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A Comprehensive Review of Benzo Alpha Pyrene (B[A]P) Toxicology.

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ABSTRACT

Benzo Alpha Pyrene (B[a]P) is a prototype Polycyclic Aromatic Hydrocarbon (PAH). It is a well known environmental pollutant and a procarcinogen. It is present ubiquitously in the environment and exposure to B[a]P occurs through air, food, water, skin contact etc. It is metabolized in the body by phase I and phase II reactions and the primary metabolites are non-toxic and easily excretable. A minor fraction of B[a]P is converted to active metabolites which are carcinogenic and adversely affect other systems of the body. This review covers all aspects of B[a]P including sources, exposure, metabolism and toxicity profile.

Keywords: Benzo alpha pyrene (B[a]P), Polycyclic Aromatic Hydrocarbon (PAH), carcinogen, Cytochrome P450

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INTRODUCTION

Polycyclic aromatic hydrocarbons (PAH)are produced by incomplete combustion of organic compounds. A 5-ring structure compound Benzo[a]Pyrene (B[a]P) is a prototype of PAH. B[a]P is an environmental pollutant, a carcinogen and, is ubiquitously present in our surroundings i.e. air, water, soil, charcoal grilled food items, certain pharmaceutical products and tobacco smoke[1,2].Sources of B[a]P in ambient air are industrial emissions, motor vehicle exhaust, tobacco smoke, cooking and residential and commercial heating of organic fuel etc. Concentration of B[a]P is found to be more than three times higher in side stream cigarette smoke than mainstream smoke.

Sources of B[a]P in the diet are barbecued/grilled/broiled and smoke cured meats, roasted and baked foods and vegetables grown in contaminated soil[2] (Figure 1). B[a]P is also present soil. The levels of B[a]P in the soil vary depending on proximity to roads, combustion sources, use of sewage or sludge derived amendments on agricultural lands etc. Exposure can also occur via the use of dermally applied pharmaceutical products which contain coal tars (including formulations used to treat eczema and psoriasis) [2]. Use of contaminated water (by petroleum spills, road run off, industrial wastewater) also increases the risk of exposure to B[a]P [3, 4, 5].

Occupational exposure occurs through inhalation and skin contact. B[a]P exposure has been reported in occupations related to coal liquefaction, coal gasification, coke production and coke ovens, coal-tar distillation, roofing and paving (involving coal-tar pitch), aluminium production (including anode manufacture), carbon-electrode manufacture, chimney sweeping, and power plants[2].

METABOLISM

B[a]P enters the body mainly through inhalation and ingestion. It is then transported to various organs through blood and lymph [6]. B[a] Pis metabolized by both phase-Iand phase-II enzymes to form epoxides, dihydrodiols, phenols, and quinones and their polar conjugates with glutathione, sulphate and glucuronide [7].

After ingestion, absorption, and transport, the initial oxygenation of B[a]P is catalysed by the microsomal mixed-function oxidases (MFOs), which contains multiple formsof cytochrome P-450 [8, 9]. The major cytochrome P450s involved in the B[a]P metabolism are CYP1A1, CYP1A2 and CYP1B1[10, 11]. Cytochrome P450s are inducible by B[a]P and other PAHs through binding to the aryl hydrocarbon-receptor (AhR) nuclear complex, resulting in changes in gene transcription of CYPs and phase-II enzymes.

Primary metabolites of B[a]P metabolism (Figure 2):

1. Three epoxides: 4,5-epoxide, 7,8-epoxide and 9,10-epoxide[9, 12, 13].
2. Three dihydrodiols: (-)-trans-4,5-diol, (-)-trans-7,8-diol and (-)-trans-9,10-diol [14, 15, 16, 17].
3. Five phenols: 1-OH, 3-OH, 6-OH, 7-OH and 9-OH. These phenols can be converted to quinones [18, 19, 20, 21, 22].

