Int Arch Occup Environ Health
DOI 10.1007/s00420-011-0687-7

ORIGINAL ARTICLE

(2-Methoxyethoxy)acetic acid: a urinary biomarker of exposure for jet fuel JP-8

Clayton B'Hymer · Patricia Mathias · Edward Krieg Jr. · Kenneth L. Cheever · Christine A. Toennis · John C. Clark · James S. Kesner · Roger L. Gibson · Mary Ann Butler

Received: 1 March 2011 / Accepted: 19 July 2011

Abstract
Purpose To demonstrate the utility of the urinary metabolite (2-methoxyethoxy)acetic acid (MEAA) as a biomarker of exposure. 2-(2-methoxyethoxy)ethanol [diethylene glycol monomethyl ether] is an anti-icing agent used in the formulation of JP-8, and it is added at a known uniform 0.1% (v/v) concentration to each batch lot. JP-8 is a kerosene-based fuel containing different compounds that vary in the content of every batch/lot of fuel; thus, MEAA has the potential to be a more specific and a consistent quantitative biomarker for JP-8 exposure.
Methods MEAA was used to measure exposure of jet propulsion fuel 8 (JP-8) in United States Air Force (USAF) personnel working at six airbases within the United States. Post-shift urine specimens from various personnel including high ($n = 98$), moderate ($n = 38$), and low ($n = 61$) exposure workgroup categories were collected and analyzed by a gas chromatographic-mass spectrometric test method. The three exposure groups were evaluated for the number per group positive for MEAA, and a statistical analysis consisted of pair-wise t-tests for unequal variances was used to test for the differences in mean MEAA concentrations between the exposure groups.
Results The number of samples detected as positive for MEAA exposure, that is, those above the test method's limit of detection (LOD = 0.1 μg/ml), were 92 (93.9%), 13 (34.2%), and 2 (3.3%) for the high, moderate, and low exposure workgroup categories, respectively. The mean urinary MEAA level was significantly greater in the high exposure category (6.8 μg/ml), compared to the moderate (0.42 μg/ml) and the low (0.07 μg/ml) exposure categories. The maximum concentration of urinary MEAA was 110 μg/ml for the high exposure category, while 4.8 μg/ml and 0.2 μg/ml maximum levels were found in the moderate and low exposure categories, respectively.
Conclusion This study demonstrated that urinary MEAA can be used as an accurate biomarker of exposure for JP-8 workers and clearly distinguished the differences in JP-8 exposure by workgroup category.

Keywords Urinary biomarkers · Jet propulsion fuel 8 · JP-8 · (2-methoxyethoxy)acetic acid · MEAA

Disclaimers The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health (NIOSH) or the Centers for Disease Control and Prevention (CDC). Mention of company names and/or products does not constitute an endorsement by NIOSH.

C. B'Hymer (✉) · P. Mathias · E. Krieg Jr. · K. L. Cheever · C. A. Toennis · J. C. Clark · J. S. Kesner · M. A. Butler
U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, Division of Applied Research and Technology, Taft Laboratory, 4676 Columbia Parkway, Cincinnati, OH 45226, USA
e-mail: cbhymer@cdc.gov

R. L. Gibson
Preventive Medicine and Biometric Department, Uniform Services University, Room A1040H, 4301 Jones Bridge Road, Bethesda, MD 20814, USA

Introduction

Jet propulsion fuel 8 (JP-8) is the main battlefield fuel for all military operations in the United States Armed Forces and represents the single largest chemical exposure in Department of Defense personnel. JP-8 is the major jet fuel used throughout the air forces of the North Atlantic Treaty Organization (NATO) countries as well, which collectively

Published online: 02 August 2011

consume an estimated twenty billion liters per year (Zeiger and Smith 1998; NRC 2003). JP-8 has been recognized as a significant source of chemical exposure, both by inhalation and dermal routes, for aircraft fuel-tank maintenance workers, their assistants, and aircraft fuel handlers. Complaints by aircraft ground crew members have included skin irritation, dizziness, and the persistence of the taste of jet fuel hours after work shift exposure. Exposure to JP-8, either by inhalation or dermal contact, occurs commonly during aircraft fueling and especially during fuel-tank or fuel-cell maintenance, when workers are required to work inside the tank or cell.

Possible health effects of acute exposure to JP-8 vapors have included immune, neurological, and reproductive system effects as well as respiratory problems (ATSDR 1998; NRC 2003; Zeiger and Smith 1998). Human epidemiological studies have reported that individuals exposed to jet fuel sometimes do poorly on neurobehavioral tests (Knave et al. 1978). Chronic exposure to jet fuel and its effect on postural balance has been studied (Smith et al. 1997), and the health effects of kerosene-based jet fuels have been reviewed in the literature (Ritchie et al. 2003). A comprehensive review of the toxicity of jet fuel has been recently published (Mattie and Sterner 2011). Although systemic dermal toxicity by JP-8 fuel components is considered unlikely (McDougal et al. 2000), correlations between dermal exposure and urinary naphthols have been reported (Chao and Nylander-French 2004). Dermal exposure and toxicity is a concern owing to the fact that some of the components contained in JP-8 have been reported to cause skin cancer in mice (NTP 1986). A subchronic dermal study of JP-8 using rats demonstrated that proliferative, degenerative, and inflammatory changes were significantly greater in the fuel-exposed skin versus non-exposed control skin sites on the same animal (Baker et al. 1999). Although there are few systemic toxicity studies from skin exposure to JP-8, one study with mice, where the animals were dosed three times a week, (41.5 mg/kg per day) resulted in changed organ and body weights (Schultz et al. 1981). A review of cutaneous exposures concluded that JP-8, like most kerosene-based fuels, causes skin irritation with prolonged or repeated contact to the skin (McDougal and Rogers 2004).

