APPENDIX A6.1.3.15 BENZO[A]PYRENE - FACTSHEET

	HPHC	BoExp assessed by PMI (matrix)
IUPAC name	Benzo[a]pyrene	Total 3-hydroxybenzo[a]pyrene (in urine)
Usual abbreviation	B[a]P	Total 3-OH-B[a]P
Other names	3,4-benzopyrene, 6,7-benzopyrene, 3,4-benzpyrene, benzo[def]chrysene	
CAS number	50-32-8	3-OH-B[a]P: 13345-21-6
Formula and molecular weight	$C_{20}H_{12}$ MW: 252.31	$C_{20}H_{12}O$ MW 268.31

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Main characteristics

Chemical class	Polycyclic aromatic hydrocarbon (PAH)
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 in mainstream smoke (3R4F): 6.73 ng/cigarette (ISO); 16.2 ng/cigarette (HCI) [7] Smoke phase: particulate phase [8] Presence in tobacco / pyrosynthesis (T°): can be found in uncombusted tobacco, due to ambient air contamination and generation of BaP in the curing process [9] / Pyrosynthesis in ranges 500-800°C [10], but major production between 400-600°C [11]. There are many precursors of B[a]P in tobacco. Among the most important are terpenoids (solanesol, phytosterols), cellulose and carbohydrates [12].
Other sources [6]	B[a]P and other polycyclic aromatic hydrocarbons (PAHs) are environmental contaminants formed during incomplete combustion or pyrolysis of organic material. Benzo[a]pyrene is a prototypic representative of the PAH class of carcinogens, which are products of the incomplete combustion of organic matter and occur in cigarette smoke and the general environment in a mixture with other PAHs. Major sources of PAHs, including B[a]P, in ambient air (both outdoors and indoors) include residential and commercial heating with wood, coal or other biomasses (oil and gas heating produce much lower quantities of PAH), other indoor sources such as cooking and tobacco smoke, and outdoor sources like motor-vehicle exhaust (especially from diesel engines), industrial emissions and forest fires. These substances are found in air, water, soils and sediments, generally at trace levels except near their sources. PAHs are present in some foods and in a few pharmaceutical products based on coal tar that are applied to the skin [13, 14]. PAHs can contaminate foods by three major routes, i.e. through pyrolysis during the grilling/barbecuing of meat products, by methods of preserving meat and other food products [15] (p. 102), or by ingestion of leafy vegetables (contaminated by PAH from ambient air) [16]. Dietary intake of B[a]P was reported from 40 to 2800 ng per day in the US [15] (p. 105). Industrial processes that involve the pyrolysis or combustion of coal and the production and use of coal-derived products are major sources of PAHs [15] (p. 35).

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Toxicity class (FDA) [1]	CA
Toxicity – CA [6, 13, 17]	IARC: group 1: carcinogenic to humans [13] B[a]P is considered as a surrogate of PAHs in mainstream smoke and is referred as evidence that would support an important role for PAHs as causes of lung cancer and other cancers in smokers [6, 17]. No epidemiological data on benzo[a]pyrene alone are available. In humans, occupational exposures to benzo[a]pyrene-containing mixtures have been associated with several different cancers: coke production: lung; coal gasification: lung, bladder; paving and roofing: lung; coal tar distillation: skin; soots: lung, esophagus, hematolymphatic system, skin; aluminum smelting: lung, bladder; tobacco smoking: lung, lip, oral cavity, pharynx, esophagus, larynx, bladder [13]. There is sufficient evidence for the carcinogenicity of B[a]P in experimental animals [13]. The current understanding of mechanisms underlying B[a]P-induced carcinogenesis in experimental animals is almost solely based on two pathways: metabolism to diolepoxides and radical cations.
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) [18]

ADME properties

Absorption and distribution [19] - B[a]P is readily absorbed from the intestinal tract and tends to localize primarily in body fat and fatty tissues such as breast.

The distribution kinetics of B[a]P after intravenous administration to rats can be used as a model for inhalation exposure, as after the first passage through the heart, the lungs will be exposed before systemic distribution [20]. B[a]P was found to be retained in the lungs of rats with slow elimination kinetics compared to other organs (except the adipose tissue). Based on these data, the elimination half-time from the lungs to the blood was estimated to be approximately 200 hours, while the metabolic elimination rate in the lungs was estimated to be about five times faster.

