APPENDIX A6.1.3.16 PYRENE - FACTSHEET

	HPHC	BoExp assessed by PMI (matrix)
IUPAC name	Pyrene	Total 1-hydroxypyrene (in urine)
Usual abbreviation		1-OHP; 1-HOP
Other names	Benzo[def]phenanthrene; pyrene[def]phenanthrene	1-Pyrenol; 3-hydroxypyrene
CAS number	129-00-0	5315-79-7
Formula and molecular weight	$C_{16}H_{10}$ MW: 202.25	$C_{16}H_{10}O$ MW: 218.25

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Main characteristics

Chemical class	Polycyclic aromatic hydrocarbons (PAH)
HPHC lists	None. Pyrene has often been used in occupational and environmental studies, as an indicator of exposure to PAH chemicals in general. As it is often part of PAH mixtures, data often refer to PAHs in general, and not only to pyrene.
Source in cigarettes	Amount in MSS (3R4F): 38.0 ng (ISO); 92.5 ng (HCI) [1] Smoke phase: particulate phase [2] Presence in tobacco / pyrosynthesis (T°): present in uncombusted tobacco [3] / main generation range 700–800°C [3]. There are many precursors of B[a]P in tobacco. Among the most important are terpenoids (solanesol, phytosterols), cellulose and carbohydrates [4].
Other sources [5, 6]	Pyrene occurs ubiquitously in products of incomplete combustion and in fossil fuels (mineral oils, petrol, etc…). It is found in relatively high quantities in coal tar (2% of the weight) [7]). It is commonly found in PAH mixtures, and its urinary metabolite, 1-hydroxypyrene, has been used as an indicator (surrogate) of exposure to PAHs [8]. Broiled and smoked foods are also sources of pyrene [9].
Toxicity class (FDA) [10]	-
Toxicity – CA [10, 11]	IARC: group 3: not classifiable as to their carcinogenicity to humans [5, 6, 11]
Toxicity – RT	-
Toxicity – CT	-
Toxicity – RDT	-
Toxicity – AD	-
Toxicity - others	All available toxicity data can be found on the Website Aggregated Computational Toxicology Resource (ACToR) [12].

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ADME properties

Absorption and distribution [5] – PAHs, including pyrene, are absorbed through ingestion, inhalation, and dermal contact. The percent absorbed varies in these studies for several reasons, including the vehicle (transport medium) in which the PAHs are found. In general, PAHs not bound to particulate matter may be absorbed in the lungs better than the same dose found on the surface of airborne particulate matter.

Once absorbed, PAHs enter the lymph, circulate in the blood, and are metabolized primarily in the liver and kidney. PAHs differ with respect to distribution patterns and lipophilic properties. Because of their lipophilic nature, PAHs can accumulate in breast milk and adipose tissue. However, biliary and urinary excretion of PAHs is relatively efficient because of the wide distribution of enzymes that transform PAHs into polar metabolites.

Metabolism [6] – PAHs, including pyrene, are predominantly metabolized via CYP enzymes in the liver. In addition to the liver and kidneys, metabolism of PAHs occurs in the adrenal glands, testes, thyroid, lungs, skin, sebaceous glands, and small intestines. Pyrene is primarily (ca 90%) metabolized to 1-hydroxypyrene (1-OHP) by CYP1A enzymes and is renally eliminated as glucuronide and sulfate conjugates (total 1-OHP) with a half-life of about 20 h [13].

Excretion [5, 14] - Metabolism is a prerequisite for hepatobiliary excretion and elimination through the feces, regardless of route of entry. The hydroxylated metabolites of the PAHs are excreted in human urine both as free hydroxylated metabolites and as hydroxylated metabolites conjugated to glucuronic acid and sulfate. A commonly measured urinary metabolite of pyrene is 1-OHP, together with its glucuronide and sulfate conjugates (i.e. total 1-OHP). The conjugate of 1-OHP with glucuronide is the predominant form of pyrene metabolite in urine [15]. Excretion half-lives in feces and urine have been reported in animal studies as 22 hours and 28 hours, respectively [16].