JP-8 is a kerosene-based complex chemical mixture containing hundreds of aliphatic and aromatic hydrocarbons along with various isomer forms plus several additives (Ritchie et al. 2003). Because the fuel is formulated to meet military specifications for performance, the overall chemical composition may vary from batch to batch (NRC 2003). Performance specifications are set for a boiling point of 260°C, a maximum olefins content of 5%, a maximum aromatic content of 22%, and the maximum amount of sulfur at 0.3%. On average, the composition is approximately 33–61% alkanes, 10–45% naphthenes, 12–22% aromatics, and 0.5–5% olefins (Vere 2003). This makes JP-8 a scientific challenge with respect to choosing an appropriate biomarker of exposure, which is both specific and having a concentration for relating a measurable exposure. Exposure to JP-8 has been evaluated in the past using a variety of measures. These have included breathing zone and exhaled breath measurement of benzene and naphthalene (Egeghy et al. 2003), the JP-8 fingerprint of volatile organics in end-exhaled breath (Pleil et al. 2000), dermal exposure using a tape-stripping technique (Chao and Nylander-French 2004; Chao et al. 2005; Kim et al. 2006; Mattorano et al. 2004; Smith et al. 2010), and the measurement of urinary metabolites of naphthalene (Chao et al. 2006; Serdar et al. 2003; Serdar et al. 2004). Because naphthalene, benzene, and toluene are encountered in other fuels and house-hold products, and benzene urinary metabolites are found in those who smoke, a more specific biomarker of JP-8 was preferred for this study. Also, a direct biomarker of exposure was desired for the current study, rather than an indirect method such as tape-stripping or work environment monitoring.

Although the compound 2-(2-methoxyethoxy)ethanol [also known as diethylene glycol monomethyl ether (DiEGME) and under the trade name of methyl carbitol™, CAS 111-77-3] has a limited number of other industrial uses, it is used as an anti-icing agent in the formulation of JP-8 at a fixed concentration of 0.1% (v/v) (NRC 2003). This glycol ether is encountered much less frequently in the general environment, whereas toluene, benzene, and naphthalene are commonly used in fuels and many home products. The corresponding metabolite of 2-(2-methoxyethoxy)ethanol is (2-methoxyethoxy)acetic acid (MEAA). MEAA was selected for use as a biomarker of exposure for this study for a number of reasons. MEAA has been shown to be the urinary metabolite best suited for use as a short-term biomarker for exposure to 2-(2-methoxyethoxy)ethanol in mice (Richards et al. 1993). Additionally, urinary MEAA has been demonstrated to be an accurate biomarker of exposure in mice by both oral and dermal administration of JP-8 (B'Hymer et al. 2005b). The metabolism of 2-(2-methoxyethoxy)ethanol is complex, but a simplified illustration based on animal studies (Cheever et al. 1988; Sumner et al. 1992) is shown in Fig. 1. In general, 2-alkylethanol compounds are rapidly metabolized via alcohol dehydrogenase (ADH) to the corresponding alkoxyacetic acids, although some alkoxyacetic acids can be further metabolized by various mechanisms to glycine, sulfate, glutamate, and glucuronide conjugates of alkoxyacetic acids (Sumner et al. 1992). Although MEAA can be further metabolized by means of acyltransferase and dealkylase carboligase, MEAA is the predominate metabolite, and urinary levels are fairly abundant. In a previous study from this laboratory, male rats dosed with ^{14}C- labeled