CYP450 acts on B[a]Pleading to formation of epoxides atthe 4,5-, 7,8-, and 9, 10-positions. Further epoxidehydratase acts on the epoxide intermediates [23, 24] to form corresponding dihydrodiols. Few epoxide intermediates get converted to phenols. The major phenol metabolite of B[a]P is 3-hydroxybenzo[a]pyrene (3-OHBP)[25, 26] formed in presence of an enzyme aryl hydrocarbon hydroxylase. The 7- and 9-phenols are rearrangement products of the unstable 7,8- and 9,10-epoxide intermediates [23, 27, 28]. Other phenols may be formed either by direct hydroxylation or rearrangement of the unstable epoxide intermediates.

The primary epoxides can be conjugated to glutathione S-conjugates [29] and the phenols and diols can be conjugated to either sulphate [30] or glucuronide [31] to form water-soluble compounds. The formation of the water-soluble glutathione, glucuronide, and sulphate conjugates is catalysed by glutathione S-epoxide transferase, UDPglucuronate transferase, andsulfotransferase, respectively. The majority of B[a]P is converted to these water-soluble, easily excretable non-toxicmetabolites.

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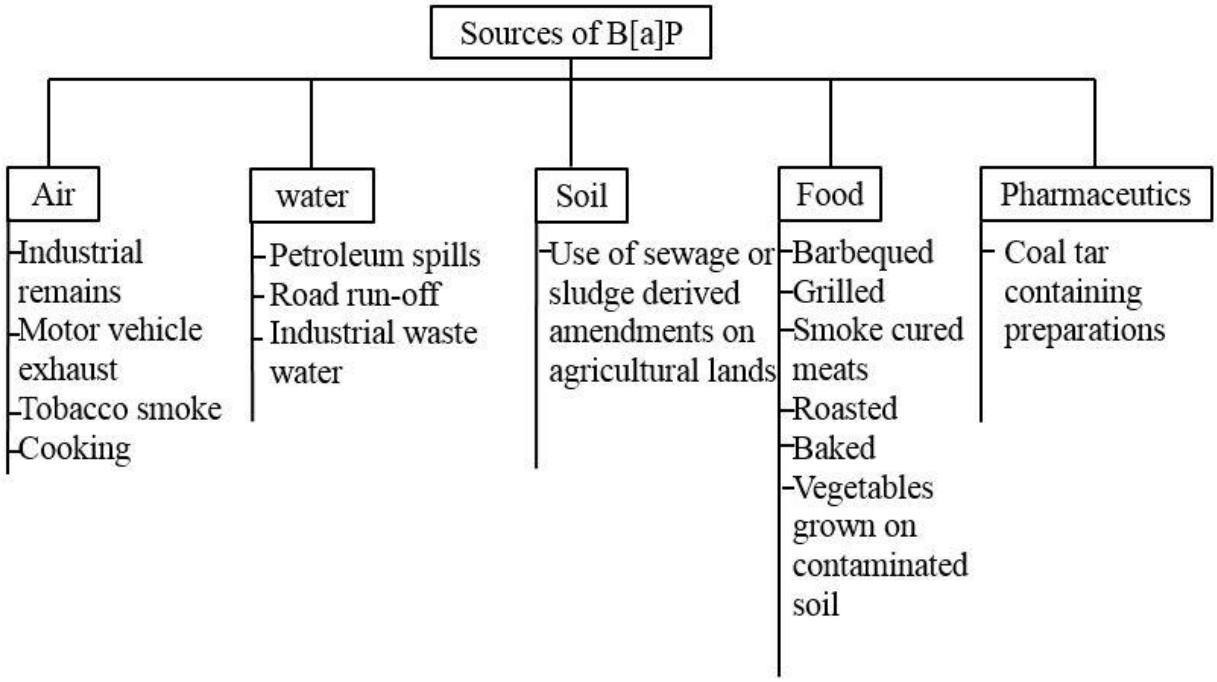

