Urinary Biomarkers of Environmental Health: Jet Fuel

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Contents

Abstract

Urinary biomarkers of exposure have become an important tool for use in exposure assessment for occupations involving the use of jet fuel. Aircraft mechanics, fuel handlers, and workers on air bases and airports are exposed to jet fuel. The health effects of jet fuel exposure include skin irritation and dermatitis for direct skin contact and various neurophysiological problems including headache, general fatigue, and poor concentration from vapor exposure.

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A number of immunosuppressive effects have been seen after exposure to some formulations of jet fuel. The health effects of the many components of jet fuel have been described extensively in the toxicology literature. Since jet fuel is a complex mixture of chemicals, it also represents a challenge in choosing appropriate and accurate biomarkers of exposure. Urinary metabolic biomarkers are the best choice for exposure assessment; metabolites of naphthalene, benzene, and toluene are particularly well suited for accurate exposure biomarkers. (2-Methoxyethoxy)acetic acid is also well suited for JP-8 specifically owing to its unique formulation package. Jet fuel health hazards are a continuing health concern from the fuel's widespread use in modern society; thus, jet fuel urinary biomarkers of exposure are vital tools to study the occupational environment and reduce worker exposure.

List of Abbreviations

BEI	Biological Exposure Indices
BMA	*S*-Benzylmercapturic Acid
CYP2E1	Cytochrome P450, Family 2, Subfamily E, Polypeptide 1
DNA	Deoxyribonucleic Acid
FID	Flame Ionization Detector
GC	Gas Chromatography
GST	Glutathione *S*-Transferase
GT	Glucuronyl Transferase
HPLC	High-Performance Liquid Chromatography
MBMA	*S*-Methylbenzylmercapturic Acid
MEAA	(2-Methoxyethoxy)acetic Acid
MPO	Myeloperoxidase
MS	Mass spectrometry or Mass Spectrometer
NQO1	NAD(P)H Dehydrogenase, Quinone 1
PAH	Polycyclic Aromatic Hydrocarbon
PMA	*S*-Phenylmercapturic Acid
SPE	Solid-Phase Extraction
t,t-MA	*Trans,trans*-Muconic Acid

Key Facts of Jet Fuel and Its Urinary Biomarkers

- Jet fuel is a mixture of hundreds of chemical components; each can exhibit a different health effect, toxicity, or disease.
- Jet fuel exposure can occur from inhalation of vapors or dermal contact in the occupational setting.
- Jet fuel mixtures contain alkanes, aromatic, and polycyclic aromatic compounds (PAHs).
- Naphthalene, a PAH, metabolically forms 1- and 2-naphthol which are used as urinary biomarkers of jet fuel exposure.

- Benzene, a known carcinogen, metabolically forms *S*-phenylmercapturic acid (PMA) and *trans*,*trans*-muconic acid (*t*,*t*-MA) which have been used as urinary biomarkers of jet fuel exposure.
- Toluene, another chemical component of jet fuel, metabolically forms *S*-benzylmercapturic acid (BMA) which has been used as a urinary metabolite of jet fuel exposure.
- (2-Methoxyethoxy)acetic acid (MEAA) is a metabolic product of 2-(2-methoxyethoxy)ethanol, a deicing agent found in some military formulations of jet fuel.
- MEAA was found to be a more accurate biomarker of the jet fuel JP-8 exposure over PMA or BMA during a study of US Air Force personnel.

Definitions of Words and Terms

Biological Exposure Indices (BEI) BEIs are the levels set by the American Conference of Governmental Industrial Hygienists (ACGIH) for biomarkers of certain occupational chemical exposures.

Biomarker of Effect or Disease A biomarker of effect is a metabolite, protein, or chemical substance that is indicative of the development of a disease or health effect.

Biomarker of Exposure A biomarker of exposure is a metabolite, protein, protein adduct, or DNA adduct that is derived from the parent chemical and is indicative of exposure to the parent chemical.

CYP2E1 or Cytochrome P450, Family 2, Subfamily E, Polypeptide 1 CYP2E1 is an enzyme encoded by the human CYP2E1 gene and functions in the oxidation of small molecules in metabolism.

Cytochrome P450 (P450) P450 is the group of enzymes that are catalysts for a number of oxidation reactions of small molecules in metabolism.

Chromatography Chromatography is the term for the set of laboratory techniques or science for the separation of chemical compounds from complex mixtures.

Gas Chromatography (GC) GC is an instrumental chromatographic technique in analytical chemistry used to separate volatile compounds in a mixture with the purpose of identifying or quantifying a specific compound or multiple compounds within the mixture. GC utilizes heating samples into their vapor phases, which are carried onto a sorbent packed or coated column by means of a carrier gas. Different compounds travel through this column at different rates and are thus separated.

Glucuronyl Transferase (GT) GT is an enzyme that catalyzes the conjugation of glucuronic acid to different chemicals as a metabolic step in detoxification. This process makes the conjugated molecule more soluble and easier for excretion.

Glutathione *S*-Transferase (GST) GST is a class of enzymes that catalyze the conjugation of the reduced form of glutathione to different chemicals as a metabolic step in detoxification.

High-Performance Liquid Chromatography (HPLC) HPLC is an instrumental chromatographic technique in analytical chemistry used to separate a liquid mixture of compounds with the purpose of identifying or quantifying a specific compound or multiple compounds within the mixture. HPLC utilizes a pressurized liquid solvent to inject a sample through an analytical column containing a sorbent. Different compounds travel through this column at different rates and are thus separated.

Mass Spectrometry (MS) MS is an instrumental technique in analytical chemistry used to identify or quantify a specific chemical by means of transforming it into a charged state or ion and measuring the mass of the parent ion or the daughter ions or fragments of the parent ion. The charged ion fragments are measured by a mass-to-charge ratio by the mass spectrometer.

Myeloperoxidase (MPO) MPO is a peroxidase enzyme that is encoded on the human MPO gene.