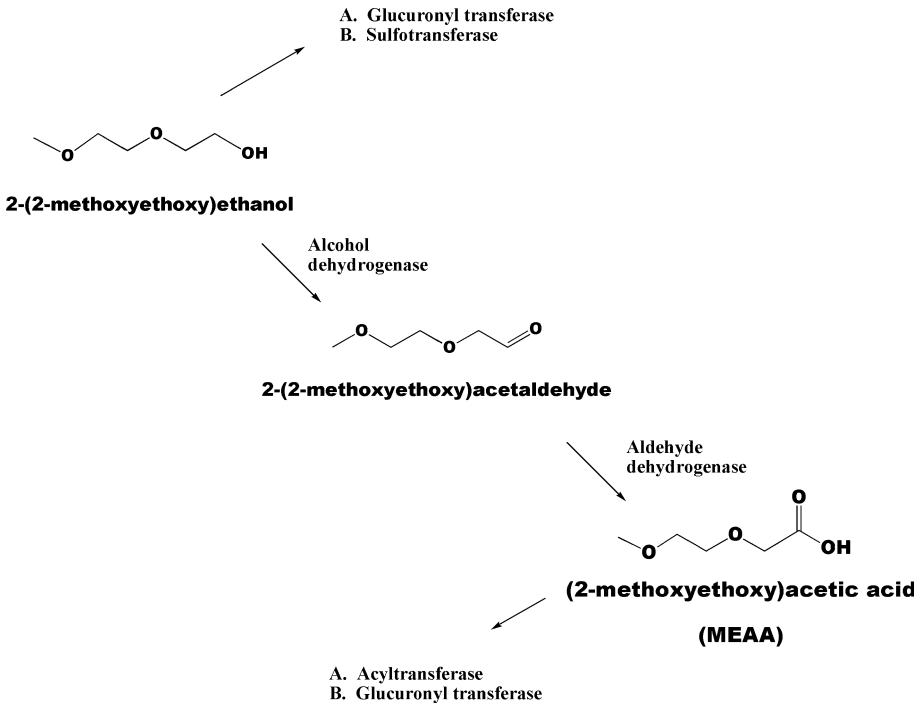

Fig. 1 Metabolic formation of (2-methoxyethoxy) acetic acid (MEAA) from the glycol ether 2-(2-methoxyethoxy)ethanol, which is used as an anti-icing agent in JP-8. MEAA is the major metabolite as determined in animal studies (Sumner et al. 1992; Cheever et al. 1988), and it is the biomarker of exposure to JP-8 for this study

2-(2-methoxyethoxy)ethanol were found to excrete 68–70% of the label as the MEAA metabolite by means of ADH conversion (Cheever et al. 1988). Only trace levels of 2-(2-methoxyethoxy)ethanol or methoxyethoxyacetaldehyde were detected in urine in this same study. Furthermore, cumulative excretion of ^{14}C-tagged compounds showed rapid 50% excretion in 8 h, during rat oral dosing studies (Cheever et al. 1988). Other animal studies have shown similar rapid conversion of 2-(2-methoxyethoxy)ethanol to MEAA (Daniel et al. 1991; Richards et al. 1993), making it a suitable acute biomarker of exposure for JP-8. Additionally, MEAA was an ideal candidate for use as a biomarker, since unpublished results from this laboratory demonstrated that urinary MEAA is not readily conjugated from dosed rat urine; the majority of the metabolite is in the free form. Hydrolysis of dosed rat urine by the use of glucuronidase and sulfotransferase did not appreciably increase the amount of free MEAA. Therefore, MEAA represents a viable target analyte for analytical quantitation.

Materials and methods

Study participants

This study was conducted on six USAF bases located within the continental United States housing various aircrafts including F-15 fighter and C-130 transport aircraft. The participants were recruited with jobs rated as high, moderate, or low potential for JP-8 exposure. Aircraft fuel–system maintenance workers, that is, those whose jobs included fuel-cell "entrant," "attendant", or "runner," were assigned to the high exposure group. The "entrant" had the job of actually entering the aircraft fuel tank or fuel cell wearing a supplied air respirator. The "attendant" assisted the entrant and stayed outside the tank at all times. The "runner" stayed outside the tank and handled fuel-soaked foam that had been removed from the tank to a storage location or obtained tools for the "entrant." Protective equipment was limited to forced air masks, gloves, and cotton overalls for the "entrant"; thus, the major route of JP-8 exposure was dermal, not by inhalation. The moderate exposure group consisted of personnel who did not perform fuel-tank maintenance, but conducted work that involved regular contact with jet fuel such as fueling aircraft, maintaining fuel storage facilities, or other fuel handling operations. The low exposure group consisted of workers whose jobs covered a wide variety of activities that did not require exposure to the jet fuel on the air force bases; examples of this group included medical technicians, base administrative personnel, military police, and similar positions. The low exposure group may be considered the control group for the nature of this study. The mean age of the participants was 25 [$\sigma = \pm 5$] years. The participants were >90% White and African, in ethnic background, and <10% Asian or other. The Human Subjects Review Board at NIOSH, as well as the institutional review boards of all participating agencies and investigators involved in other aspects of this study, approved the study protocol.

Collection of urine samples

Urine samples were collected from participants in the low ($n = 61$), moderate ($n = 38$), and high ($n = 98$) groups at the end of a 4-h work shift for urinary measurements of MEAA. The work shift was limited to the morning hours only, owing to the warm climate and warm season of the air bases at the time of this study. Subjects were excluded from this study if they consumed alcohol within 24 h of urine collection. The urine samples were immediately cooled by Blue Ice® (Rubbermaid, Atlanta, Georgia, USA) to maintain a temperature of 4°C and then shipped to the laboratory within 24 h. In the laboratory, all samples were aliquoted and stored at −80°C until analysis.

Chemicals and reagents

Standard compounds of (2-methoxyethoxy)acetic acid (MEAA, CAS no. 16024-59-9) and the deuterated 2-butoxyacetic acid (d-BAA) used as an internal standard were synthesized and described previously (Cheever et al. 1988; Brown et al. 2003). All other reagents used were analytical grade and are regularly available for laboratory use.