Due to its high lipophilicity, B[a]P may be retained in the lipophilic membranes of the cells first encountered and only slowly distribute systemically. The toxicokinetic data found in the various sources are complex and not entirely consistent. Nevertheless, it can

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be expected that inhaled B[a]P is retained in the respiratory tract tissue long enough for local metabolic activation, and the parent compound is only slowly released to the circulation. Due to its high affinity to adipose tissue, the circulating concentration of B[a]P seems to be relatively small. Accumulation of B[a]P upon repeated inhalation exposures does not seem to be very likely for most tissues, but this may be relevant for the lungs (and the adipose tissue).

Metabolism [13, 19, 21-23] - The complete sequence of steps in the metabolic activation pathway of B[a]P to mutagenic and carcinogenic diol epoxides has been demonstrated in experimental animals, in human tissues and in humans.

Benzo[a]pyrene is metabolized by both phase-I and phase-II enzymes to form a series of arene oxides, dihydrodiols, phenols, and quinones and their polar conjugates with glutathione, sulfate, and glucuronide. Benzo[a]pyrene-7,8-diol is a key metabolite that is formed by the action of epoxide hydrolase on benzo[a]pyrene-7,8-epoxide. This dihydrodiol can be further metabolized by cytochrome P450s (CYPs) to a series of benzo[a]pyrene-7,8-diol-9,10-epoxides (BPDE), which form one class of ultimate carcinogenic metabolites of benzo[a]pyrene. Both CYPs and peroxidases (e.g. prostaglandin-H synthase) can oxidize benzo[a]pyrene. The major cytochrome P450s involved in the formation of diols and diolepoxides are CYP1A1, CYP1A2 and CYP1B1. Both CYPs and peroxidases can form radical cations by one-electron oxidation. These cations comprise another class of ultimate carcinogenic metabolites. Some polymorphisms in human CYPs and phase-II enzymes (glutathione S-transferases, uridine 5′-diphosphate glucuronosyltransferases and sulfotransferases modulate susceptibility to cancer [24]. In another metabolic pathway, benzo[a]pyrene-7,8-dihydrodiol is oxidized to benzo[a]pyrene-7,8-quinone by enzymes of the aldo-keto reductase (AKR1) family. Among these, gene polymorphisms that influence susceptibility have been identified. NAD(P)H: quinone oxidoreductase-1 (NQO1) catalyzes the reduction of benzo[a]pyrene quinones to hydroquinones, which may be re-oxidized and generate reactive oxygen species (semi-quinone radicals). Polymorphisms in this gene have also been described. The current understanding of mechanisms underlying benzo[a]pyrene-induced carcinogenesis is almost solely based on two complementary pathways, i.e those of the diolepoxides and the radical cations.

Individuals with the (linked) CYP1A1*2 or *3 variant alleles have a greater capacity to activate PAHs, including Benzo[a]pyrene from tobacco smoke and occupational exposure and, as a result, are at greater risk for PAH-related cancers, especially certain respiratory cancers [25].

B[a]P is metabolized to approximately 20 primary and secondary oxidized metabolites and to a variety of conjugates (Figure 1). 3-Hydroxybenzo[a]pyrene (3-OH-B[a]P) represents about 40% of the metabolite fractions [26, 27], and exists as sulphate and glucuronide conjugates (30-40 % of the total metabolites) or in free form (4 % of total metabolites) [27]. Several metabolites can induce mutations, transform cells and/or bind to cellular macromolecules, however, only the 7,8-diol-9,10-epoxide is presently considered to be the ultimate carcinogenic metabolite. Following exposure to B[a]P, humans metabolically activate B[a]P to form DNA adducts with deoxyguanosine, which

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have been measured in populations (e.g., coke-oven workers, chimney sweeps) exposed to PAH mixtures that contain B[a]P [14, 15]. BPDE is not the only B[a]P derived metabolite that can form adducts, but it generates the adduct shown to be associated with cancer [28]. The reactive anti-benzo[a]pyrene-7,8-diol-9,10-oxide induces mutations in rodent and human cells, in the K-RAS proto-oncogene and in TP53 . Similar mutations were found in lung tumors from non-smokers exposed to PAH-rich coal combustion products that are known to contain benzo[a]pyrene (as well as many other PAHs) [29].