NQO1 or NAD(P)H Dehydrogenase, Quinone 1 NQO1 is an enzyme that reduces quinones to the corresponding hydroquinones.

Polycyclic Aromatic Hydrocarbon (PAH) PAHs are a class of chemical compound having fused aromatic ring structures. They are often found as combustion products and at low levels in petroleum products.

Introduction

Biomarkers of Exposure

Biomarkers of exposure are an important field of study in environmental health. Although biomarkers can be useful for other purposes, such as those measuring the effects of exposure or disease and those of susceptibility to exposure, biomarkers of exposure can be used to monitor and then minimize exposure by incorporating improvements in personal protective equipment, in engineering controls, or in work protocols. Thus, one of the desired goals in using biomarkers of exposure for biomonitoring is in improving the environmental conditions before any disease occurs in an individual. Molecular biomarkers of chemical exposure from

environmental toxicants can be the parent chemical, itself, or blood or urinary metabolites. Molecular biomarkers can also be used as early signs of exposure by the use of protein adducts or DNA adducts derived from the parent chemical or from various metabolites. Advances in chemical analysis have greatly improved the capability to detect biomarkers; thus, an individual's exposure to environmental or occupational toxicants can be characterized and assessed by the measurement of the collected biological media. These general principles have been applied to jet fuel studies by various researchers over the past few years. Urinary biomarkers of exposure offer the least invasive biological sample for use in field studies and the greatest ease of analysis, thanks to the use of various chromatographic analyses, many of which incorporate mass spectrometric detection.

The History of Jet Fuel and Human Exposure

Jet propulsion fuels are chemical mixtures with various performance formulations with specific designations such as JP-4, JP-5, JP-8, JP-8 +100, Jet A, Jet A-1, etc. All are essentially liquid hydrocarbon mixtures containing hundreds of aliphatic and aromatic hydrocarbons along with various additives (NRC 2003; Ritchie et al. 2003); the composition and major components of various formulations are displayed in Table 1. Table 1 shows only the major components. Historically, the specification for JP-4 called for a wide-cut fuel, and JP-4 was first issued by the US Air Force in 1951. The wide cut was taken from the petroleum distillate as to include roughly half gasoline and half kerosene as fractions. The higher flash point kerosene-based JP-5 was developed for the US Navy to reduce the fire risk on aircraft carriers and is currently used by most of the world's naval aviation. Combat experience gained during the Vietnam War indicated that the US Air Force aircraft using the highly volatile JP-4 had a higher combat loss rate than the US Navy aircraft that used the less volatile JP-5. Also, JP-4 was found to have a higher fire crash risk than JP-5. Conversion to the full kerosene-based JP-8 was started by the US Air Force in 1979 and completed in the 1990s. The chemically similar Jet A and Jet A-1 fuels are also narrow-cut kerosene fuels and do not contain many of the performance and anti-icing additives used in the JP-8 formulation. JP-8 +100 is a JP-8 fuel with the addition of a thermal stability performance package.

Jet fuel is obviously used in the jet and turbine aviation, but since 1990, JP-8 has been used in most fuel applications by the US military including diesel vehicles, tanks, power generators, and even cookstoves. JP-8 is used to suppress environmental sand or dust, for decontamination or cleaning of military vehicles and equipment, and as a carrier for herbicide applications. JP-8 is the main battlefield fuel for all military operations in the United States and represents the single largest chemical exposure to personnel in the US Department of Defense. JP-8 is also the major fuel used throughout the air forces of the North Atlantic Treaty Organization (NATO). The US and NATO allies collectively consume an estimated 20 billion liters a year of JP-8 (Zeiger and Smith 1998). Jet A is commonly used for commercial aviation within the United States while the similar Jet A-1 is

Table 1 The chemical composition of jet fuel formulations JP-5, JP-8, and Jet A-1 (% v/v)

JP-5	JP-8	Jet A-1
Range C_7–C_{17}	**Range C_6–C_{18}**	**Range C_6–C_{16}**
Olefins: mean 1.6 ± 0.8 %	Olefins: mean 1.2 ± 0.7 %	
Alkanes	**Alkanes**	**Alkanes**
Dodecane 22.5 %	Dodecane 22.5 %	Dodecane 4.7 %
Tetradecane 16.9 %	Tetradecane 16.9 %	Tridecane 4.4 %
Hexadecane 12.2 %	Hexadecane 12.2 %	Undecane 4.4 %
Decane 16.1 %	Decane 16.1 %	Tetradecane 3.0 %
Cyclooctane 4.5 %	Cyclooctane 4.3–4.4 %	Pentadecane 1.5 %
Isooctane 3.7 %	Isooctane 3.7 %	2,6-Dimethylundecane 2.1 %
Methylcyclohexane 3.5 %	*n*-Hexane <0.1 %	2-Methylundecane 1.2 %
n-Hexane <0.1 %		Heptylcyclohexane 1.0 %
		n-Hexane <0.1 %
Aromatics and PAHs	**Aromatics and PAHs**	**Aromatics and PAHs**
Butylbenzene 4.7 %	Butylbenzene 4.7 %	*p*-, *m*-, *o*-Xylenes 3.5 %
Tetramethylbenzene 4.3 %	Tetramethylbenzene 4.3 %	1-Methylnaphthalene 1.8 %
Tetrahydronaphthalene 4.1 %	Tetrahydronaphthalene 4.1 %	2-Methylnaphthalene 1.5 %
m-Xylene 4.0 %	*m*-Xylene 4.0 %	2,6-Dimethylnaphalene 1.3 %
Methylnaphthalenes 3.5 %	Methylnaphthalenes 3.5 %	Naphthalene 1.1 %
p-, *o*-Xylenes 0.6 %	*p*-, *o*-Xylenes 1.4 %	Tetramethylbenzene 1.3 %
Naphthalene 0.4–3 %	Naphthalene 1.5 ± 0.7 %	Toluene 0.8–0.9 %
Toluene 0.1 %	Toluene <1 %	Benzene 0.5–0.8 %
Trimethylbenzenes 1–2 %	Trimethylbenzenes <6.6 %	

Information in the table from Ritchie et al. (2003). The table only displays the major components or those of interest

used worldwide. Therefore, jet fuel has been recognized as a significant source of chemical exposure by both dermal and inhalation routes for aircraft mechanics, fuel handlers, aviation workers, as well as most military personnel. Exposure to jet fuel occurs most frequently during fuel tank or fuel cell maintenance, when mechanics are required to physically work inside the tank or cell. Civilian exposure is also likely from both dermal and inhalation routes by workers in similar occupations. It has been estimated that over two million military and civilian personnel a year are occupationally exposed to some type of jet fuel (Ritchie et al. 2003).