MEAA analysis

Urinary MEAA levels were measured using a method that was validated and previously described in the literature (B'Hymer et al. 2003, 2005a). Briefly, a 4-ml aliquot of urine is acidified with 12 N hydrochloric acid to pH 1–1.5 and spiked with deuterated 2-butoxyacetic acid (d-BAA) to act as an internal standard. The MEAA and internal standard are extracted by liquid–liquid extraction (LLE) using ethyl acetate, and the extraction solvent is evaporated to a 1-ml volume. Ethanol and concentrated sulfuric acid are used to react with the target analytes to form the corresponding ethyl esters. These products are extracted by LLE using methylene chloride, and the methylene chloride solvent is reduced to a 1-ml volume by evaporation. Standard MEAA is used to spike unexposed urine at various levels and treated similarly to create the calibration curves for quantitation. A gas chromatograph (GC) equipped with a mass spectrometric detector and an HP-1 capillary column (Agilent Technologies, Santa Clara, California, USA) was used to analyze the sample and standard solutions. The limit of detection (LOD) for this method was 0.1 μg MEAA per ml of urine. The analysis of each urinary extract was conducted using duplicate sample injections into the gas chromatograph. A methylene chloride blank injection run was used in between all sample injections to avoid any possibility of injection carryover of the MEAA ethyl ester. The accuracy and precision of this method was determined to be 99% with a coefficient of variation 8.6% or less at the time of its validation (B'Hymer et al. 2003). Spiked control samples run at the time of the analysis were within 10% of theory.

Creatinine determination

Separate aliquots of the urine samples were diluted 1:20 to measure creatinine concentrations using a Vitros 250 Chemistry Analyzer (Ortho-Clinical Diagnostics, Rochester, New York, USA), which employs a slide composed of a dry, multilayered analytical element that includes a leuco dye coated on a polyester support (Findlay et al. 1985; Mauck et al. 1986). Creatinine measurements were calibrated with a 3-level set of standards, the highest being 17 mg/dl, which corresponds to a creatinine concentration of 340 mg/dl for urine samples diluted 20-fold. Urine control pools (Bio-Rad Laboratories, Hercules, California, USA) and instrument Vitros Performance Verifier pools (Ortho-Clinical Diagnostics) were assayed in duplicate at the front, middle, and rear of each analytical run. Control pool within and among test run coefficients of variation were <2.5 and 1.25%, respectively. Coefficients of variation for all sample duplicate values were <10%.

Statistical analysis

Pair-wise t-tests for unequal variances (Cochran and Cox 1950) were used to test for differences in mean MEAA concentrations between the exposure workgroups. Analyses of the concentrations were performed both unadjusted and adjusted for creatinine. Measurements below the limit of detection (LOD, 0.1 μg/ml) were assigned a value of the limit of detection divided by the square root of two. Hornung and Reed (1990) recommend this method for data that are lognormally distributed and not highly skewed. All calculations were made using SAS® (Version 9.2, SAS Institute, Inc., Cary, North Carolina, USA).

Results

The mean urinary post-shift MEAA level was much greater in personnel in the high exposure category as well as having a higher frequency of results above the limit of detection, which is the most significant result of this study. This data is summarized in Table 1. The percentage of measurements greater than the limit of detection (0.1 μg/ml) was 93.9% (92 samples), 34.2% (13 samples), and 3.3% (2 samples) for the high, moderate, and low exposure categories, respectively. The high exposure category had a mean value of 6.8 μg/ml (standard deviation (SD) = 15.5 μg/ml); the moderate had a mean of 0.42 μg/ml (SD = 0.89 μg/ml),

and the low had a mean of 0.07 μg/ml (SD = 0.02 μg/ml). The maximum concentration of urinary MEAA for individuals within each exposure category was 110 μg/ml, 4.8 μg/ml, and 0.2 μg/ml for the high, moderate, and low JP-8 exposure groups, respectively (see Fig. 2). It should also be noted in Table 1 that means and the standard deviations for the low and medium work groups are biased owing to the substitution process of using 0.07 μg/ml for the values below the limit of detection. When values below the LOD (0.1 μg/ml) are replaced by the LOD divided by the square root of two (0.07 μg/ml), the maximum absolute bias of an individual measurement is 0.07 μg/ml. The maximum absolute bias of the mean occurs when all the values below the limit of detection are equal to zero. Estimates of the maximum absolute bias of the mean for the low, medium, and high groups are 0.07, 0.05, and 0.00 μg/ml, respectively. In this case, all zeros, estimates of the bias of the standard deviation for the low, medium, and high groups, are −0.0127, −0.0196, and −0.0019, respectively, and estimates of the bias of the variance for the low, medium, and high groups are −0.000751, −0.035205, and −0.059584, respectively, and thus, are underestimated.

For the statistical comparison of the mean MEAA levels of the exposure workgroup categories, participants with measurements below the limit of detection were assigned value of 0.07 μg/ml [the LOD divided by the square root of two] for the statistical comparison between workgroups. Hornung and Reed (1990) recommend this method for data that are lognormally distributed and not highly skewed. Although other methods of how to report non-detects in science have been reviewed in the literature (Helsel 2010), the method chosen is appropriate for statistical comparison of the exposure categories done in this study. Table 1 also displays the urinary creatinine-adjusted dose data; this data showed similar statistical results for the high, moderate, and low exposure categories. The individual MEAA concentrations (μg/ml) per exposure category are represented graphically in Fig. 2. [It should be noted that the MEAA levels in the high exposure workgroup are distributed over a very wide range; thus, the scale of Fig. 2 is based on log base 10 to more easily show the complete span of concentration levels]. Results of the statistical analysis of the data shown in Table 1 were similar for MEAA concentrations, whether dose adjusted or not dose adjusted for creatinine. For MEAA (μg/ml), the mean of the high exposure category was statistically significantly greater than the means of the low ($p = 0.0001$) and the moderate ($p = 0.0001$) categories. The mean of the moderate exposure category was statistically significantly greater than the mean of the low category ($p = 0.0193$). Similarly, for MEAA levels adjusted for creatinine (μg/mg creatinine), the mean of the high category was statistically greater than the means of the low ($p = 0.0001$) and the moderate ($p = 0.0001$) categories. The mean of the moderate category was statistically significantly greater than the mean of the low category ($p = 0.0151$).

