APPENDIX A6.1.3.7 BENZENE - FACTSHEET

	HPHC	BoExp assessed by PMI (matrix)
IUPAC name	Benzene	S-phenyl-mercapturic acid (in urine)
Usual abbreviation	-	S-PMA; SPMA; PMA
Other names	(6)-Annulene; benzine1; benzole; benzolene; bicarburet of hydrogen; carbon oil; coal naphtha; cyclohexatriene; mineral naphtha; motor benzol; phene; phenyl hydride; pyrobenzol; pyrobenzole	
CAS number	71-43-2	4775-80-8
Formula and molecular weight	C_6H_6 MW: 78.1	$C_{11}H_{13}NO_3S$ MW: 239.30

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Main characteristics

Chemical class	Monocyclic aromatic hydrocarbon
HPHC lists	FDA-93 [1]; FDA-18 [2]; HC [3, 4]; PMI-58; WHO-39; [5] WHO-18 [6]; WHO-9 [6]
Source in cigarettes	Amount: more than half of the US population exposure to benzene results from smoking tobacco or being exposed to tobacco smoke [7] Amount in mainstream smoke (3R4F): 45.7µg (ISO); 69.7µg (HCI) [8] Smoke phase: gas/vapor phase [9, 10] Presence in tobacco / pyrosynthesis (T°): present in uncombusted tobacco [11]. Precursors in tobacco are organic acids (in particular stearic acid), terpenes and polyphenols [12-14] / 500-800°C [15]; majority formed 450-600°C [16].
Other sources [17]	Sources of benzene include rubber industry emissions, oil refineries, chemical plants, shoe manufacturers, gasoline, automobile exhaust fumes, automobile service stations, glues, solvents, paints, art supplies. Natural sources include oil seeps and forest fires. Individuals may also be exposed to benzene by consuming contaminated water.
Toxicity class (FDA) [1]	CA, CT, RDT
Toxicity – CA	IARC: group 1: carcinogenic to humans [18] Benzene causes acute myeloid leukemia (acute non-lymphocytic leukemia), and may also cause acute and chronic lymphocytic leukemia, non-Hodgkin's lymphoma and multiple myeloma. Individuals who have experienced benzene poisoning requiring treatment show a substantially increased risk of mortality from leukemia [19]. Since 1987, there have been numerous reports from cohort studies in populations exposed to benzene, including updates of earlier reports, and new case–control studies of leukemia or its subtypes, non-Hodgkin lymphoma (NHL), multiple myeloma, and to a lesser extent other tumors in adults. There have also been several case–control studies of childhood leukemia with data on benzene, solvents, gasoline, and other related exposures. In addition, several meta-analyses have been published of one or more tumor sites. These data have all been summarized in the

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	report of IARC on chemical agents and related occupations [18].
Toxicity – RT	-
Toxicity – CT	Cardiovascular effects, particularly ventricular fibrillation, have been suggested as the cause of death in fatal exposures to benzene vapor. Cardiovascular effects such as arrhythmias have also been demonstrated in animals after inhalation exposure [17].
Toxicity – RDT	Epidemiological studies implicating benzene as a developmental toxicant have many limitations, and thus, it is not possible to assess the effect of benzene on the human fetus. Results of inhalation studies conducted in animals are, however, fairly consistent across species and demonstrate that benzene can be fetotoxic as evidenced by decreased fetal weight and/or minor skeletal variants. Benzene has also been shown to reduce pup body weight in mice [17].
Toxicity – AD	-
Toxicity - others	Eye and skin irritant [19]. All available toxicity data can be found on the Website Aggregated Computational Toxicology Resource (ACToR) [20].

ADME properties

Absorption and distribution [17, 21] - Benzene is readily absorbed via the lungs, with 40-50% retention of the inhaled amount. It is taken up preferentially by fatty and nervous tissues. Lesser concentrations are found in the kidney, liver and spleen. Benzene crosses the human placenta, and levels in cord blood are similar to those in maternal blood. It is expected to readily bind to plasma proteins [17].

