Polycyclic aromatic hydrocarbons
(Benzo[a]pyrene)
Toxicological Overview

Key Points

Kinetics and metabolism
- PAHs are absorbed by all routes of exposure
- PAHs are distributed widely throughout the body, with fatty tissues tending to show higher amounts
- metabolites of PAH’s are generally excreted as conjugates of GSH, glucuronic acid or sulphate in the urine, faeces and via biliary excretion

Health effects of acute exposure
- few studies were identified that reported the effects of BaP alone in humans following acute inhalation, ingestion or dermal exposure

Health effects of chronic exposure
- chronic exposure to mixtures of PAHs in air have resulted in a range of respiratory effects, ischemic heart disease, chronic dermatitis, depressed immune system and cancer of the skin and lungs
- BaP amongst other PAHs is able to form DNA adducts which are likely key to their mutagenic potential
- complex mixtures of PAHs which include BaP are considered to be carcinogenic to humans
Summary of Health Effects

PAHs typically occur in complex mixtures and not as individual compounds, BaP is considered to be one of the most toxic PAHs and has been extensively studied. For the general public, the main route of exposure to poly aromatic hydrocarbons (PAHs) is ingestion of food. For smokers, the contribution of smoking to total PAH exposure will be similar to that of food. Inhalation and skin absorption are the main routes of occupational exposure.

PAHs are rapidly distributed throughout the body following all routes of exposure; they may be detected in most tissues minutes to hours following exposure. Benzo[a]Pyrene (BaP) is metabolised by cytochrome P450 enzymes and epoxide hydrolase resulting in a number of metabolites being formed. These metabolites include the reactive epoxide BaP 7,8 diol-9,10-epoxide (BPDE), which is believed to be play a role in the carcinogenicity of BaP. Following metabolism PAHs are excreted in the urine and/or faeces depending on their molecular weight.

No data on the acute effects of BaP in humans were identified and few studies were reported in animals. Following acute oral exposure of rats to BaP, effects on the liver were observed.

Following chronic exposure to PAHs in an occupational setting a decrease in lung function was reported, as well as chest pain, respiratory irritation, cough, dermatitis and depressed immune system; although in most cases it was not possible to evaluate the contribution of BaP to such effects. Few adverse effects were observed in rats or hamsters exposed to BaP via inhalation. Following ingestion, myelotoxicity was observed in poor affinity aryl hydrocarbon-receptor (AhR) mice but not in high affinity mice. Hepatotoxicity was also reported.

BaP can cross the placenta; BaP-DNA adducts have been found in foetal cord blood and also in the sperm. Presence of these adducts has been associated with reduced sperm count in men and decreased motor development in infants. BaP was found to cause adverse developmental and reproductive effects in mice and rats. Dietary administration during gestation reduced fertility and fetal abnormalities whereas administration by gavage caused an increase in fetal death and decreased fertility.

Biomarkers of exposure to BaP are seen concurrently with biomarkers of genotoxic effect. The International Agency for Research on Cancer (IARC) has stated that BaP likely contributes to the genotoxic action of complex PAH mixtures that individuals may be exposed to occupationally.

IARC has classified BaP as carcinogenic to humans (Group 1). No epidemiological studies of exposure to BaP alone in man can be identified; however sufficient data exists for exposure to complex PAH mixtures containing BaP.
Introduction

PAHs are a large group of compounds consisting of hydrocarbons containing two or more benzene rings fused together or to other hydrocarbon rings. They are formed during the combustion of carbonaceous material at high temperatures [1]. PAHs typically occur in complex mixtures and not as individual compounds, the process generating the mixture may define its composition. BaP is considered to be one of the most toxic PAHs and has therefore been extensively studied; hence the majority of the data in this document refers to BaP. Unless otherwise specified, “PAHs” will refer to a mixture of PAH compounds (which may include BaP).

Kinetics and Metabolism

PAHs are lipophilic compounds that are readily absorbed from the lungs following inhalation, the gastrointestinal (GI) tract following ingestion and the skin following dermal exposure [2].