Toxicity, Health Effects, and Disease Associated with Jet Fuel Exposure

The early research studies of jet fuel toxicity noted that many of the effects could be attributed to the components of the fuel. These include alkane and aromatic components such as benzene, toluene, and xylenes. Various nervous system and neurological disorders including fatigue, anxiety, headaches, and polyneuropathy have been noted in civilian and military aircraft workers exposed to JP-4 (Knave et al. 1978; Struwe et al. 1983). Government reviews of the literature describing the health effects of jet fuel exposure have been published (ASTDR 1998; DOE 1989). In addition, the health effects and toxicity of the more modern JP-8 have also been

extensively studied and described. Human chronic exposure to jet fuel and its effect on postural balance (Smith et al. 1997) as well as comprehensive reviews of the toxicity of jet fuel have been published (Mattie and Sterner 2011; Ritchie et al. 2003). Although systemic dermal toxicity by jet fuel components is considered unlikely (McDougal and Rodgers 2004), correlation between dermal exposure and urinary naphthols has been reported (Chao and Nylander-French 2004). Dermal exposure and toxicity are a concern owing to the fact that some of the components contained in jet fuel have been reported to cause skin cancer in mice (NTP 1986). A subchronic dermal study of JP-8 in rats demonstrated that proliferative, degenerative, and inflammatory changes were significantly greater in fuel-exposed skin versus non-exposed skin sites on the same animal (Baker et al. 1999). Although there are several systemic toxicity studies from skin exposure to jet fuel, in one study where mice were dosed three times a week (41.5 mg/kg/day), decreased organ and body weights were observed (Schultz et al. 1981). Jet fuels, as well as kerosene, have been shown to cause various forms of skin irritation, especially with prolonged or repeated contact with the skin (McDougal and Rodgers 2004). Jet fuel has also been demonstrated to have immunosuppressive effects in animals. In a study of mice dosed with JP-8, immunosuppression in this species was thought to occur through a mechanism related to the aryl (aromatic) hydrocarbon receptor (Dudley et al. 2001).

Since jet fuel formulations are generally kerosene-based complex chemical mixtures (C_6–C_{18}), their individual components have known health effects on humans. Most jet fuels contain some polycyclic aromatic hydrocarbons (PAHs) (0.3 to 3 % v/v); the predominate ones being naphthalenes. Aromatics compounds including benzene (< 1 % v/v), toluene (approximately 1 %) and xylenes (3 to 4 %) as well as various alkanes including n-hexane are other major jet fuel components. The military formulations of JP-5 and JP-8 contain icing inhibitor 2 (2-methoxyethoxy)ethanol (also known as diethylene glycol monomethyl ether (DiEGME) CAS no. 111-77-3) which is unique in that it is added at constant 0.1 % (v/v) for every batch lot of fuel. The health effects of each of these components will be briefly described.

Exposure to respirable PAHs has been thought to be a significant human cancer risk (Holland et al. 1981) especially for oral surfaces, lungs, skin, and possibly the kidneys. The carcinogenicity of PAHs is based on their bioactivation to yield carcinogenic intermediates that can penetrate cells. Benzo[a]pyrene and fluoranthene have been ranked by the ASTDR as among the most hazardous substances in the environment (Ostrowski et al. 1999). Naphthalene, another PAH found in jet fuel at low levels (Smith et al. 2010), is classified as Group 2B or "possibly carcinogenic to humans" by the International Agency for Research on Cancer on the basis of sufficient evidence of cancer in experimental animals (IARC 2002).

Aromatic compounds have a long history of health effects. Exposure to benzene has relatively clear health effects including aplastic anemia and acute myelocytic leukemia (AML). A link between benzene exposure and leukemia was first reported by Delore and Brogomano in 1928 (Delore and Borgomano 1928). It is generally

accepted today that the primary health effect of significant benzene exposure is the depression of bone marrow that may result in aplastic anemia or certain forms of leukemia (Synder 2000). Pancytopenia, the reduction in red blood cells, white blood cells, and platelets, is the result of depressed bone marrow function and is a characteristic of aplastic anemia. There is significant epidemiological research that suggests the connection between the development of this disease and exposure to benzene. Another health effect associated with benzene exposure is myelofibrosis, a disease where the bone marrow is replaced with fibrous tissue (Zoloth et al. 1986). Although benzene, itself, may directly produce some to these health effects, current belief is that the majority of the problems are produced by benzene metabolites (Gad-El-Karim et al. 1985; Gad-El-Karim et al. 1986). Although there is often concomitant exposure to benzene and toluene in jet fuel and other occupational situations, toluene exposure has generally been associated with neurological effects, not hematopoietic effects as is the case with benzene. The acute effects of toluene exposure include a narcotic effect, as well as impairment of some neuromuscular and cognitive functions. These effects have been known to persist beyond clearance of the parent toluene and its metabolites from the body. In human volunteer tests, the observed neurobehavioral effects of toluene exposure were fatigue, headaches, lack of coordination, and muscle weakness (von Oettingen et al. 1942a, b). Cases of uncontrolled studies of the health effects of toluene have been recorded with drug abusers who have "huffed" the chemical. The health effects of abusers of toluene have generally been the same as those previously mentioned under controlled studies. Toluene has also been implicated in reproductive problems. In females exposed to toluene, an increase in spontaneous abortions was reported (Ng et al. 1992). Xylenes, or dimethylbenzenes, have been found to have neurological effects. Increased reaction time, impaired postural equilibrium, and reduced short-term memory have been documented for *p*-xylene exposure (Olsen et al. 1985). Chronic occupational exposure to xylenes has been associated with chest pain with electrocardiogram abnormalities, dyspnea, thrombocytopenia, leukopenia, and cyanosis in addition to central nervous system problems (Ritchie et al. 2001). In animal studies, xylene exposure has been observed to cause hearing loss predominately in the high frequencies (Pryor et al. 1987). Neither toluene nor xylenes have been associated with cancer.