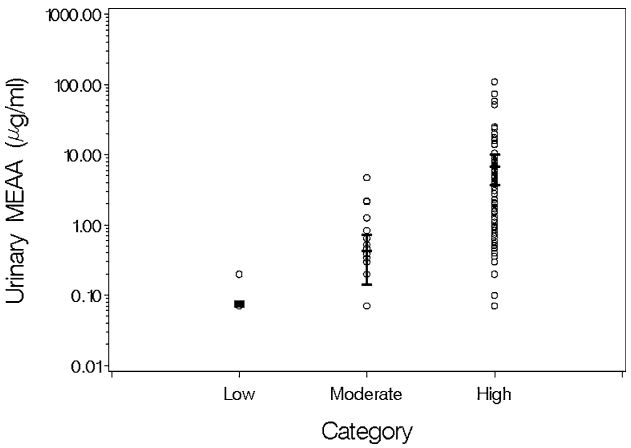

Fig. 2 Comparisons of urinary MEAA levels in low, moderate, and high jet fuel exposure work group categories. MEAA concentrations are in μg/ml on a $\log_{10}$ scale

Discussion

Urinary biomarkers have been widely used for assessing exposure from inhalation and dermal exposure routes. Urinary 1- and 2-naphthols have previously been used as biomarkers for JP-8 exposure (Chao et al. 2006; Serdar et al. 2003, 2004). Metabolites of the aromatic compounds benzene and toluene have also been used to assess

Table 1 Post-shift MEAA urinary measurements of U.S. Air Force Personnel

Exposure Group	*n*	MEAA (μg/ml)					MEAA (μg/mg creatinine)			
		Mean	SD	Min	Max	*n* > LOD (%)	Mean	SD	Min	Max
Low	61	0.07	0.02	0.07	0.2	2 (3.3)	0.08	0.07	0.02	0.30
Medium	38	0.42	0.89	0.07	4.8	13 (34.2)	0.26	0.44	0.02	2.3
High	98	6.8	15.2	0.07	110	92 (93.9)	2.9	6.4	0.02	45

SD standard deviation, *Max* maximum detected concentration, *Min* minimum concentration or 0.07 μg/ml, *LOD* limit of detection (0.1 μg/ml), samples less than the LOD are coded as 0.07 μg/ml = LOD divided by the square root of 2 (see the text for a full explanation)

exposure (Li et al. 2006; Maestri et al. 2005; Manini et al. 2006; Marchese et al. 2004). However, these compounds are encountered in the general environment through household products, cigarette smoke, fuels, and other products or factors outside the work environment. The metabolite MEAA is specific for 2-(2-methoxyethoxy)ethanol, which is blended at a fixed level in the formulation of jet fuel and is less likely to be encountered outside the work environment. These two factors, plus the high abundance of the urinary MEAA formed through the metabolic process (Fig. 1), made MEAA a good biomarker of exposure for this study.

A well-chosen biomarker of exposure should have several qualities, and these qualities have been discussed elsewhere in the literature (B'Hymer and Cheever 2010). One is that a well-chosen biomarker should be specific for the exposure of interest, and MEAA reasonably fits that criterion. Second, a good biomarker should be easily detectable at levels to distinguish exposure group categories. The high exposure category from this study had significantly higher levels of MEAA. Statistically, the means of the high, moderate, and low exposure categories differ significantly from each other, as described in the results section of this work. The test used in this study appears to have adequate MEAA sensitivity for its use as a biomarker of exposure, even for the low exposure work category. MEAA, therefore, is clearly associated with the type of workgroup and has the potential for good predictive value, a desirable characteristic of a well-chosen biomarker of exposure. Also, the GC/MS test (B'Hymer et al. 2003, 2005a) is relatively inexpensive to use; liquid–liquid extraction was used with low cost solvents, and acid-catalyzed esterification avoided expensive chemical derivatization. Finally, urinary MEAA testing is non-invasive, a positive attribute for measuring any biomarker of exposure. Most participants are not as reluctant to donate urine samples versus blood or tissue samples, which can increase participation rates during field studies. This, in turn, can lead to the accomplishment of more meaningful field studies.

Since there appears to be such good predictive value of urinary MEAA and such good sensitivity of the MEAA measuring test used during this study, MEAA would be a useful biomarker to evaluate the effectiveness of engineering controls and personal protective equipment in future intervention studies. Fuel-cell maintenance workers encounter an environment of highly concentrated fuel vapors in a confined space, as well as have direct skin contact with residual liquid fuel. In addition to their supplied air and other respirators used to minimize inhalation exposure, the fuel-cell worker wore cotton overalls during some of their operations at the time of this study. The cotton overalls were worn to avoid generation of static electrical sparks and reduce the risk of fire or explosion. Unfortunately, the wicking properties of cotton tend to enhance dermal exposure and adsorption of JP-8; this can lead to erythema and irritation of the skin. JP-8 has been demonstrated to have significant dermal uptake in mice (B'Hymer, et al. 2005b) and represents a significant health concern. Therefore, personal protective equipment changes, especially in clothing materials and the type of clothing used, could easily be evaluated in future studies by measuring urinary MEAA.