PAHs may adsorb onto particulate matter in air. In humans, it was reported that BaP measured in the lungs following inhalation of soot particles was much lower than expected. This may be due to the ability of the pulmonary epithelial cells to metabolise BaP thereby facilitating its absorption and clearance from the lungs [3]. Occupational studies have inferred that inhaled PAHs are absorbed, as urinary metabolites were present in workers exposed to PAHs [2]. The absorption of BaP following inhalation is highly dependent on the type of particles onto which it is adsorbed and the site of deposition in the respiratory tract [1]. Pulmonary absorption often occurs in parallel with mucociliary clearance, by which PAHs that are absorbed onto inhaled particulates are cleared out of the pulmonary tree and subsequently swallowed [3, 4].

PAHs are well absorbed in the GI tract by passive diffusion. The extent of absorption may vary depending of the bioavailability of particles, diet and PAH size (highest for smaller molecule PAHs) [1]. In rats, approximately 40% of absorption occurred through the GI tract following administration of 0.5 µg/kg BaP for 90 minutes directly into the duodenum; 38-58% absorption occurred following administration of BaP given by gavage or in the diet [2].

BaP is efficiently absorbed through the skin of animals [1]. Extensive skin absorption has been demonstrated in mice, as almost all of an applied dose of BaP appeared in the faeces following application to the skin [4]. Similarly, rapid absorption was demonstrated in rats, monkeys and guinea pigs [2].

PAHs are rapidly distributed throughout the body by all routes of exposure; they may be detected in most tissues minutes to hours following exposure [1]. Fatty tissues generally contain more PAHs than other tissues [1, 5]. However, PAHs do not accumulate in the body [1].

BaP can readily cross the placenta following oral, inhalation or dermal exposure. One study reported that when pregnant rats were exposed to BaP via inhalation, an increase in BaP and metabolites was measured in both maternal and fetal blood and tissues. Similarly, BaP
was measured in the fetus when rats were given oral BaP on day 21 of pregnancy [2]. BaP has been detected in human breast milk, particularly in smokers, however animal studies suggest that it does not accumulate in the offspring [6].

Cytochrome P450 enzymes (CYPs) and epoxide hydrolase are the main enzymes involved in PAH metabolism. Many studies have investigated the metabolism of PAHs in tissues and cells following ingestion of food containing PAHs, or inhalation or ingestion of environmental PAHs. The liver has the greatest capacity to metabolise PAHs followed by the lungs, intestinal mucosa, skin and kidneys. BaP stimulates its own metabolism by inducing CYP enzymes via the activation of the AhR. PAHs can also inhibit CYP enzymes [1].

PAHs are initially metabolised to several epoxides by CYP enzymes. The epoxides may spontaneously rearrange to phenols or are converted to dihydrodiols. PAHs are also metabolised to a number of quinones by CYPs. The dihydrodiols are further metabolised by CYPs to 4 optically active isofoms of dihydrodiol epoxides, notable amongst these is the anti-BaP 7,8 diol-9,10-epoxide (anti-BPDE). The stereoisomer (+) anti-BPDE is considered to be the most tumorigenic and predominant metabolite of BaP that forms DNA adducts in mammalian tissues. In addition, PAHs and their reactive metabolites undergo conjugation with sulphate, glutathione (GSH) and glucuronic acid [1, 7].

The route of exposure may influence the toxicity of PAHs. Following oral exposure the compound undergoes first-pass metabolism which reduces the levels of PAHs and metabolites that reach systemic circulation. Inhalation or dermal exposure may result in higher levels of PAHs reaching peripheral tissues as the compounds may bypass the first-pass effect of the liver [1, 2]. Genetic polymorphisms may affect the capacity of individuals to metabolise PAHs [1].

Studies suggest that metabolites of PAH’s are generally excreted as conjugates of GSH, glucuronic acid or sulphate in the urine, faeces and via biliary excretion [7]. High molecular weight PAHs and their metabolites are mainly excreted via the faeces [1].