Jet fuel formulations contain multiple alkane compounds. *n*-Hexane, one of the lowest carbon chains found in jet fuel, is considered the most toxic of the alkanes. Direct ingestion of even small quantities of hexane induces nausea, vertigo, and severe intestinal and bronchial irritation. Chronic exposure to *n*-hexane is known to induce peripheral neuropathy in humans and laboratory animals. It has been reported in *Patty's Toxicology* (Bingham et al. 2000) that 50 g is an oral fatal dose in humans. The neurotoxicity of *n*-hexane is due to its oxidized metabolite, 2-5-hexanedione. Hexane metabolites have also been demonstrated to be cytotoxic to rat Schwann cells reducing DNA synthesis by a concentration-dependent relationship (Kamijima et al. 1996). No known published reports have linked leukemia or lymphoma with hexane exposure. Fortunately the composition of kerosene jet fuel consists of extremely low levels of the lower boiling point alkanes

such as hexane. The major constituents of JP-8 include the longer carbon-chain alkanes such as decane, undecane, dodecane, tetradecane, and hexadecane (NRC 2003). Inhaled long-chain hydrocarbons generally show poor blood uptake as a result of their lower blood solubility. Conversely, they have relatively high lipid/blood partition coefficients which can lead to accumulation in high lipid tissues such as fat and the brain. Most of these alkanes show some enhancement of skin carcinogenicity as was demonstrated in a study of benzo[a]pyrene-dosed mice (Van Duuren and Goldschmidt 1976).

2-(2-Methoxyethoxy)ethanol, a glycol ether and anti-icing agent added to the military formulations of jet fuel (JP-5 and JP-8), is highly water soluble and, thus, has a high capacity for systemic absorbance by dermal or pulmonary exposure. Dermal exposure of JP-8 containing this compound was conducted on mice (B'Hymer et al. 2005b). Few human toxicity studies have been conducted with 2-(2-methoxyethoxy)ethanol, but numerous animal studies have been reported. For example, it has been demonstrated that 2-(2-methoxyethoxy)ethanol had teratogenic and developmental toxicity in rats (Hardin et al. 1986). Observed fetotoxicity in the rabbit from dermal exposure to this glycol ether has also been reported (Scortichini et al. 1986).

The Commonly Utilized Urinary Metabolites: Their Application as Biomarkers of Jet Fuel Exposure

Since current jet fuel formulations are generally kerosene-based complex chemical mixtures, they represent a considerable challenge with respect to toxicity of the individual components and analysis for specific biomarkers of exposure. A well-chosen biomarker of exposure should have several important qualities (B'Hymer and Cheever 2010). First, it should be specific for the exposure of interest; some metabolites are common to multiple chemical substances and, therefore, are not specific biomarkers. Second, the biomarker should be easily detectable even at a small quantity. High sensitivity is important to distinguish the levels of exposure in the environment. Also, the biomarker must be associated with the exposure. In the ideal case, a biomarker should provide good predictive values to a specific level of exposure or specific health status. Reference values to the general population are very desirable for a proper assessment of exposure. Low overall analysis expense is another desirable characteristic of biomarker analysis for use in biomonitoring. The availability of suitable economical reference standards and low-cost sample preparation are important aspects for extensive field studies. This also implies that the analytical measurement procedure for the biomarker of exposure should have high-throughput capability and low pre-analysis sample treatment. Finally, ideal biomarkers found by means of noninvasive sampling techniques are highly desirable. Generally, urine sampling fits this last quality since human study participants are more likely to donate urine samples over some other biological sources such as blood or tissue. These characteristics have limited biomarkers of jet fuel exposure to metabolites of naphthalene, benzene, toluene, possibly xylenes, and, in specific cases, 2-(2-methoxyethoxy)ethanol (a component in JP-5 and JP-8 formulations). Since a low detection capability is a desired quality of an ideal biomarker, the mass

spectrometer (MS) coupled with some form of chromatographic separation technique dominates the literature for biomarker quantification analysis. Generally, the chromatographic separation has been either gas chromatography (GC) or high-performance liquid chromatography (HPLC). Less frequently, GC analysis has been reported using the less expensive flame ionization detector (FID) (Bieniek 1996). It should be noted that the FID has lower analytical method "specificity" than the MS detector; specificity is the ability to determine a target analyte without interference from other sample components.

Metabolic Biomarkers from Naphthalene

The metabolites of naphthalene have been the primary PAH biomarkers used to assess exposure to jet fuel. The metabolic route for naphthalene is fairly simple; naphthalene is metabolized by means of P450 and is oxidized to produce 1,2-naphthalene oxide, which in turn can spontaneously rearrange to form 1- and 2-naphthol (see Fig. 1). Urinary 1- and 2-naphthol have been reported to have been used as biomarkers of total absorbed dose of JP-8, reflecting an accurate measure of dermal exposure (Chao et al. 2006). Additionally, inhalation of naphthalene also produces urinary naphthols (Serdar et al. 2003b). It has been clearly demonstrated by occupational studies that jet fuel exposure does contribute to the production of naphthols (Chao et al. 2006; Smith et al. 2012).