Metabolism [17, 21-23] - Available data indicate that benzene metabolism occurs mainly in the liver, and metabolites are carried to the bone marrow where benzene toxicity is expressed. However, benzene metabolism may occur, at least in part, in the bone marrow. Data regarding metabolism of benzene in humans are derived primarily from studies using inhalation exposures. Metabolism of benzene (Figure 1) includes oxidation, dehydrogenation or conjugation with glucuronic acid.

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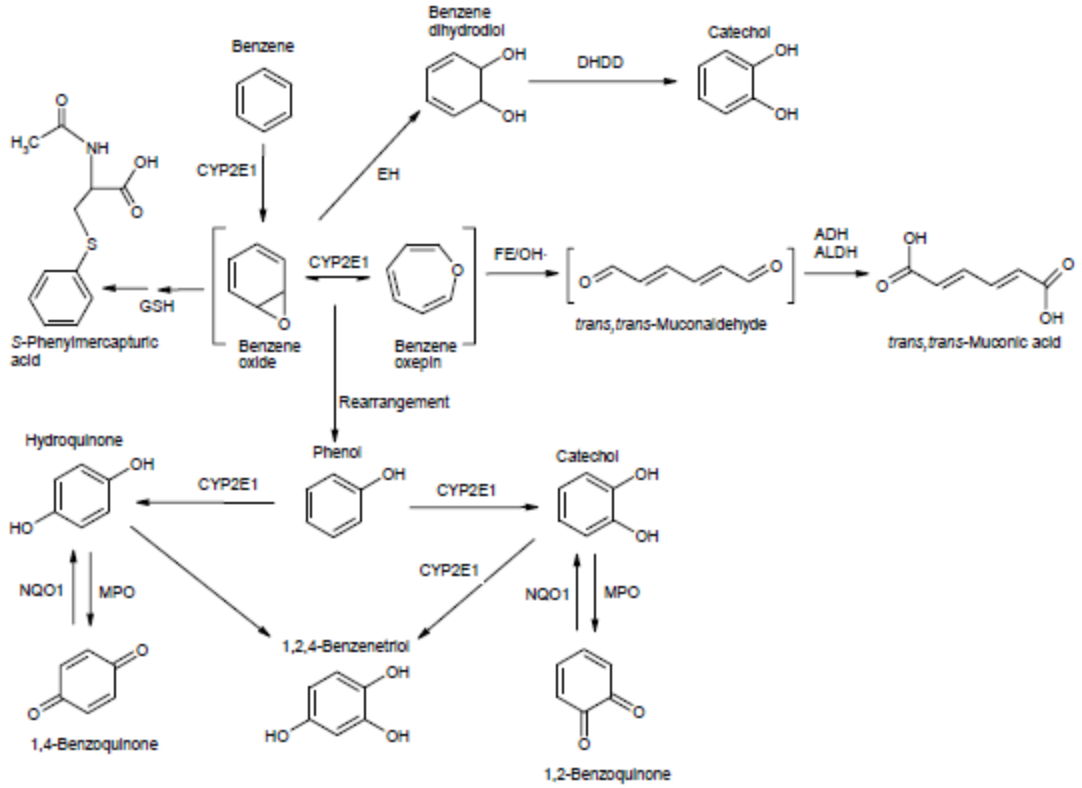


Figure 1 Metabolic pathways for benzene [17]

ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase; CYP2E1, cytochrome P-450 2E1; DHDD, dihydrodiol dehydrogenase; EH, epoxide hydrolase; GSH, glutathione; MPO, myeloperoxidase; NQO1, NAD(P)H:quinone oxidoreductase 1

Figure source: Agency for Toxic Substances and Disease Registry.