Sources and Route of Human Exposure

The major route of exposure to PAHs for non-smokers in the general population is food, with a minor contribution from inhalation of ambient air. For smokers, the contribution of smoking to total exposure is likely to be similar to that of food [8].

Food may become contaminated with PAHs from environmental sources, industrial preparation or during home cooking [8]. Various foods such as vegetables, meat and fish have been shown to contain PAHs, but they are largely formed due to cooking at high temperatures such as charbroiling, grilling and frying. Smoked and barbequed food are particularly important sources of exposure, although the largest contributors to PAH intake are “cereals and cereal products” and “vegetable fats and oils” [3, 4, 9-11]. The maximum estimated daily intake of BaP for a 70 kg person is 6-8 ng/kg [8, 11]. After evaluating a recent food survey, the Food Standards Agency (FSA) concluded that PAHs were typically...
found in low levels in food and that consumers do not need to change their eating habits [12].

In the past, metal production and agriculture were responsible for the majority of total BaP emissions in the UK. Likely effected by the Environmental Protection Act 1990 and the ban on burning agricultural stubble, total emissions in 2011 had reduced to roughly 1/20th (now 3 tonnes) of those in 1991. Natural sources in 2011 accounted for 47.2% of the total emissions, while residential and commercial sources contributed the greatest portion of the anthropogenic emissions at 76% [13].

Annual mean air concentrations of BaP in the UK are generally below the EU target value of 1 ng/m³, with the vast majority of monitoring sites showing levels below the UK air quality objective of 0.25 ng/m³. Locations with point sources (e.g. industrial installations and domestic solid fuel burning) are an exception to this. The main sources of BaP in the UK are domestic coal and wood burning, outdoor fires and industrial processes [14, 15].

Indoor air may be contaminated with PAH's by infiltration of outdoor air or from indoor emissions, which include smoking, cooking, and heating with fuel stoves and fireplaces and to a lesser extent from incense and candle burning. Levels of BaP within the home appear to vary seasonally, with the highest concentrations found in winter. BaP levels in European homes were found to be between 0.01 to 0.65 ng/m³ [1].

Mainstream tobacco smoke contains high concentrations of PAHs, levels in the range of 1-1.6 µg per cigarette have been measured and as such this represents a major source of exposure for smokers. BaP levels in sidestream smoke have been reported to range from 52-95 ng per cigarette, more than three times higher than that seen in mainstream smoke [5]. In a smoker's home more than 87% of total PAH's in air may be introduced by cigarette smoke; in a room heavily polluted with cigarette smoke, BaP levels may be as high as 22 ng/m³ [1].

PAHs are commonly detected in surface waters, due to urban runoff and industrial activities [3]. Contamination of drinking water with PAHs is usually associated with coal tar linings of distribution pipes. However, drinking water contributes only a minor amount to the total intake of PAHs [4, 16].

PAHs are found in the majority of surface soils due to atmospheric deposition or urban runoff. Soils near industrial sources such as coal coking often contain high concentrations of PAHs [3, 9]. BaP in English soils comprises approximately 5-7% by weight of the total PAH content [17]. The British Geological Survey defined the normal background concentrations for BaP in England and Wales to be 3.6 mg/kg in urban areas and 0.5 mg/kg in all other areas [17, 18].

Occupational exposure is largely through inhalation and skin absorption. The greatest levels of occupational exposure to BaP are in aluminium production (up to 100 µg/m³), with lesser exposure in roofing and paving (10-20 µg/m³) and lesser still in coal processing, wood impregnation, chimney sweeping and in power plants (at or below 1µg/m³) [5].
Health Effects of Acute/Single Exposure

Human data

Inhalation
No studies were identified that reported the effects of BaP in humans following acute inhalation exposure.

Ingestion
Data on acute oral toxicity of BaP in humans are not available.

Dermal/ocular exposure
No studies were identified that reported effects of BaP in humans following acute dermal exposure.

Animal and in-vitro data

Inhalation
No studies were identified that reported effects of BaP in animals following acute inhalation exposure.