Analysis of urine for the presence of 1- and 2-naphthol is fairly simple and frequently reported in the literature (Bieniek 1996, 1997; Erdem et al. 2011; Ramsauer et al. 2011; Serdar et al. 2003a). Generally, the analytical procedure starts with urine sample treatment. Naphthols can be in conjugated forms, as glucuronides or sulfates when in urine. Deconjugation of the naphthols using either mineral acid or enzymatic treatment is generally performed first although analysis of untreated urine has been reported (Andreoli et al. 1999). Solid-phase extraction (SPE) can be used to collect the 1- and 2-naphthol from the urine sample. A number of analysis techniques have been reported. Analysis using GC with a FID detector

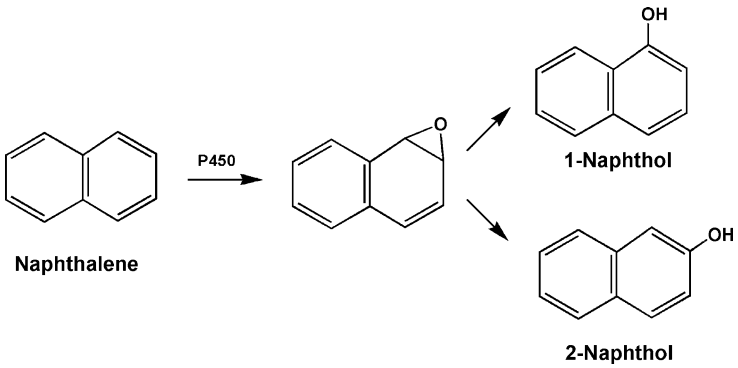

Fig. 1 The metabolic pathway of naphthalene to produce the biomarkers 1- and 2- naphthol

has been described (Bieniek 1996, 1997) and used for jet fuel exposure determination elsewhere (Erdem et al. 2011). A procedure utilizing GC-MS where the extracted naphthols were derivatized with trimethylsilane (TMS) has been described (Serdar et al. 2003a). Other researchers have used this GC-MS procedure for occupational studies to determine urinary levels of naphthols (Smith et al. 2010, 2012). HPLC-MS has also been used as a technique to detect and quantify naphthols in urine samples (Ramsauer et al. 2011). Detection techniques utilizing the MS have the advantage of being very specific and not likely to have interferences from other substances found in urine.

Metabolic Biomarkers from the Aromatic Compounds Benzene, Toluene, and Xylenes

Metabolic biomarkers from benzene, toluene, and xylenes have been well studied and often reported in the scientific and industrial hygiene literature since these compounds are widely used as general industrial solvents. In the case of benzene, not only is it a component in fuel, but it is also a constituent of engine emissions and general combustion. Toluene is a component of many commonplace items including lacquer-based paints, styrene glue, and a major component in gasoline. Toluene vapor is a typical pollutant in urban areas as a result of automobile emissions. Xylenes are commonly used component of industrial solvents. Since these compounds are also components of jet fuel, much of the analytical methodology and general biomarker knowledge has been carried over from industrial hygiene or general environmental research to assessing jet fuel exposure.

Benzene exposure, like the other components of jet fuel, can be either by the inhalation or dermal route. When inhaled, only about a tenth of the absorbed benzene is exhaled from the human lungs and very little is excreted unchanged in the urine. Therefore, the remainder is metabolized in the body and excreted through the urine. Dermal exposure is expected to be similarly metabolized and excreted through the urine. Some of the common urinary metabolites of benzene are *S*-phenylmercapturic acid (PMA), *trans*,*trans*-muconic acid (*t*,*t*-MA), hydroquinone, catechol, phenol, and 1,2,4-trihydroxybenzene (Qu et al. 2000; Sabourin et al. 1988). A condensed metabolic scheme for benzene is displayed in Fig. 2. Briefly, benzene is oxidized by means of CYP2E1 to benzene epoxide, which is further metabolized by several routes. Phenol and its conjugates are the main biotransformation products for benzene, which includes hydroquinone and catechol. Hydroquinone can be further metabolized to 1,4-benzoquinone and 1,2,4-trihydroxybenzene. Catechol can be metabolized to 1,2-benzoquinone. However, compounds produced along the phenol route are not suitable as specific urinary biomarkers for benzene as some of these compounds are the by-products of other chemicals. Phenol and trihydroxybenzene were shown to not be statistically different between low exposure and unexposed population groups in field studies (Qu et al. 2000). Benzene epoxide (see Fig. 2) can also eventually be converted to *t*,*t*-MA which has more extensive use as a urinary biomarker of benzene

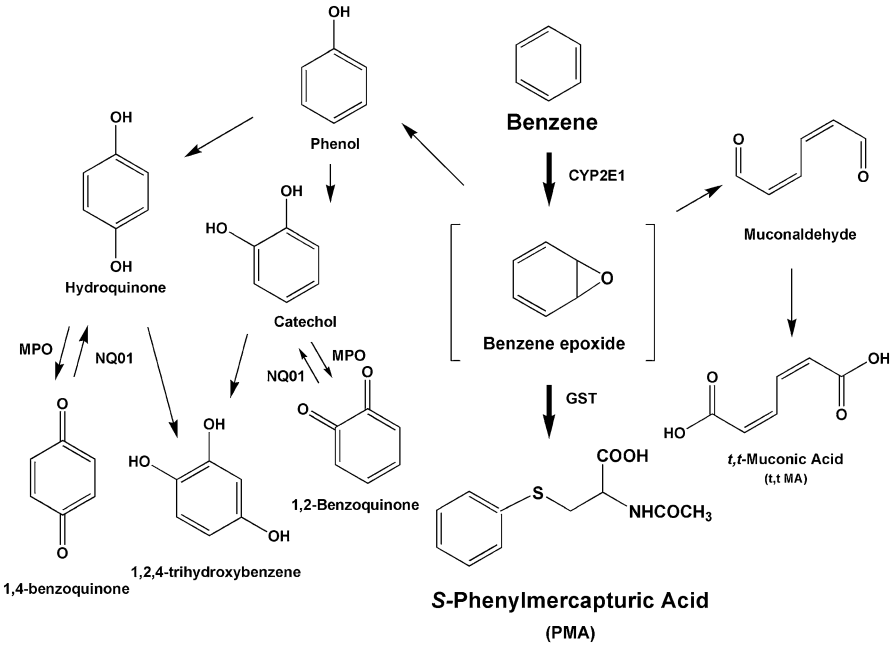

Fig. 2 The metabolic pathway of benzene to produce biomarkers *S*-phenylmercapturic acid (PMA) and *trans*,*trans*-muconic acid (*t*,*t*-MA) as well as other metabolites