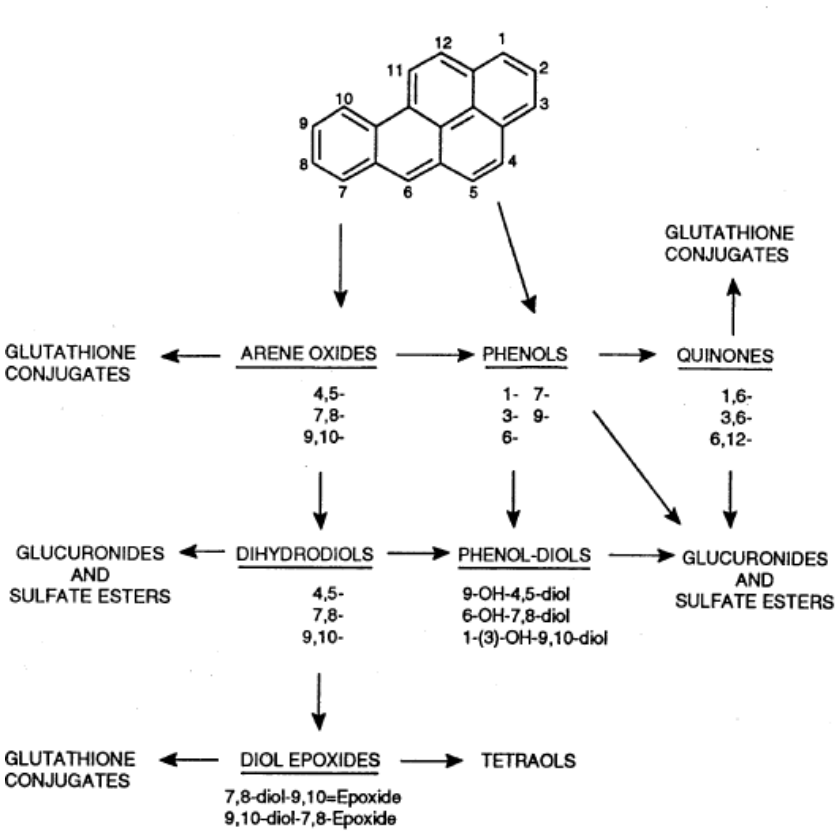


Figure 1 Proposed metabolic scheme for B[a]P [14]

CYP, cytochrome P450; EH, epoxide hydrolase; GSH, glutathione; PS, prostaglandin H synthase; QR, quinone reductase

Figure source: Agency for Toxic Substances and Disease Registry.

Excretion [19] - Disappearance of B[a]P from blood and liver of rats following single i.v. injections is very rapid, having a half-life in blood of less than 5 min and a half-life in liver of 10 min. In blood and liver the initial rapid elimination phase is followed by slower disappearance phase, lasting 6 h or more. A rapid equilibrium is established between B[a]P in blood and that in liver and the compound fast disappearance from blood is due to metabolism and distribution in tissues.

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The half-life of the B[a]P-deoxyribonucleoside adducts and the total radioactivity bound to the DNA is about 5 days. Half-life of 3-OH-B[a]P in urine is 3-4 h (humans) [30]. Reference values for animals and humans can be found in the paper by Chien et al. [30].

There are only very few data measuring levels of total 3-OH-B[a]P (i.e., total of the free and conjugated forms of 3-OH-B[a]P) in the urine smokers and non-smokers [31-34]. Instead, a specific metabolite of pyrene, 1-hydroxypyrene, and its conjugates in urine are commonly used as a surrogate biomarker of human exposure to PAHs [35, 36]. More than twenty monohydroxy PAH metabolites have now been described [37], but the metabolite of pyrene, 1-OHP, has been most often used for biomonitoring exposure to PAHs.

Surrogate marker

Pyrene, although not carcinogenic, is one of the PAHs found in cigarette smoke and is often used as an indicator of total PAH exposure [38]. It 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 [39].

1-Hydroxypyrene (1-OHP) is generally considered an appropriate biomarker of exposure to PAHs, as it is rapidly distributed, metabolized and eliminated from the body, at least in rats [40]. In addition, 1-hydroxypyrene in urine represents a constant fraction (2%) of total pyrene intake and the half-life of urinary 1-hydroxypyrene excretion in humans ranged from 4 to 35 h [41].

CYP1A2, CYP1A1, and GSTM1 gene–gene interactions could be important confounders in the interpretation of molecular epidemiology studies [42].The highest 1-OHP levels were observed in individuals carrying the CYP1A1 Ile/Val genotype who were also of the GSTM1 null genotype [43]. Individuals with the (linked) CYP1A1*2 or *3 variant alleles have a greater capacity to activate PAHs from tobacco smoke and occupational exposure and, as a result, are at greater risk for PAH-related cancers, especially certain respiratory cancers [25].

For more details on pyrene, refer to the “pyrene factsheet” [Appendix 6.1.3.16].