The predominant metabolic pathway of benzene, in the liver, involves non-enzymatic rearrangement of benzene oxide to form phenol, the major initial product of benzene metabolism. Phenol is oxidized in the presence of P450 CYP2E1 [23] to catechol or hydroquinone, which are oxidized via myeloperoxidase (MPO) to the reactive metabolites 1,2- and 1,4-benzoquinone, respectively (Figure 1). The reverse reaction (reduction of 1,2- and 1,4-benzoquinone to catechol and hydroquinone, respectively) is catalyzed by NAD(P)H:quinone oxidoreductase (NQ01). Both catechol and hydroquinone may be converted to the reactive metabolite 1,2,4-benzenetriol via CYP2E1 catalysis. Alternatively, benzene oxide may undergo epoxide hydrolase-catalyzed conversion to benzene dihydrodiol and subsequent dihydrodiol dehydrogenase (DHDD)-catalyzed conversion to catechol (Figure 1). Each of the phenolic metabolites of benzene (phenol, catechol, hydroquinone, and 1,2,4-benzenetriol) can undergo sulfonic or glucuronic conjugation; the conjugates of phenol and hydroquinone are major urinary metabolites of benzene. Other pathways of benzene oxide metabolism include: (1) reaction with glutathione (GSH) to form S-phenylmercapturic acid (S-PMA), and (2) iron-catalyzed ring-opening conversion to trans,trans-muconic acid (tt-MA), presumably via the reactive trans,trans-muconaldehyde (Figure 1). Although the metabolism of

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benzene has been studied extensively, the steps leading to benzene toxicity are not yet fully understood. The relative contribution that each benzene metabolite makes to the adverse health effects of benzene, both alone and in various combinations, is still a subject of some debate. Several studies have shown that benzene metabolites related to muconic acid and hydroquinone are more genotoxic than phenol or compounds related to catechol [24]. Benzene metabolites have been found to form covalent adducts with macromolecules such as albumin from blood in humans and mice which could contribute to the mechanism of toxicity, although the relationship between adduct formation and toxicity is not clear, thus remaining unlikely [17]. So far, no evidence has been found of DNA adducts of benzene in humans [25].

Excretion - The main route of excretion of unmetabolized benzene is by expired air, while urine is the major route of excretion of benzene metabolites (with very little fecal excretion) [26]. The excretion phase of unmetabolized benzene is in three phases, at 0.7-1.7 hour, 3-4 hour and 20-30 hour [27]. When administered to mice subcutaneously, 72% of the dose is recovered in expired air. In humans, benzene is still detected in expired air 24 hour after inhalation. In men and women exposed to 52-62 ppm benzene for 4 hr, a mean of 46.9% was taken up, 30.2% was retained and the remaining 16.8% excreted as unchanged benzene in expired air [28] (p. 117). The most frequent route by which humans are exposed to benzene is via inhalation. Toxic effects observed in humans have generally been attributed to a combination of inhalation and skin exposure.

Subjects who inhaled concentrations of 340 mg/m^3 (106 ppm) benzene in air for 5h excreted 29% as phenol, 3% as catechol and 1% as hydroquinone in the urine [27]. Most of the phenol and catechol was excreted within 24 hr, and the hydroquinone within 48h. In monkeys dosed intra-peritoneally with 5-500 mg/kg radiolabeled benzene, the proportion of radioactivity excreted in the urine decreased with increasing dose, whereas more benzene was exhaled unchanged, indicating saturation of benzene metabolism at higher doses [17].

Trans,trans-muconic acid (t,t-MA), phenol and S-PMA represent 3.9, 40 and 0.11%, respectively, of the retained dose excreted in urine [22]. Catechol and hydroquinone represent 3 and 1%, respectively, of the excretion of benzene, with most catechol excreted within 24h, and most hydroquinone within 48h [27]. Both catechol and hydroquinone being smoke constituents themselves, they would be unreliable indicators of exposure to benzene.

Although t,t-MA generally shows a good correlation with medium to high levels benzene exposure, it has been found in subjects not exposed to benzene. Also, the ratio of t,t-MA to total benzene metabolites in urine decreased with increased exposure to benzene [24]. In addition, the levels t,t-MA in the urine of smokers or non-smokers was not consistently different, making it unsuitable for studies on exposure to smoke [29], even though other studies found differences (reviewed in [30]). Half-life of t,t-MA is of around 5 hours [22]. Sorbic acid, a food additive, can be metabolized to t,t-MA and can contribute to background levels of t,t-MA levels in urine, thereby decreasing its specificity as a biomarker of exposure to benzene [30, 31].