There are some obvious limitations for the use of MEAA as a biomarker of exposure to JP-8. As was previously noted in this work, the metabolic conversion of 2-(2-methoxyethoxy)ethanol to MEAA is fairly rapid; the half-life was determined to be approximately 8 h in various species from previously reported studies (Cheever et al. 1988; Daniel et al. 1991; Richards et al. 1993). MEAA, therefore, represents a biomarker of acute exposure to JP-8. Also, MEAA only represents a metabolite of an additive component of JP-8; it does not represent a metabolite of any of the other toxic components within the fuel, itself, and it has little clinical value for an individual. Since the rate of skin penetration would be different between 2-(2-methoxyethoxy)ethanol and the various toxic components of JP-8, it could not be used to assess the exposure of those other components of JP-8 to the individual worker. In other words, MEAA is a direct marker of exposure to 2-(2-methoxyethoxy)ethanol, a compound added consistently at a 0.1% (v/v) level in each batch of JP-8, and MEAA is an indirect marker of exposure to the other components of JP-8. Finally, MEAA production is based upon alcohol dehydrogenase (ADH) conversion metabolism. In the current study, over 90% of the participants were ethnic White or African and had not consumed alcohol 24 h before testing. The results, therefore, were not significantly biased on ethnic metabolisms or drinking habits, which could be a problem for either some ethnic backgrounds or lifestyles. Individuals with reduced ADH metabolism would not be useful for use in a MEAA monitored study. MEAA can obviously still be used, within the previously mentioned constraints, to assess worker practices, personal protective equipment, and engineering controls for workers exposed to JP-8.

Both the results of MEAA concentration in urine and those adjusted for creatinine show the statistically similar results for the high, moderate, and low exposure category workers. The statistical analysis showed that the mean of the high category was significantly greater than the means of the low and the moderate levels, when non-adjusted or adjusted for creatinine ($p = 0.0001$ for all cases). The mean of the moderate category was significantly greater for both non-adjusted MEAA concentration ($p = 0.0193$) and the adjusted concentration for creatinine ($p = 0.0151$). This was not an unexpected result; the use of creatinine to

normalize analyte concentrations in urine has been extensively reported to not necessarily improve the correlation of dose to exposure for many urinary components (Alessio et al. 1985; Boeniger et al. 1993; Carrieri et al. 2001). Therefore, the use of MEAA for a biomarker of exposure for JP-8 is valid either as concentration in urine with or without the creatinine adjustment. Although other researchers (Gaines et al. 2010) have suggested that other urine level normalization schemes are better than creatinine adjustment, the differences in the MEAA levels between exposure workgroup categories are so large that it would not change the statistical significance of this study. Some additional comments about the statistics used in this presentation and its obvious limitations are necessary. The low and medium work groups had a large proportion of results below the LOD of the test method (0.1 μg/ml). In this study, values below the LOD were replaced by the LOD divided by the square root of two (0.07 μg/ml), and the maximum absolute bias of an individual measurement would be 0.07 μg/ml. The maximum absolute bias of the mean occurs when all the values are below the LOD. Estimates of the maximum absolute bias of the mean for the low, medium, and high groups are 0.07, 0.05, and 0.00 μg/ml, respectively, for our presentation as described in the results section. Therefore, the most significant data described in Table 1 are the results of the groups with individuals (n) with MEAA above the LOD.

Future work with MEAA and JP-8 is ongoing at this laboratory and is beyond the scope of this manuscript. Planned future work is in two areas. Firstly, other common biomarkers of exposure for toluene, benzene, and naphthalene will be used for comparison to MEAA urinary levels. Again, the lack of specificity for JP-8 exposure for metabolites of these other chemicals due to their prevalence in the general environment should make MEAA the better choice. Future studies would have to take this factor into account. Finally, and more importantly, future studies involving the use of urinary MEAA levels to evaluate personal protective equipment are planned. At the time of this study, cotton overalls were used that did not adequately protect the high exposure group from dermal contact with JP-8. Currently, the US Air Force is evaluating suits of various materials and designs to minimize dermal exposure by the fuel cell "entrants".

Conclusions

This laboratory observed that urinary MEAA levels were significantly elevated in USAF personnel who worked in potentially high exposure workgroup categories involving jet fuel JP-8. MEAA represents a more specific biomarker of JP-8 exposure than other urinary metabolites previously used in other studies, since the fuel is consistently formulated at a 0.1% (v/v) level with the parent compound, 2-(2-methoxyethoxy)ethanol. Mean MEAA levels were demonstrated to distinguish between low, moderate, and high exposure categories of the workers tested during this study. The percent of measurements as positive for MEAA [greater than the test method's limit of detection (LOD = 0.1 μg/ml)] was 93.9, 34.2, and 3.3% for the high, moderate, and low exposure categories, respectively. Urinary creatinine adjustment did not affect the predictive capability of MEAA as a biomarker of JP-8 exposure.