Two human volunteers were exposed to pyrene by ingestion and by dermal application, in two separate experiments. Urinary measurements of 1-OHP were performed for a total period of 48 h after dosing. Following the absorption phase, 1-OHP was excreted with a first order apparent half-life of approximately 12 h for both volunteers and both exposure routes [17]. Similar half-lives were found in a study where volunteers had five consecutive daily dermal application of a coal tar-based shampoo or creosote, a coal-tar derivative that contains PAHs. The excretion peaked between 10 and 15 h after application and first-order apparent half-lives of 11.5-15 h could be calculated for the elimination phase [18].

Exposure levels in smokers and non-smokers

Pyrene, although not carcinogenic, is one of the PAHs found in cigarette smoke and is often used as an indicator of total PAH exposure [19]. 1-OHP is generally considered an appropriate biomarker of exposures to PAH, as it is rapidly distributed, metabolized and eliminated from the body [20]. In addition, 1-OHP in urine represents a constant fraction (2%) of total pyrene intake and the half-life of urinary 1-OHP excretion in humans ranged from 4 to 35 h [8, 21]. Concentration levels of the urinary 1-OHP varies

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throughout the day within individuals, with an apparent diurnal pattern (i.e. higher concentration in the early and late hours of the day and lower concentration during the middle of the day). Creatinine normalization of spot urine collections should remove or at least reduce this daily pattern, but it is unlikely that all published half-lives have been calculated on the basis of adjusted levels. Also, 24h-urine collection shows less variability in the biomonitoring of PAH, but this is more labor intensive and costly. Background-adjusted half-life of 1-OHP is likely to be of around 4 hours [22-24].

A substantial variability in biologic response to PAHs, including pyrene, is also expected because of inter-individual differences in the activity of the enzyme systems involved in their metabolism. Enzyme polymorphisms have been suggested to explain inter-individual differences in the rate of metabolism and activation/deactivation of PAH-derived carcinogens, which can be of up to two-fold [25]. CYP1A2, CYP1A1, and GSTM1 gene–gene interactions could be important confounders in the interpretation of molecular epidemiology studies [26]. The highest 1-OHP levels were observed in individuals carrying the CYP1A1 Ile/Val genotype who were also of the GSTM1 null genotype [25].

In smokers, 2- to 5-fold higher levels of 1-OHP were reported compared to non-smokers (Table 1). Also, a reduction by 70% in mean 1-OHP values in groups using an electrically heated cigarette compared to a group continuing to smoke conventional cigarettes was reported [27-30]. The levels of urinary 1-OHP can be influenced by the diet, but also by environmental and occupational exposures [9]. Mean levels of urinary 1-OHP appeared to be strongly associated with cigarette consumption, increasing with higher consumption [31]. After adjustment for urinary creatinine excretion, there also appeared to have a gender effect on 1-OHP levels, with levels being higher in females. Furthermore, LS mean, values of total 1-OHP excretion per day were lower with lower tar yield categories [32], but this was not demonstrated in a study by Melikian et al. [33]. 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 1-OHP (i.e. 251, 167, 127 and 114 mg/24h, respectively, even though the unit reported should certainly be ng/24h) [34]. In smoking cessation studies, the levels of urinary 1-OHP decreased by 2- to 4-fold in studies of 5 to 8 days abstinence (Table 2).