exposure. *S*-Phenylmercapturic acid (PMA) is a glutathione *S*-transferase (GST) product of the benzene epoxide intermediate, and PMA has often been studied as a biomarker of benzene exposure and jet fuels containing benzene (B'Hymer et al. 2012a; Qu et al. 2000; Sabourin et al. 1988). As a result, PMA and *t*,*t*-MA have been recognized as the most sensitive biomarkers for low-level benzene exposure.

The urinary metabolites of benzene have generally been analyzed by means of HPLC-MS with the exception of phenol. Qu (Qu et al. 2000) reported using GC-MS for the quantification of urinary phenol, and an HPLC-MS method was used for the quantification of PMA and *t*,*t*-MA (Melikian et al. 1999). In the same study, hydroquinone, catechol, and trihydroxybenzene were quantified by a separate HPLC-MS method. A fully validated HPLC-MS method for the quantification of PMA and the corresponding toluene mercapturic acid metabolite, BMA, has recently been reported in the literature (B'Hymer 2011).

Benzene has been found to have some limitations for use as an accurate biomarker of jet fuel exposure. Not only is it a component in jet and other fuels, but it is also a constituent of automobile engine emissions and general combustion; therefore, exposure from the general urban environment is possible (Bono et al. 2005). In addition, benzene has also been found to be a small combustion component in cigarette smoke. In a comparison to other biomarkers of JP-8 exposure, PMA was found to be less accurate for measuring exposure among

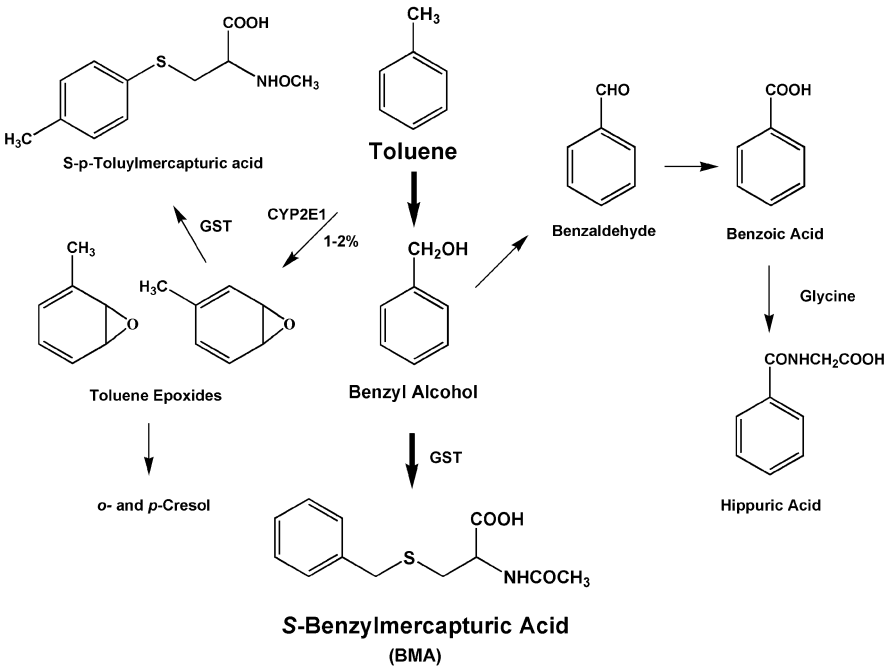

Fig. 3 The metabolic pathway of toluene to produce the preferred biomarker *S*- benzylmercapturic acid (BMA) as well as other metabolites

work groups than other metabolic biomarkers. This was believed to be due to the low concentration of benzene in jet fuel, resulting in low exposures for workers and a general lack of sensitivity for the PMA metabolite with the methodology used (B'Hymer et al. 2012a).

Toluene exposure follows a metabolic route much like the benzene exposure described previously. Two of the most common urinary metabolites of toluene are *S*-benzylmercapturic acid (BMA) and hippuric acid (Marchese et al. 2004). A condensed metabolic scheme for toluene is displayed in Fig. 3. Briefly, in vivo toluene is oxidized in a series of steps [toluene to benzyl alcohol to benzaldehyde to benzoic acid] and finally conjugated with glycine to form the hippuric acid. The benzyl alcohol intermediate can also be metabolized by GST to form BMA. A small percentage of toluene is metabolized by CYP2E1 to eventually form *o*- and *p*-cresol and *S*-*p*-toluymercapturic acid. The metabolism of toluene can be concentration dependent and a full description of these pathways have been reported previously (Angerer et al. 1998; Rietveld et al. 1983).

As for urinary benzene metabolite analysis, typical methodologies involve HPLC-MS for the analysis of toluene metabolites. The traditional urinary metabolites used as biomarkers to toluene exposure have been *o*-cresol and hippuric acid (one of the most abundant metabolites of toluene exposure). The American Conference of Governmental Industrial Hygienists (ACGIH) has set biological

exposure indices (BEI) levels for *o*-cresol and hippuric acid. The BEI level for *o*-cresol is 0.5 mg/L of urine, and the BEI level for hippuric acid is 1.6 mg/mg creatinine in urine samples collected at the end of a work shift (ACGIH 2011). In addition, studies have shown that BMA is one of the preferred metabolites for use as biomarker of toluene exposure (Angerer et al. 1998; Inoue et al. 2002). *S*-Toluylmercapturic acids have been found in toluene-exposed animals (Cosnier et al. 2012) and used in human exposure studies (Angerer et al. 1998).