Acknowledgments The authors would like to thank the Department of Defense and the United States Air Force for their cooperation and help with this study. The authors would also like to thank Mr. Dennis Lynch, Dr. Anne Vonderheide, Dr. Jeanette Krause, and Dr. David Mattie for their help in reviewing and editing this manuscript.

Conflict of interest The authors declare that they have no conflict of interest and receive no benefits in any form from a commercial party directly or indirectly to the subject of this manuscript.

References

Alessio L, Berlin A, Dell'Orto A, Toffoletto F, Chezzi I (1985) Reliability of urinary creatinine as a parameter used to adjust values of urinary biological indicators. Int Arch Occup Env Health 55:99–106

ATSDR (1998) Toxicological profile for jet fuels (JP-5 and JP-8). U.S. Department of Health and Human Services, Agency for Toxic Substances and Disease Registry, Atlanta, Georgia:

B'Hymer C, Cheever KL (2010) Biomarkers and metabolites: HPLC/MS analysis. In: Cazes J (ed) Encyclopedia of chromatography, 3rd edn. CRC Press/Taylor & Francis, Boca Rotan, Florida, pp 238–246

B'Hymer C, Cheever KL, Butler MA, Brown KK (2003) Procedure for the quantification of the biomarker (2-methoxethoxy)acetic acid in human urine. J Chromatogr A 795:145–150

B'Hymer C, Butler MA, Cheever KL (2005a) A comparison and evaluation of analysis procedures for the quantification of (2-methoxyethoxy)acetic acid in urine. Anal Bioanal Chem 383:201–209

B'Hymer C, Keil DE, Cheever KL (2005b) A test procedure for the determination of (2-methoxyethoxy)acetic acid in urine from jet fuel-exposed mice. Toxicol Mech Methods 15:367–373

Baker W, Miller T, Dodd D, McDougal J. (1999) Repeated dose skin irritation study on jet fuels—a histopathology study. Operational Toxicology Branch, Wright-Patterson AFB, OHIO, AFRl-HE-WP-TR-1999-022

Boeniger MF, Lowry LK, Rosenberg J (1993) Interpretation of urine results used to assess chemical exposure with emphasis on creatinine adjustments: a review. Am Ind Hyg Assoc J 54: 615–627

Brown KK, Cheever KL, Butler MA, Shaw P, McLaurin JL (2003) Synthesis, characterization and use of 2-[(2H9)butoxy]acetic acid and 2-(3-methylbutoxy)acetic acid as an internal standard and instrument performance surrogate, respectively, for the gas chromatographic-mass spectrometric determination of 2-butoxyacetic acid, a human metabolite of 2-butroxyethanol. J Chromatogr B 792:150–159

Carrieri M, Tevisan A, Bartolucci GB (2001) Adjustment to concentration-dilution of spot urine samples: correlation between specific gravity and creatinine. Int Arch Occup Env Health 74:63–67

Chao YE, Nylander-French LA (2004) Determination of keratin protein in a tape-stripped skin sample from jet fuel exposed skin. Ann Occup Hyg 48:65–73

Chao YE, Gibson RL, Nylander-French LA (2005) Dermal exposure to jet fuel (JP-8) in U.S. Air Force personnel. Ann Occup Hyg 49:639–645

Chao YE, Kupper LK, Serdar B, Egeghy PP, Rappaport SM, Nylander-French LA (2006) Dermal exposure to jet fuel JP-8 significantly contributes to the production of urinary naphthols in fuel-cell maintenance workers. Environ Health Perspect 114:182–185

Cheever KL, Richards DL, Weigel WW, Lal JB, Dinsmore AM, Daniel FB (1988) Metabolism of bis(2-methoxethyl)ether in the adult male rat–evaluation of the principle metabolite as a testicular toxicant. Toxicol Appl Pharmacol 94:150–159

Cochran WG, Cox GM (1950) Experimental designs. Wiley, New York

Daniel FB, Cheever KL, Begley KB, Richards DE, Weigel WW (1991) Bis(2-methoxyethyl) ether: metabolism and embryonic disposition of a development toxicant in the pregnant CD-1 mouse. Fundam Appl Toxicol 16:567–575

Egeghy PP, Hauf-Cabalo L, Gibson RL, Rappaport SM (2003) Benzene and naphthalene in air and breath as indicators of exposure to jet fuel. Occup Environ Med 60:969–976

Findlay JB, Wu AL, Knott V, Mauck L, Frickey PH, Norton GE (1985) Development of a Kodak Ektachem® clinical chemistry slide for CK-B activity. Clin Chem 31(Abstract 509):1000

Gaines LGT, Fent KW, Flack SL, Thomasen JM, Ball LM, Zhou H, Whittaker SG, Nylander-French LA (2010) Effect of creatinine and specific gravity normalization on urinary biomarker 1, 6-hexamethylene diamine. J Environ Monit 12:591–599

Helsel D (2010) Much ado about next to nothing: Incorporating nondetects in science. Ann Occup Hyg 54:257–262

Hornung RW, Reed LD (1990) Estimation of average concentration in the presence of non-detectable values. Appl Occup Environ Hyg 5:46–51