Ingestion
Exposure of rats (intragastric administration) to 100 mg/kg bw/day BaP for four days increased relative liver weight by 27% and induced aldehyde dehydrogenase. Limited evidence suggested that acute ingestion of BaP (50-150 mg/kg bw/day for 4 days) does not cause adverse GI effects in rats, although enzyme activity was altered. It was suggested that more serious effects may occur at higher concentrations [2].

Dermal/ocular exposure
BaP applied dermally caused allergen specific contact hypersensitivity reactions in mice after acute applications of 120 µg. A dose dependent contact hypersensitivity response to dermal application of BaP has been observed in guinea pigs; two applications of 0.001% BaP over 2-3 weeks gave slight hypersensitivity while a 1% dose gave a more severe response [2].

Acute topical application of BaP (concentration and duration of exposure not stated) to the backs of shaved mice suppressed sebaceous glands, although it was not possible to determine if such effects were due to the solvent or BaP, as a control group was not used [2].
Health Effects of Chronic/Repeated Exposure

Human data

Inhalation

A large number of epidemiological studies have been carried out considering a variety of occupations in which the workforce is chronically exposed to a mixture of PAHs [4]. These studies have demonstrated that such exposures result in symptoms including respiratory distress, decreased ventilatory function, chest pain, chest and throat irritation, cough, haematemesis, chronic dermatitis, depressed immune system and cancer of the skin and lungs [2, 4]. It is not possible to determine with any certainty the contribution of individual PAHs to these effects [4].

One study investigated the respiratory effects of inhaled BaP in employees working in various areas of a rubber factory. The authors reported a decrease in ventilatory function following prolonged exposure, as assessed by duration of employment, the greatest effects being observed in workers that had the highest exposure to particulate matter and BaP. No attempt was made to identify other possible chemical exposures or to separate effects due to BaP or particulates [2].

Ischemic heart disease was observed to increase in a dose dependent manner in asphalt workers exposed to BaP. Mean exposure for the cohort was 273 ng/m$^3$ and exposure at or above this level was associated with a 1.64 fold greater risk of ischemic heart disease mortality compared to those exposed to below 68 ng/m$^3$ [1]

Ingestion

Data on chronic oral toxicity of BaP in humans are not available.

Dermal/ocular exposure

Few data are available pertaining to BaP alone.

Regressive verrucae (warts) were reported in humans following up to 120 applications of 1% BaP over a four month period [2].

Genotoxicity

The formation of DNA adducts is believed to be a key event in the mutagenicity and carcinogenicity of PAH’s; adducts may lead to misrepair and result in mutations [1]. Anti-BPDE has been demonstrated to form DNA adducts in man and as such acts as a biomarker for exposure to BaP. Molecular epidemiological studies in individuals exposed to complex mixtures of PAHs have shown that BaP adducts are seen concomitantly with biomarkers of genotoxic effect. The observed effects include chromosomal aberrations, sister chromatid exchange, DNA damage and formation of 8-oxo-deoxyguanosine. These same markers of exposure and effect are also observed in experimental animals, with association. IARC considers that BaP contributes to the genotoxic effects seen in complex
PAH mixtures; with the anti-BPDE-DNA adduct being the most mechanistically relevant adduct [19].

Smoking and diet (major sources of BaP) have been highly correlated with levels of the anti-BPDE-DNA adduct. The metabolite responsible for these adducts is seen to cause a unique array of mutations in the TP53 tumour suppressor gene, in cancers associated with smoking [19].

Evidence suggests that inhalation exposure to BaP at levels over 1 ng/m$^3$ is predictive of greater genomic frequency of translocation, micronuclei and DNA fragmentation [1].

**Carcinogenicity**

No epidemiological studies on exposure to BaP alone are available for evaluation [19]. There is however extensive literature on the epidemiology of workforces exposed to complex mixtures of PAHs which include BaP. Studies include asphalt works, coke production plants, aluminium smelters and occupations where exposure to coal tar, coal tar pitches and soot occurs. Such studies clearly showed an elevated incidence of lung tumours following inhalation and skin tumours following chronic skin contact. It is difficult to assess with any confidence the contribution of BaP or any other individual PAH to such findings [3, 4, 20].