Figure 1: Sources of B[a]P exposure

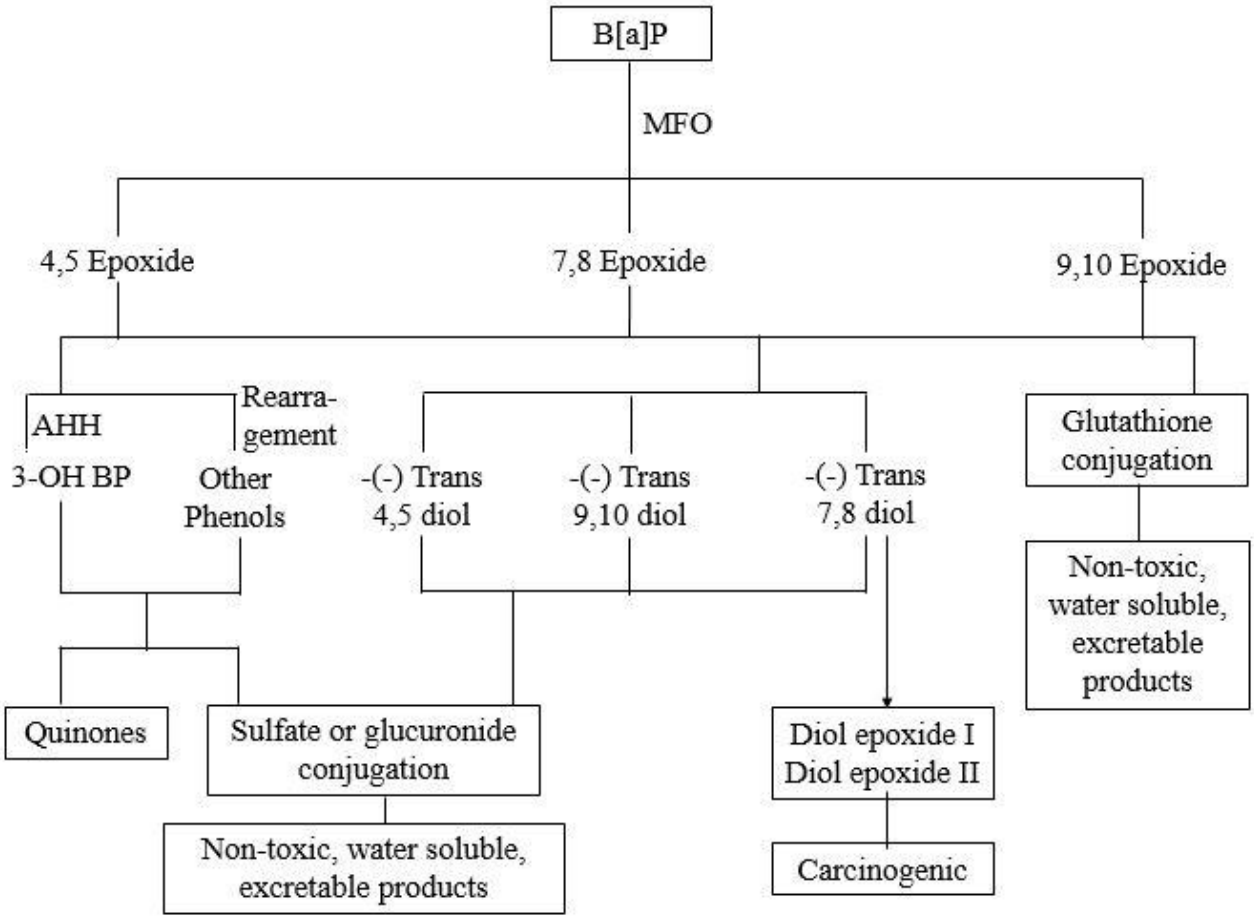

Figure 2: Overview of B[a]P metabolism

A small fraction of B[a]P is converted to active metabolites which are genotoxic and carcinogenic [8] by further metabolism or "recycling" of the phenol and dihydrodiol metabolites throughthe MFO system. Thus, the (-)-trans.7,8-diol is converted to two stereoisomers of 7,8-diol-9,10-epoxides: diol epoxide I and diol epoxide II [32, 33] by the MFOs. There are four possible stereoisomeric diol epoxides derived from the trans-7,8-diol. Each diol epoxide is further hydrolysed to two tetrols and reduced to onetriol.