Kim D, Anderson ME, Nylander-French LA (2006) Dermal absorption and penetration of jet fuel component in humans. Toxicol Lett 165:11–21

Knave B, Olsen BA, Elofsson S, Gamberale F, Isaksson A, Mindus P, Persson HE, Struwe G, Wennberg A, Westerholm P (1978) Long-term exposure to jet fuel. II. A cross-sectional epidemiologic investigation on occupationally exposed industrial workers with special reference to the nervous system. Scand J Work Environ Health 4:19–45

Li Y, Li AC, Shi H, Junga H, Jiang H, Jiang X, Naidong W, Lauterbach JH (2006) Determination of *S*-phenylmercapturic acid in human urine using an automated sample extraction and fast liquid chromatography-tandem mass spectrometry method. Biomed Chromatogra 20:597–604

Maestri L, Negri S, Ferrari M, Ghittori S, Imbriani M (2005) Determination of urinary S-phenylmercapturic acid as a specific metabolite of benzene by liquid chromatography/single quadrupole mass spectrometry. Rapid Commun Mass Spectrom; 19:1139–1144

Manini P, Andreoli R, Mutti A (2006) Application of liquid chromatography-mass spectrometery to biomonitoring of exposure to industrial chemicals. Toxicol Lett; 162:202–210

Marchese S, Cruini R, Gentili A, Perret D, Rocca LM (2004) Simultaneous determination of urinary metabolites of benzene, toluene, xylene and styrene using high-performance liquid chromatography/hybrid quadrupole time-of-flight mass spectrometry. Rapid Commun Mass Spectrom 18:265–272

Mattie DR, Sterner TR (2011) Past, present and emerging toxicity issues for jet fuel. Toxicol Appl Pharmacol (article in press)

Mattorano DA, Kupper LL, Nylander-French LA (2004) Estimating dermal exposure to jet fuel (naphthalene) using adhesive tape strip samples. Ann Occup Hyg 48:139–146

Mauck JC, Mauck L, Novros J, Norton GE, Toffaletti J (1986) Development of a single slide Kodak Ektachem® thin-film assay for serum and urine creatinine. Clin Chem 32(Abstract 735): 1197–1198

McDougal JN, Rogers JV (2004) Local and systemic toxicity of JP-8 from cutaneous exposures. Toxicol Lett 149:301–308

McDougal JN, Pollard DL, Weisman W, Garrett CM, Miller TE (2000) Assessment of skin absorption and penetration of JP-8 jet fuel and its components. Toxicol Sci 55:247–255

NRC (2003) Toxicologic assessment of jet-propulsion fuel 8. National Research Council of the National Academies. The National Academies Press, Washington

NTP (1986) Toxicology and carcinogenesis study of marine diesel fuel and JP-5 Navy Fuel in B6C3F1 Mice (Dermal Studies). Technical Report 310. NIH Publication no. 86–2566. Research Triangle Park. National Toxicology Program, North Carolina

Pleil JD, Smith LB, Zelnick SD (2000) Personal exposure to JP-8 jet fuel vapors and exhaust at air bases. Environ Health Perspect 108:183–192

Richards DE, Begley KB, DeBord DG, Cheever KL, Weigel WW, Tirmenstein MA, Savage RE (1993) Comparative metabolism of bis(2-methoxethyl)ether in isolated rat hepatocytes and in the intact rat–effects of ethanol on in vitro metabolism. Arch Toxicol 67:531–537

Ritchie GD, Still KR, Rossi J, Bekkedal MYV, Boff AJ, Arfsten DP (2003) Biological and health effects of exposure to kerosene-based jet fuels and performance additives. J Toxicol Environ Health Part B 6:357–451

Schultz TW, Eppler JL, Witschi H (1981) Health effects research in oil shale development. Oak Ridge National Laboratory, Oak Ridge, Tennessee, ORNL/TM-8034

Serdar B, Egeghy PP, Waidyanatha S, Gibson R, Rappaport SM (2003) Urinary biomarkers of exposure to jet fuel (JP-8). Environ Health Perspect 111:1760–1764

Serdar B, Egeghy PP, Gibson R, Rappaport SM (2004) Dose-dependent production of urinary naphthols among workers exposed to jet fuel (JP-8). Am J Ind Med 46:234–244

Smith LB, Bhattacharya A, Lemasters G, Succop P, Puhala E, Medvedovic M, Joyce J (1997) Effect of chronic low-level exposure to jet fuel on postural balance of U.S. Air Force personnel. J Occup Environ Med 39:623–632

Smith KW, Proctor SP, Ozonoff A, McClean MD (2010) Inhalation exposure to jet fuel (JP-8) among U.S. Air Force personnel. J Occup Environ Hyg 7:563–572

Sumner SC, Stedman DB, Clarke DO, Welch F, Fennell TR (1992) Characterization of urinary metabolites from [1, 2-methoxyl-13C]-2-methoxyethanol in mice using 13C nuclear magnetic resonance spectroscopy. Chem Res Toxicol 5:553–560

Vere RA (2003) Aviation fuels. In: Hobson GD (ed) Modern petroleum technology, part 2. Wiley, Chinchester, pp 723–771

Zeiger E, Smith L (1998) The first international conference on the environmental health and safety of jet fuel. Environ Health Perspect 106:763–764