Some toluene metabolites used as biomarkers for biomonitoring have limitations. Hippuric acid is not specific to toluene exposure and can be derived from metabolic conversion of other parent chemicals. Although BMA has been used as a specific biomarker for toluene derived from occupational jet fuel exposure (B'Hymer et al. 2012a), toluene, itself, can be a problem in an exposed population. Toluene is a major component in gasoline and is found in automobile emissions; urban populations often have toluene exposure from the general environment, which could be a confounder in determining jet fuel exposure from urinary levels.

Xylenes, or dimethylbenzenes (*o*-, *m*-, and *p*-isomers), are another minor component of jet fuel but can be monitored by means of urinary metabolites. Xylene metabolites are formed along similar routes as benzene and toluene. A condensed metabolic scheme for xylenes is displayed in Fig. 4. In vivo, the xylene positional

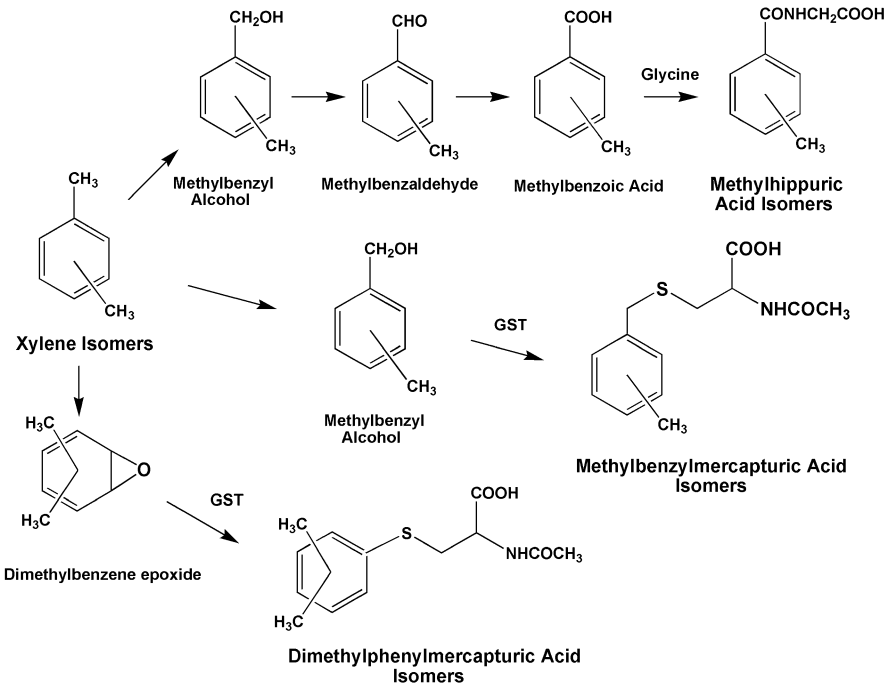

Fig. 4 The metabolic pathway of xylene isomers to produce various biomarkers (The specific positional isomers are not shown in this figure)

isomers can be oxidized through a number of steps to form *o*-, *m*-, and *p*-methylhippuric acid in a similar manner as described for toluene. The methyl benzyl alcohol intermediate can also be metabolized by GST to form *S*-methylbenzylmercapturic acid (MBMA) isomers. Epoxide formation on the aromatic ring followed by GST can also produce dimethylphenylmercapturic acid isomers. Analysis of the urinary biomarkers for xylenes has usually been accomplished by HPLC-MS (Manini et al. 2004). Although these urinary metabolites have been found in workers exposed to xylene-based solvents, they have been infrequently pursued in occupational studies of jet fuel exposure.

Metabolic Biomarker from 2-(2-Methoxyethoxy)ethanol

As described previously, 2-(2-methoxyethoxy)ethanol is a deicing agent added to the formulation package of JP-5 and JP-8. Being water soluble, 2-(2-methoxyethoxy)ethanol exhibits a high capacity for systemic absorbance from dermal exposure. A metabolic scheme for this compound is shown in Fig. 5. It is initially metabolized by alcohol dehydrogenase and then aldehyde dehydrogenase to form (2-methoxyethoxy)acetic acid (MEAA). Although MEAA can form other metabolites, MEAA is the primary urinary metabolite from

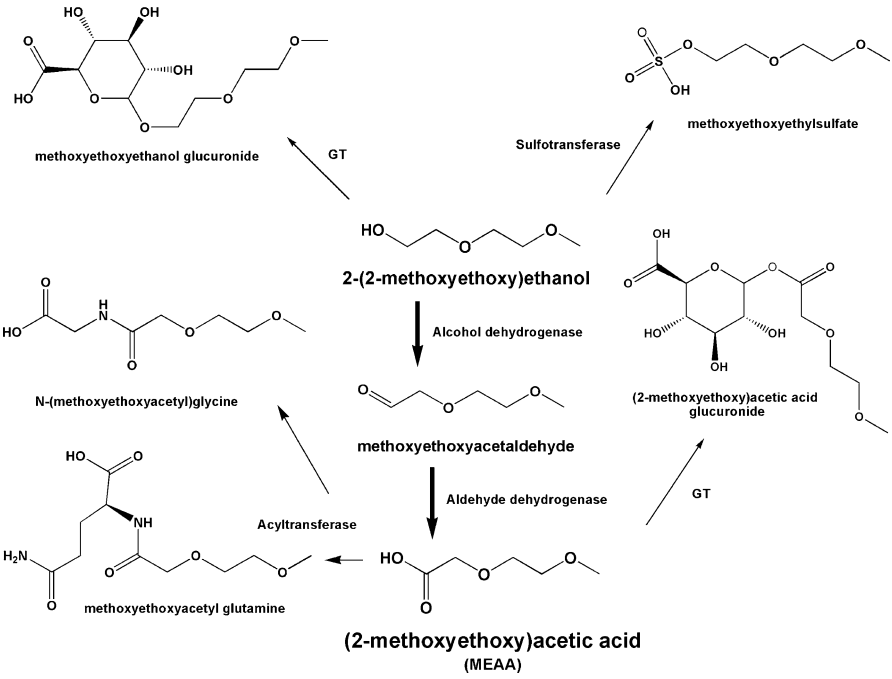

Fig. 5 The metabolic pathway of jet fuel deicing agent 2-(2-methoxyethoxy)ethanol to form (2-methoxyethoxy)acetic acid (MEAA)