Exposure levels in smokers and non-smokers

The qualification data for both 1-OHP and total 3-OH-B[a]P remains equivocal [44]. They give approximately a two-fold difference between smokers and non-smokers in some studies [31, 45], but the ranges of values for smokers and non-smokers overlapped in other studies [32, 33, 46] (Table 1). More recently, cigarette filter analysis for B[a]P has been used to estimate B[a]P uptake from smoking (mouth-level exposure [47]). A new method measuring three monohydroxylated polycyclic aromatic hydrocarbons in human urine, detected mean levels of 1.30 ng/g creat of total 3-OH-B[a]P for non-smokers and 2.83 to 6.00 ng/g creat for smokers of 8 and 13 mg tar yield cigarettes, respectively [34]. The use of other metabolites of PAH in the context of smoking vs non-smoking has not yet been extensively studied. Ramsauer et al. suggested that measuring a profile of PAH metabolites is more informative than metabolites of a single PAH [48]. They developed and validated a high-performance liquid chromatographic method with

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tandem mass spectrometric detection (LC-MS/MS) for the determination of phenolic metabolites of naphthalene, fluorene, phenanthrene and pyrene in urine of smokers and non-smokers. Their method applied to urine samples of 100 smokers and 50 non-smokers showed significant differences between both groups for all measured PAH metabolites, and strong correlations with daily cigarette consumption.

The determination of 1-OHP together with its glucuronide and sulfate conjugates has become the most prevalent urinary marker in more recent studies of PAH exposure from MS. In smokers, 2- to 5-fold higher levels were reported compared to non-smokers. Also for 1-OHP, a reduction by 70% in mean values in groups using an electrically heated cigarette compared to a group continuing to smoke conventional cigarettes was reported [49-52], but another study did not report an alteration in mean values on smoking cessation [53]. Only few studies have assessed levels of total 3-OH-B[a]P in smokers [31, 32, 34, 44, 45], thus its utility as biomarker of exposure has not yet been fully demonstrated. However, 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 total 3-OH-B[a]P (i.e. 204.0, 127.0, 89.6, and 67.8 pg/24h, respectively) [54]. In the same study, smoking abstinence for 5 days showed reduction of urinary total 3-OH-B[a]P levels by 3.5-fold. Furthermore, a reduction by about 3-fold of total 3-OH-B[a]P levels was observed when switching from conventional cigarettes to snus oral tobacco consumption or after smoking cessation for 8 days [31], but long-term data comparisons in tobacco product switching or smoking cessation studies and other properties like urinary accumulation kinetics were not found.

Table 1. Representative urinary 3-OH-B[a]P levels in smokers and non-smokers or quitters

Author (year)	Levels in smokers	Levels in non-smokers (or quitters)	Units; variability
Lafontaine (2006) [32]	<0.01-0.084 (0.030; 0.023)	<0.01-0.045 (0.014; 0.011)	nmol/mol creat; arithmetic mean (median)
Zhang (2015) [34]	2.83 ± 1.78 (8mg tar) [2830 ± 1780] [a] 6.00 ± 4.44 (13mg tar) [6000 ± 4440]	1.30 ± 0.20 [1300 ± 200] [a]	ng/g creat [fg/mg creat]; mean ± SD
Sarkar (2010) [31]	162.70 ± 126.33	55.93 ± 21.71 [b]	pg/24h; mean ± SD
Theophilus (2015) [54]	242.0 ± 138	67.8 ± 36.7 [c]	pg/24h; mean ± SD

[a] Reported values are strikingly high, for unknown reasons

[b] After 8 days of smoking cessation

[c] After 5 days of smoking cessation

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List of abbreviations

3R4F	Kentucky 3R4F reference research cigarette [55]
ACToR	Aggregated Computational Toxicology Resource
AD	Addictive
ADME	Absorption and Distribution, Metabolism, and Excretion
CA	Carcinogen
CAS number	Chemical Abstracts Service number
Creat	Creatinine
CT	Cardiovascular toxicant
FDA	Food and Drug Administration
HC	Health Canada
HCI	Health Canada Intense regime [56]
IARC	International Agency for Research on Cancer
ISO	International Organization for Standardization smoking regime [57]
i.v.	Intravenous
IUPAC	International Union of Pure and Applied Chemistry
MW	Molecular weight
PAH	Polycyclic aromatic hydrocarbon
PMI	Philip Morris International
RDT	Reproductive or developmental toxicant
RT	Respiratory toxicant
WHO	World Health Organization

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