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Phenol is not a suitable BoExp for benzene exposure as the background levels are very variable caused by endogenous production of phenol as well as dietary and environmental exposure to phenol [22]. The half-life of phenol in humans is of around 5 hours [22, 32].

The half-life of S-PMA is of around 9 hours [22, 33, 34]. Its excretion is nearly constant during one day, both in smokers and in nonsmokers, suggesting no influence of the collection time [35].

The urinary excretion of unchanged benzene was also reported to be suitable BoExp to benzene, showing an exposure-related increase [36]. Blood and breath benzene levels have biological half-lives of seconds to minutes, while urinary benzene levels reflect exposures since the previous one of two voids for single exposure [37](p. 90-91). Urinary benzene levels were shown, however, to not always be able to distinguish smokers from non-smokers, making it less reliable than other markers[37](p. 91).

Exposure levels in smokers and non-smokers

As discussed before, not all metabolites of benzene can be useful as biomarkers of exposure to tobacco smoke due to their lack of specificity. With the increased sensitivity of the determination method, S-PMA is found to be significantly different between smokers and non-smokers [22], but an older study had not found any difference [33]. An important step for the determination of S-PMA in urine is to acidify the sample prior to analytical determination in order to convert the pre-S-PMA to S-PMA [38]. SPMA values could otherwise be too low.

Levels of the urinary benzene metabolite S-PMA have been reported to be higher in smokers than in nonsmokers, with fold-difference going from 2- to 12-fold difference (Table 1).

A recent study in which smokers consuming 20 cigarettes per day (cpd) or more were forced to smoke exactly 20, 15, 5 or 0 cpd during a 5-day confinement period showed a dose-relationship between the number of cigarettes and the levels of urinary S-PMA (i.e. 1.0, 0.7, 0.6 and 0.4 µg/24h, respectively) [39]. Furthermore, a highly significant correlation between urinary cotinine and S-PMA was observed by Schettgen et al. in German smokers [40].

After smoking abstinence of 3 to 8 days, levels of urinary S-PMA were found to decrease by a bit more than 2-fold up to more than 20-fold (Table 2).

In a cigarette brand-switching study, subjects smoked for one week either cellulose acetate filter cigarettes or charcoal filter cigarettes and then switched to the corresponding brand with the other filter type for the second week [41]. Determination of various biomarkers of exposure showed that, among others, the urinary excretion rate of S-PMA was significantly lower (about 30%) when smoking charcoal compared to cellulose acetate filter cigarettes. In another study, urinary S-PMA levels were even reduced about 70 to 80% by switching to smoking test cigarettes with efficient charcoal filtration, and within three days after smoking cessation the S-PMA levels markedly decreased towards non-smoking levels (Table 2) [42]. The latest report of the Institute of Medicine listed S-PMA as a representative biomarker of exposure for the tobacco toxicant benzene [43].

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S-PMA

In non-smokers Ranges: 0.17-2.14 nmol/24h [40.68-512.10 ng/24h] [43]

In smokers Ranges: 3.2-32.1 nmol/24h [765.76-7681.53 ng/24h] [43]