In the 2012 evaluation, IARC classified BaP as carcinogenic to humans (Group 1). It was concluded that BaP contributes to the genotoxic and carcinogenic effects resulting from occupational exposure to complex PAHs mixtures. The robust animal evidence and consistent and coherent mechanistic evidence from experimental and human studies provide biological plausibility to support the overall classification [19].

Estimated cumulative exposure to BaP of 100µg/m$^3$ (equivalent to 3.3 µg/m$^3$ for 30 years) in the aluminium smelting industry has been associated with a 2.68 fold increase in the incidence of lung cancer [1].

**Reproductive and developmental toxicity**

There is evidence to suggest that exposure to PAHs may cause developmental effects in humans. This is supported by evidence in animal developmental studies.

PAH-DNA adducts have been found in fetal cord and maternal blood after maternal exposure to PAHs in ambient air; in light of this the World Health Organisation states that prenatal exposure could increase cancer risk from PAHs [1].

Studies show a dose-response relationship between exposure to PAHs during pregnancy and effects related to intrauterine growth restriction. A study of neonates showed that those with increased levels of PAH-DNA adducts had significantly lower birth weight, length and head circumference [1].

High cord blood levels of BaP-DNA adducts has been associated with decreased birth weight and a reduction in postnatal weight [6].
An association between dietary BaP intake and decreased birth weight, decreased birth length and having a small for gestational age infant was found in women with low vitamin C intake [6].

There is evidence to suggest that BaP may cause developmental neurotoxic effects. Human studies of prenatal environmental PAH exposure (determined by personal air monitoring and measuring BaP-DNA adduct levels in cord blood) have reported neurodevelopmental effects including impaired cognitive ability, impaired neuromuscular function and increased attention problems and anxious/depressed behaviour following prenatal exposure [6]. Infants born close to a coal-fired power station in China had 0.32±0.14 BaP-DNA adducts per 10^8 nucleotides, this level was associated with a decreased motor development at age 2. A 0.1 unit increase in BaP DNA adducts per 10^8 nucleotides, at birth, was associated with a 2 fold greater chance of developmental delay at age 2. After closure of this plant, lower adduct levels were seen in the cord blood of a new cohort and was no longer associated with reduced motor development [1].

Evidence from human studies indicates that PAH or BaP exposure may cause reproductive toxicity in males and females. Studies in adult men exposed to PAH mixtures via occupational exposure or smoking have reported an association between higher levels of BaP-DNA adducts in sperm and male infertility [6, 21]. In a case control study in a Chinese population a strong association was reported between maternal blood BaP-DNA adducts and risk of miscarriage. In a study addressing the probability of conception in women undergoing IVF (in vitro fertilisation), follicular fluid BaP levels were significantly higher in women who did not conceive [6].

Animal and in-vitro data

Inhalation

Rats exposed to BaP dust via inhalation (7.7 mg/m^3, 2 hours per day, 5 days per week for 4 weeks) showed no treatment related lesions in the lungs or nasal cavities. No dose-response relationship could be demonstrated as only one concentration of BaP was tested [3]. In the same study, kidney sections were also examined and no adverse effects were noted [2, 4]. Similarly, male hamsters did not show any adverse effects following exposure via inhalation to 9.8 mg/m^3 or 44.8 mg/m^3 BaP for 4.5 hours per day, five days per week for 16 weeks [3].

Ingestion

Few data on chronic oral toxicity of BaP in animals are available. Daily oral administration of 120 mg/kg bw BaP to poor affinity AhR mice (DBA/2N) for one to four weeks caused deaths due to myelotoxicity, whereas high affinity mice (C57B1/6N) remained unaffected during the 6 month treatment. Hepatotoxicity, as well as effects on liver and kidney enzymes have also been reported at this concentration [3, 4].