The diol epoxides are highly carcinogenic metabolites of B[a]P. The amount of recycling of B[a]P metabolites by the MFOs to further oxidized products dependson the experimental conditions. Factors that control recycling depends on the availability of phenol or diol available to the MFO. Less recycling occurs when large amounts ofB[a]P substrates are present and a large amount of recycling is observed when very small amounts of B[a]P are used as substrate [34].

Second factor governing the frequency of recycling is the presence of competing enzyme systems with the appropriate cofactors. High levels of conjugating enzymes compete with the MFOs for the oxygenated metabolites of B[a]P, thus decreasing the probability of oxygenated metabolites for recycling.

EFFECTS OF B[a]P METABOLISM

Binding of B[a]P Metabolites with Macromolecules: The metabolic intermediates of B[a]P metabolism bind covalently to nucleic acids[35], resulting in cytotoxicity, mutagenicity [36], cell transformation in vitro and cancer induction in experimental animals.

B[a]P diol epoxides I and II form covalent adducts primarily with guanosine residues of RNA and DNA. Upto a limited extent, they can also form covalent adductswith adenosine and cytidine [37]. The carbon at the l0thposition of the B[a]P diol epoxide is linked to the N-2-amino group of guanines.

Studies done to decipher the carcinogen nature of B[a]P indicate that out of the four stereoisomers of the 7,8-diol-9,10 epoxide, the (+) diol epoxide I have higher tumorigenicity as compared to other 3 stereoisomers[38]. Also, the tumor initiating activity of (+) diol epoxide I was much higher as compared to other stereoisomers. Diol epoxides are the predominant metabolites as carcinogen and tumor initiating forms but other routes of activation of B[a]P to other metabolites may also contribute to carcinogenesis.

B[a]P diol epoxides also binds covalently to cellular proteins. Weinstein et.Al.,(1976) demonstrated the interaction between B[a]P diol epoxides and protein components of chromatin.

Cancer: Even after extensive literature review, we could not find any epidemiological data of B[a]P carcinogenesis in humans. B[a]P is known to cause tumor in experimental animals following exposure through many different routes which are summarized below.

1. **Oral administration:** There was an increase in incidence of tumors in various organs like tongue, liver, lung, forestomach, oesophagus, lymphoid tissue and haematopoietic tissues after oral administration of B[a]P either by gavage or in the diet of mice [39-47].
2. **Inhalation:** B[a]P induced increase in incidence of papilloma and squamous cell carcinoma in both upper respiratory tract and upper digestive tract in male hamsters[48].
3. **Skin application:** Benign (Squamous cell papillomas and Keratoacanthomas) and malignant (Squamous cell carcinoma) tumors were observed in different strains of mice when B[a]P was applied directly on skin[49-58].
4. **Subcutaneous (s.c.) injection:**Fibrosarcomas (malignant tumors) developed at the injection site in mice[59-62].
5. **Intraperitoneal (i.p.) injection:**Intraperitoneal injection of B[a]P in newborn and adult mice lead to an increased incidence of liver (adenomas and carcinomas), lung (adenomas and carcinomas) and lymphoreticular tumors[63-73].
6. **Intrapulmonary injection:** After injection of B[a]P in the lung of rats, there was an increase in the incidence of malignant lung tumors (mainly squamous cell carcinomas)[74-77].
7. **Intratracheal administration:** Intratracheal administration of B[a]P resulted in benign and malignant respiratory tumors in mice, rats and in hamsters[78-80].

8. **Buccal pouch application:** A higher incidence of forestomach papillomas was observed in male hamsters after repeated application of B[a]P to the buccal pouch mucosa[81].
9. **Intramammilary administration:** Benign and malignant mammary gland tumors were observed after intramammilary injection of B[a]P in rats[51, 82, 83].
10. **Intracolonic instillation:** Intracolonic instillation of B[a]P induced lymphomas in forestomach and various other organs in mice[84, 85].
11. **Intravaginal application:** In mice, intravaginal application of B[a]P produced invasive cervical carcinoma[86].
12. **Intrafetal injection:** One study conducted by Rossi et al.,[87] showed that intrafetal injection of B[a]P produced lung adenomas in male and female Swiss mice.