Table 1 Representative urinary S-PMA levels in smokers and non-smokers

Author (year)	Levels in non-smokers	Levels in smokers	Units; variability
Melikian et al. (1999) [44]	4.8 ± 1.1 [4800 ± 1100]	9.1 ± 1.7 [9100 ± 1700]	µg/g creat [pg/mg creat]; mean ± SE
Scherer et al. (2007) [45]	294 ± 156	1909 ± 2029	ng/24h ; mean ± SD
Mendes et al.(2008) [46]	Not reported	7.2 ± 4.0 [7200 ± 4000]	µg/24h [ng/24h]; mean ± SD
Ding et al. (2009) [47]	nd-0.45 [nd-450]	nd-18.4 [nd-18400]	µg/g creat [pg/mg creat]; range
Lovreglio et al. (2010) [29]	1.00 ± 2.46 [1000 ± 2460]	1.51 ± 1.48 [1510 ± 1480]	µg/g creat [pg/mg creat]; mean ± SD
Lindner et al. (2011) [48]	0.39 ± 0.27 [390 ± 270]	4.13 ± 3.36 [4130 ± 3360]	µg/24h [ng/24h]; mean ± SD
Alwis et al. (2012) [49]	0.60 ± 0.40	0.92 ± 2.11	ng/mL; mean ± SD
Pluym et al. (2015) [50]	0.018 (<LOQ-0.097) [18 (<LOQ-97)]	1.1 (0.2-3.5) [1100 (200-3500)]	µg/g creat [pg/mg creat]; median (min-max)

Table 2 Representative urinary S-PMA levels after smoking cessation

Author (year)	Levels in quitters	Levels in smokers	Units; variability
Feng et al. (2006) [51]	0.32 ± 0.26 [a] [320 ± 260]	4.49 ± 4.29 [4490 ± 4290]	µg/24h [ng/24h]; mean ± SD
Sarkar et al. (2008) [42]	0.27 ± 0.05 [a] [270 ± 50]	4.98 ± 2.89 [4980 ± 2890]	µg/g creat [pg/mg creat]; mean ± SD
Frost-Pineda et al. (2008) [52]	0.4 ± 0.1 [a] [400 ± 100]	6.0 ± 3.9 [6000 ± 3900]	µg/24h [ng/24h]; mean ± SD
Carmella et al. (2009) [53]	0.396 ± 0.345 [b] [94.76 ± 82.56]	3.20 ± 3.80 [765.76 ± 909.34]	nmol/24h [ng/24h]; mean ± SD
Tricker et al. (2012) [54]	0.53 ± 1.03 [c] [530 ± 1030]	3.75 ± 3.55 [3750 ± 3550]	µg/24h [ng/24h]; mean ± SD
Tricker et al. (2012) [55]	0.22 ± 0.08 [a] [220 ± 80]	4.72 ± 3.43 [4720 ± 3430]	µg/24h [ng/24h]; mean ± SD
Tricker et al. (2012) [56]	1.37 ± 0.42 [a] [1370 ± 420]	3.41 ± 1.88 [3410 ± 1880]	µg/24h [ng/24h]; mean ± SD
Tricker et al. (2012) [57]	0.34 ± 0.23 [a] [340 ± 230]	3.54 ± 0.34 [3540 ± 340]	µg/24h [ng/24h]; mean ± SD
Theophilus et al. (2015) [39]	0.4 ± 0.2 [c] [400 ± 200]	0.9 ± 0.4 [900 ± 400]	µg/24h [ng/24h]; mean ± SD

[a] Smoking cessation for 8 days

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[b] Smoking cessation for 3 days
[c] Smoking cessation for 5 days

List of abbreviations

3R4F	Kentucky 3R4F reference research cigarette [58]
ACToR	Aggregated Computational Toxicology Resource
AD	Addictive
ADME	Absorption and Distribution, Metabolism, and Excretion
CA	Carcinogen
CAS number	Chemical Abstracts Service number
cpd	Cigarettes per day
Creat	Creatinine
CT	Cardiovascular toxicant
FDA	Food and Drug Administration
HC	Health Canada
HCI	Health Canada Intense regime [59]
HPHC	Harmful and Potentially Harmful Constituents
IARC	International Agency for Research on Cancer
ISO	International Organization for Standardization smoking regime [60]
IUPAC	International Union of Pure and Applied Chemistry
MPO	Myeloperoxidase
MW	Molecular weight
NHL	Non Hodgkin lymphoma
PMI	Philip Morris International
RDT	Reproductive or developmental toxicant
RT	Respiratory toxicant
SD	Standard deviation
SE	Standard error
t,t-MA	Trans,trans-muconic acid
WHO	World Health Organization

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