exposure and is the predominate metabolite found in urine. MEAA has been demonstrated in animal studies to be the urinary metabolite best suited for use as a short-term biological marker of exposure to 2-(2-methoxyethoxy)ethanol (Richards et al. 1993). However, as Fig. 5 shows, other metabolites can be formed from the initial glycol ether by glucuronyl transferase (GT), dealkylase or sulfotransferase, and MEAA can be further metabolized by acyltransferase or dealkylase carboligase. These other metabolites are only minor components in the urine and MEAA is the major urinary metabolite.

MEAA has been demonstrated as a biomarker of JP-8 exposure in both animal studies (B'Hymer et al. 2005b) and humans exposed during the course of their work (B'Hymer et al. 2012a, b). A validated analysis procedure for MEAA has utilized GC-MS (B'Hymer et al. 2005a, 2003). A study of US Air Force personnel ranked as having low, moderate, and high levels of exposure to JP-8 determined that MEAA was the most accurate biomarker of exposure (B'Hymer et al. 2012a). This study compared the urinary levels of the benzene metabolite PMA, the toluene metabolite BMA, and MEAA of the exposed work groups and determined that MEAA was sufficiently accurate and sensitive to distinguish the levels of JP-8 exposure. MEAA, as a metabolite of the parent deicing agent, had the additional advantage of being derived from a component of the fuel that has a constant concentration level, 0.1 % (v/v), for all batch lots of JP-8. The other nonadditive package components of JP-8 can vary from batch to batch, thus problematic when assessing the accuracy of exposure to the fuel given the scope of JP-8 usage.

The main limitation with using MEAA as a urinary biomarker of jet fuel is that only JP-5, JP-8, and JP8 +100 use it in an additive package. For the other jet fuels, the various metabolites previously mentioned would have to be used as urinary biomarkers of exposure. Another obvious limitation for the use of MEAA as a biomarker is that it does not represent other main fuel component metabolites, which results in little clinical value for the tested individual. Obviously, the naphthalene, benzene, or toluene metabolites, naphthols, PMA, and BMA, respectively, would have a greater utility in the assessment of an individual's exposure to the respective toxic parent compounds. The rate of skin penetration and bioavailability from inhalation would be different between the toxic components in jet fuel, so that a single urinary biomarker can only be used as an indirect biomarker for the other components.

Final Measurement Considerations for Urinary Biomarkers

Some final considerations for the use of jet fuel urinary biomarkers must be mentioned. Urinary biomarkers in general can be measured as a concentration to volume of urine, a mass comparison adjusted to the level of creatinine in urine, or based upon the specific gravity of the urine sample. The level of dehydration and the level of workload of the human test subject have to be considered when utilizing

a urinary biomarker. Dehydrated subjects are likely to give higher concentration levels than fully hydrated test subjects. The traditional approach has been to adjust the urine levels based on creatinine urine levels, which is the practice used by the ACGIH, as well as other authorities, for BEI recommendations. However, the use of creatinine to normalize urinary analyte concentrations has been extensively reported to not necessarily improve correlation of dose to exposure for some urinary components (Alessio et al. 1985; Boeniger et al. 1993; Carrieri et al. 2001). Gaines et al. (2010) suggested the use of urine specific gravity for biomarker normalization as an alternative to creatinine.

Rapid metabolic conversion of the analytes used for urinary biomarkers is fairly common. Therefore, the biomarkers mentioned in this document must be considered for acute exposure having occurred very recently. For example, the conversion of 2-(2-methoxyethoxy)ethanol to MEAA is fairly rapid; its half-life has been determined to be approximately 8 h in various animal studies (Cheever et al. 1988; Daniel et al. 1991; Richards et al. 1993). The other urinary metabolites of jet fuel also have half-life periods less than 24 h; therefore, samples must usually be collected at the end of a shift or within a workday period for meaningful internal dose information.

Summary Points

- Urinary biomarkers of jet fuel exposure can be used for occupational assessment and are derived from some of the main components of the fuel mixtures.
- The toxicity and health effects of jet fuel exposure are directly related to the effects of the components of the fuel which are kerosene mixtures.
- The main components are alkane and aromatic compounds including polycyclic aromatic hydrocarbons (PAHs) such as naphthalenes.
- Some of the metabolites of the jet fuel components can be used as urinary biomarkers of exposure; these include 1- and 2-naphthol produced from naphthalene, *S*-phenylmercapturic acid and *trans,trans*-muconic acid (t,t-MA) derived from benzene, and *S*-benzylmercapturic acid (BMA) produced from toluene.
- Some of the military fuel formulations, such as JP-5 and JP-8, contain additive packages; one important anti-icing additive, 2-(2-methoxyethoxy)ethanol, is converted to the urinary metabolite (2-methoxyethoxy)acetic acid (MEAA).
- The most common analysis methods to detect and quantify urinary biomarkers of jet fuels generally utilize either high-performance liquid chromatography (HPLC) with mass spectrometric detection (MS) or gas chromatography with MS detection.
- Urinary levels of biomarkers can be expressed as concentration to the volume of urine, adjusted to a normalized level by urinary creatinine levels, or based on the specific gravity; creatinine normalization is the generally accepted traditional approach.

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