TOTAL 1-OHP

In non-smokers	Ranges: 0.18–0.50 nmol/24h [39.29-109.13 ng/24h] [35]
In smokers	Ranges: 0.50–1.45 nmol/24h [109.13-316.46 ng/24h] [35]

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Table 1 Representative urinary 1-OHP levels in smokers and non-smokers

Author (year)	Levels in smokers	Levels in non-smokers (or ex-smokers)	Units; variability
Scherer (2007) [36]	0.196 ± 0.145 [196 ± 145]	0.101 ± 0.070 [101 ± 70]	µg/24h [ng/24h]; mean ± SD
Shepperd (2013) [37]	254 ± 101 (71-511)	105 ± 65 (37-336)	ng/24h; LS means ± SD (ranges)
Barbeau (2011) [38]	162 ± 90 [313 ± 174]	42 ± 33 [81 ± 64]	nmol/mol creat [pg/mg creat]; mean ± SD
Lindner (2011) [31]	290.6 ± 352.2	122.5 ± 100.5	ng/24h; mean ± SD
Mendes (2008) [39]	232.1 ± 108.9	NM	ng/24h; mean ± SD
St. Helen (2012) [40]	2.76 [a] (2.46, 3.09) [602.37 (536.90, 674.39)]	1.12 [a] (0.96, 1.30) [244.44 (209.52, 283.73)]	pmol/mg creat [pg/mg creat]; geometric mean (95% CI)
St. Helen (2012) [40]	1.14 [c] (1.03, 1.26) [248.81 (224.80, 275)]	0.51 [c] (0.43, 0.61) [111.31 (93.85, 133.13)]	pmol/mg creat [pg/mg creat]; geometric mean (95% CI)
Buratti (2007) [41]	0.41 ± 0.30 (0.33) [410 ± 300 (330)]	0.28 ± 0.29 (0.18) [280 ± 290 (180)]	µg/L [pg/mL]; mean ± SD (median)
Melikian (2007) [33]	Low tar 378 (318-448)	NM	pg/mg creat; geometric mean (95% CI)
Melikian (2007) [33]	Medium tar 396 (334-470)	NM	pg/mg creat; geometric mean (95% CI)
Melikian (2007) [33]	High tar 347 (279-432)	NM	pg/mg creat; geometric mean (95% CI)
Jongeneelen (1988) [8]	0.28 (0.09-1.31) [540 (174-2528)]	0.26 (0.02-0.66) [502 (39-1273)]	µmol/mol creat [pg/mg creat]; median (90% CI)
Roethig (2009) [19]	317 ± 6.8	110 ± 7.1	ng/24h; weighed mean ± SE
Roggi (1997) [42]	0.408 ± 0.312 [408 ± 312]	0.233 ± 0.41 [233 ± 410]	µg/g creat [pg/mg creat]; mean ± SD
Sithisarankul (1997) [43]	1.04 ± 0.13 [227 ± 28]	0.55 ± 0.05 [120 ± 11]	pmol/mL [pg/mL]; mean ± SE
Merlo (1998) [44]	0.176 ± 0.172 [340 ± 332]	0.089 ± 0.095 [172 ± 183]	µmol/mol creat [pg/mg creat]; mean ± SD