Rats fed 1,100 mg/kg bw/day BaP in the diet for more than 100 days showed a decreased growth rate [3].
PAHs including BaP have been shown to promote atherosclerotic plaque formation in AhR responsive mice, chickens and pigeons [1].

Dermal Exposure

BaP (16, 32 or 64 g per application) was applied once a week for 29 weeks onto the skin of female mice. Dose-related epidermal thickening and a pronounced inflammatory response of the dermis, amongst other effects were reported in the first weeks of exposure in those administered the high dose, and subsequently in the lower dose groups [2].

Genotoxicity

Several PAHs are mutagenic and genotoxic and induce DNA adducts in vitro and in vivo [1, 11].

BaP has consistently been shown to be positive in in-vitro assays for point mutations in Salmonella and for chromosome damage in mammalian cells, in the presence of an exogenous source of metabolic activation. Indeed it is often used as a positive control in such assays [3].

An increase in the same biomarkers of genotoxic effect seen in man on exposure to complex PAH mixtures which may include BaP have also been seen in experimental animals exposed to BaP or anti-BPDE [19]. Such effects include point mutations, sister chromatic exchange, chromosomal aberrations, sperm abnormalities and somatic mutations. BaP induced mutations are notably found in tumour suppressor genes and proto-oncogenes [5].

There is strong evidence that the formation of DNA adducts by BaP is important in mouse lung tumorigenesis and that this mechanism and the formation of radical-cations by BaP is involved in mouse skin carcinogenesis [19].

There is some evidence for the role of BaP in bitumen-fume genotoxicity; in mice exposed to bitumen fume condensates, anti-BPDE–DNA adducts and BaP metabolites have been found in the lungs and urine respectively [7].

Carcinogenicity

IARC concluded that there is sufficient evidence that BaP is carcinogenic to experimental animals [19].

BaP applied directly to the skin of various strains of mice has been reported to induce malignant (and begin) skin tumours, predominantly squamous cell carcinomas. Oral administration of BaP to mice by gavage or diet has yielded an increase in tumour response in lymphoid and hematopoietic tissues and in several organs including the lung, liver oesophagus and tongue. An increase in mammary gland adenocarcinomas has been reported in rats administered BaP by gavage. Lifetime inhalation studies in hamsters gave dose-response related increases in papilloma’s and squamous cell-carcinomas in the upper respiratory tract and in the upper digestive tract [19].
Skin tumour development was not observed in AhR deficient mice, however squamous-cell carcinomas of the skin were present in the wild-type mice [19].

**Reproductive and developmental toxicity**

BaP exposure has been shown to have effects on the development of laboratory animals following prenatal exposure. Developmental effects reported include decreased number of pups, an increase in reabsorptions, reduced pup weight and malformations [1, 2, 6]. Studies in mice exposed to BaP via oral administration during gestation suggest that intrauterine growth restriction, stillbirths and malformations following exposure may be dependent upon the AhR status of the mother and offspring [1-3].

Evidence from animal laboratory studies indicates that gestational exposure to BaP can have an effect on the reproductive function of offspring. Decreases in testis weight, sperm production, testosterone levels and fertility have been reported in male rodents. Effects observed in female mice include decreases in ovary weight, numbers of follicles, corpora lutea and fertility [1, 6].

Persistent neurodevelopmental effects have been observed in rats and mice exposed to BaP via ingestion as neonates; such effects include deficits in learning and memory, anxiety-related behaviours, sensorimotor development and neuromuscular function [6].

Trans-placental exposure to BaP (with dibenzo[a,l]pyrene) has been shown to induce lung and livers tumours in mice [1].

Reproductive effects have been reported in adult laboratory animals exposed to BaP. Reductions in sperm count, motility and production have been reported in various strains of adult male rats and mice and across routes of exposure. Hormonal changes and histological changes in the testis have also been observed in male adult animals. Reduced fertility and reductions in ovary weight have been reported in female adult animals following oral or inhalation exposure to BaP [6].
References
