations of TAME and ETBE detected in urine of the workers were clearly lower than the parallel MTBE concentrations, even if TAME is the main component in oxygenate mixture (Vainiotalo *et al.,* 2006).

Since there might be a significant risk of dermal exposure, the gas station workers were requested to wear plastic gloves during the work shift, so no significant skin exposure occurred during the study. Oxygenates urinary levels of occupationally non-exposed group were similar to the ones asserted in the literature (Vainiotalo *et al.,* 1998). The MTBE level detected in this study was more than those obtained for tank lorry drivers (Vainiotalo *et al.,* 1998) and oil depot workers (Hakkola *et al.,* 2001), whereas it is much lower than levels which are reported for pump repair workers (Vainiotalo *et al.,* 2006). In comparison with a similar study which reported the level of MTBE in Italian workers employed in service stations (Ghittori *et al.,* 2005), greater levels of the ethers were found in Iranian gas station workers. Gas station workers and policemen were found to be probably the most exposed groups in this study. Inhalation is presumably the main route of exposure in gas station workers and policemen.

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