Epigenetic effects: The metabolites of B[a]P have been found to increase cell proliferation and increased expression of the *Cdc25B* gene (cell-division cycle25B) and reduced phosphorylation of Cdk1 (cyclin-dependent kinase1) in different human cell lines[88]. Exposure to B[a]P and its metabolites leads to alteration in DNA methylation. After treatment of immortalized bronchial epithelial cells withanti-B[a]P-7,8-diol-9,10-epoxide, the concentration of cytosine-DNA methyltransferase-1 was increased and was associated with hypermethylation of the promoters of 5–10 genes, including members of the cadherin gene-family[89].

Apoptosis: B[a]P and its metabolites alsoregulate apoptosis. B[a]P was found to induce apoptosis via theJNK1/FasL (c-Jun N-terminal kinase 1/FasLigand) and JNK1/p53 signalling pathways in human MRC-5 lung fibroblasts [90]. Apoptosis induced by anti-B[a]P-7,8-diol-9,10-epoxide in H460 human lung-cancer cells was associated with induction of Bak (BCL2-antagonist) and with activation of caspase [91].

Effects on other systems: Following oral exposure, animal studies have demonstrated developmental neurotoxicity, reproductive toxicity and immunotoxicity. Gestational exposure to mice and rats lead to neurobehavioral changes and cardiovascular effects. Oral exposure in adult animals lead to various reproductive and immune system dysfunction including decrease in sperm count, ovary weight, follicle numbers and decrease in thymus weight, B cell numbers and immunoglobulin concentration. B[a]P suppresses immunity by modulating p53-dependent signalling pathways in lymphocytes. B[a]P has been shown to induceimmuno suppression in adult mice by altering the cell-mediated responses [92]. Immune development in offspring was also altered following *in utero* exposure to B[a]P[93].

Following inhalational exposure, developmental and reproductive toxicity was observed in rats which included decreased fetal survival, nervous system defects in off springs and decreased testes weight and sperm counts in adult animals[94].

DISCUSSION

B[a]P, a well characterized procarcinogen, is a member of the PAH family. It is ubiquitously present in the environment and enters the body mainly by inhalation and ingestion. Once inside the cells, it is metabolized by P450-dependent monooxygenase system to various epoxides, phenols and dihydro diols. These metabolites are either converted to water soluble excretory products or recycled to reactive and toxic metabolites depending upon the availability of substrates and enzymes of the two pathways.

The adverse effects of B[a]P includes carcinogenesis, teratogenicity, neurotoxicity, reproductive toxicity, immunosuppression and developmental toxicity in experimental animals. It also affects lipid metabolism, apoptosis and induces epigenetic modifications. B[a]P is categorized as a human Group 1carcinogen by the International Agency for Research on Cancer [95]. The location of tumors depends on the route of exposure. Inhalation of B[a]P often induces lung cancer, while oral administration leads to tumors in various organs/tissues, including the gastro-intestinal tract, liver, lungs, and mammary glands [96].

Most of the information on B[a]P toxicity has been obtained from animal studies and this data cannot be extrapolated for human trials because of species difference. Also the higher toxicological doses which are used in animal testing might already be present at relevant concentrations in environment. These disadvantages limit the extrapolation of data from animal studies to humans.

Therefore, the US National Research Council proposed the Toxicity Testing in the 21st Century (TT21C) and encouraged the use of *in vitro* toxicity pathway-based approaches using cell lines (97), as compared to high-dose studies in laboratory animals. The toxicity pathways, or adverse outcome pathways (AOP) are evaluated by *in vitro* assays of innate cellular signalling pathways and are severely affected if disturbed[97]. However, the AOP/TT21C strategy in B[a]P toxicity testing is still under investigation.

Based on the best available literature, it is concluded that B[a]P contributes to various deleterious effects including carcinogenesis and toxicity of various organ systems. The strong and extensive experimental evidence for the carcinogenicity of B[a]P in many animal species and human cell lines support the overall classification of B[a]P as a human carcinogen (Group 1).

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