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Author (year)	Levels in smokers	Levels in non-smokers (or ex-smokers)	Units; variability
Scherer (2000) [45]	0.346 ± 0.048 [346 ± 48]	0.171 ± 0.020 [171 ± 20]	µg/24h [ng/24h]; mean ± SE
Pastorelli (1999) [46]	0.54 ± 0.31 [540 ± 310]	0.24 ± 0.24 [240 ± 240]	ng/mL [pg/mL]; mean ± SD
Jacob (1999) [47]	603 ± 483	218 ± 159	ng/24h; mean ± SD
Li (2000) [48]	0.46 ± 0.36 [888 ± 695]	0.04 ± 0.03 [77 ± 58]	µmol/mol creat [pg/mg creat]; mean ± SD
Nerurkar (2000) [49]	0.51 (0.34–0.69)	0.27 (0.22–0.32)	nmol/12h; geometric mean (95% CI)
Dor (2000) [50]	0.04 (0.02-0.06) [77 (39-116)]	0.02 (0.01-0.04) [39 (19-77)]	µmol/mol creat [pg/mg creat]; median (95% CI)
Alexandrie (2000) [25]	0.17 (0.09–0.99) [328 (174-1910)]	0.10 (0.02–0.50) [193 (39-965)]	µmol/mol creat [pg/mg creat]; median (range)
Nan (1991) [51]	0.05 ± 2.04 [96 ± 3936]	0.04 ± 2.43 [77 ± 4688]	µmol/mol creat [pg/mg creat]; geometric mean ± SD
van Delft (2001) [52]	0.70 ± 0.39 [1351 ± 752]	0.27 ± 0.22 [521 ± 424]	µmol/mol creat [pg/mg creat]; mean ± SD
Kim (2001) [53]	0.05 ± 0.04 [96 ± 77]	0.03 ± 0.02 [58 ± 39]	µmol/mol creat [pg/mg creat]; mean ± SD
Szaniszlo (2001) [54]	0.57 ± 0.38 [1100 ± 733]	0.11 ± 0.05 [212 ± 96]	µmol/mol creat [pg/mg creat]; arithmetic mean ± SD
Morin (2011) [55]	350 (315-385) [d]	91 (72-109)	ng/24h; mean (95% CI)

[a] In Polish participants
[b] Adjusted by sex and age
[c] In US participants
[d] In subjects smoking 14 cigarettes/day

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Table 2 Representative urinary 1-OHP levels after smoking abstinence or switching to a reduced toxicant prototype

Author (year)	Levels in smokers	Levels in ex-smokers or switchers	Units; variability
Feng (2006) [27]	0.14 ± 0.06 [140 ± 60]	0.04 ± 0.01 [40 ± 10] [a]	µg/24h [ng/24h]; mean ± SD
Carmella (2009) [56]	1.36 ± 0.76 [296.82 ± 165.87]	0.83 ± 1.07 [181.15 ± 233.53] [b]	nmol/24h [ng/24h]; mean ± SD
Tricker (2012) [57]	136.7 ± 50.6	37.4 ± 11.2 [c]	ng/24h; mean ± SD
Tricker (2012) [58]	191.4 ± 68.8	75.1 ± 54.2 [a]	ng/24h; mean ± SD
Tricker (2012) [59]	236.02 ± 68.18	127.04 ± 24.71 [a]	ng/24h; mean ± SD
Tricker (2012) [60]	174.7 ± 77.6	42.0 ± 15.3 [a]	ng/24h; mean ± SD
Frost-Pineda (2008) [28]	160.2 ± 82.5	41.1 ± 17.5 [a]	ng/24h; mean ± SD
Theophilus (2015) [34]	313 ± 137	114 ± 53.4 [c]	mg/24h [d]; mean ± SD

[a] After 8 days of smoking cessation

[b] After 3 days of smoking cessation

[c] After 5 days of smoking cessation

[d] Values in the publication are reported in mg/24h, but it is likely to be a typo error, and should probably read "ng/24h"

List of abbreviations

3R4F	Kentucky 3R4F reference research cigarette [61]
ACToR	Aggregated Computational Toxicology Resource
AD	Addictive
ADME	Absorption and Distribution, Metabolism, and Excretion
CA	Carcinogen
CAS number	Chemical Abstracts Service number
CI	Confidence interval
Creat	Creatinine
CT	Cardiovascular toxicant
FDA	Food and Drug Administration
GSTM1	Glutathione S-Transferase Mu 1

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HC	Health Canada
HCI	Health Canada Intense regime [62]
IARC	International Agency for Research on Cancer
ISO	International Organization for Standardization smoking regime [63]
IUPAC	International Union of Pure and Applied Chemistry
LS	Least squares
MW	Molecular weight
NM	Not measured
PAH	Polycyclic aromatic hydrocarbon
PMI	Philip Morris International
RDT	Reproductive or developmental toxicant
RT	Respiratory toxicant
SD	Standard deviation
SE	Standard error
WHO	World Health Organization

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