

**“Polycyclic aromatic hydrocarbon (PAH) and cancer risk on uncovered
& unregulated meat consumption”**

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Abstract

Particulate matter (PM) less than one-tenth of the size of a human hair coming out from vehicle emission may cause a serious health issues in a community. It is not less than endemic caused by current pollutants spit out by various transportation systems. The composition of particulate matter includes hydrocarbons, nitrogen oxides, benzene, Sulphur dioxide, 1, 3 buta diene, polycyclic aromatic hydrocarbons (PAHs), carbon monoxide (CO) and lead. Most of them can cause inflammation, allergic reactions or respiratory illnesses like apnea and short breathing. However, the polycyclic aromatic hydrocarbon present in vehicle emission can lead to severe deposition in human lung and other tissues. The consumption of poultry meat from a butcher shop present nearby emission of vehicles across the roads could be unhealthy. This may be, due to deposition and absorption of small fraction of particulate matters of emission. The consumption of such meat products causes the ingestion of polycyclic aromatic hydrocarbons (PAHs) as well. PAHs may cause lung and other cancers. Recent study highlights the importance of good practices and regulation of selling meat products for human consumption. The study explained and linked the molecular mechanism of cancer caused by cellular deposition of polycyclic aromatic hydrocarbons (PAHs). The possible methods to detect and quantify polycyclic aromatic hydrocarbons present in such meat products could be ensured to eliminate from the food chain.

Key words- Particulate matter (PM), polycyclic aromatic hydrocarbons (PAHs), meat, fuel emission, poultry, open meat products, lipophilicity and cancer.

Introduction

The polycyclic aromatic hydrocarbons (PAHs) are both salicylic and heterocyclic hydrocarbons. They are one of the many combustion products of fuel exhausted from vehicle emission. The fuel emission is a major problem in urban and high-density populated city. The emission contains not only metallic elements like lead and asbestos but also various oxide products like nitrogen oxides, Sulphur dioxides (SO₂), carbon monoxide (CO) and carbon dioxides (CO₂). The other high-density hydrocarbons are also part of fuel emission such as 1,3 butadiene, benzene and mainly polycyclic aromatic hydrocarbon (PAH) (1). Among all, due to reported genotoxicities and cancerous activities, PAH poses the main threat to human health. Chemically PAH molecules are composed of several benzene molecules condensed together. Sometimes PAH molecules also contain heteroatoms in their ring structure. PAH molecules are of several types, for example benzopyrenes, benzofluorenes & benzo-anthracene derivatives. In fuel emission of transport vehicles PAH is produced from the combustion of organic fuel (2). The emitted smoke contains several particulate matters of submicron to nanometer size. Apart from inhalation toxicity, the butcher shops that sell open and uncovered meat products might provide additional burden of in taking PAHs and causing serious health effects. The high lipid solubility of PAH derivatives makes them easily permeable to cell membrane of tissue section of consumable meat products. As reported in

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the live cell studies and in-vitro cell-based assays the cell permeability of PAH was found to be 70 folds in crossing the lipoproteins containing cell membrane than without lipoproteins containing cell membrane (3). Therefore, high lipid solubility makes PAH a preferential molecule to get absorbed inside the tissue flesh and remain imbibed into meat products. Even if meat products get washed with water before cooking or boiling, the PAH molecules remain stayed embedded within the meat products. The permeability of PAHs absorbed in tissue section could be assessed by simple artificial membrane assays. One of them is parallel artificial membrane permeability assay (PAMPA). However, quantification of amount of PAH could be performed by simple liquid-liquid micro extraction method. The simple nitration and oxidation products may also be marked as chemical identification test. In general, the PAHs present in atmosphere reacts with ozone, nitrogen dioxide, nitric acid and Sulphur dioxide. The reaction yields oxides, dinitro products and a sulphonic acid derivative respectively. Otherwise more sensitive spectroscopic techniques like gas chromatography-mass spectrometry (GC-MS), mass spectrometry (MS) and nuclear magnetic resonance spectroscopy (NMR) are the best tools to quantify PAHs even at micro and nanogram concentrations. The permissible limit of PAHs as per environmental protection agency (EPA), in the form of maximum contaminant level (MCL) is 0.2 parts per billion (ppb). The specific PAH has specific MCL value, for example 0.3 mg for anthracite, 0.06 mg for acenaphthene and 0.04 mg for fluoranthene (4). The high lipid solubility makes it a perfect candidate to extract and dissolve in organic solvent. Among all, benzopyrene is the most common PAH present in atmospheric air, soil and water (5). The occupational exposure of benzopyrene [Ba(P)] is reported to augment through air and water. The effect on human health could be several metabolic disorders and cancers (6). The most prominent health effect is lung cancer on inhalation of polluted air. However, the ingested tissue deposition of any PAHs and its toxicity has never been reported (7). The highlight of this research work includes the indirect exposure of PAHs through exposed meat consumption and their effect on human health, primarily cancer. The carcinogenic properties of benzopyrene are attributed to its metabolic product formed by the cytochrome P450 (CYP P450) group of enzymes. The CYP P450 mediated 7 β ,8 α -dihydroxy-9 α ,10 α -epoxy-tetrahydro benzo[a]pyrene (BPDE) acts as an intercalator to form DNA adduct and induce carcinogenesis. Benzo[a]pyrene and other PAHs have common structural features of five fused rings of hexagonal structure. The maximum permissible limit for benzopyrene [Ba(P)] as per EPA (environmental protection agency) & EU (European union) is in the range of 0.00082 $\mu\text{g L}^{-1}$ to 0.0022 $\mu\text{g L}^{-1}$ in any water sample (8-9). However, 0.2 mg of PAH per cubic mm of air has been set as a permissible limit by occupational safety health administration (OSHA). These limits are high when absorbed or imbibed by any food products like cut and open tissue section of meat products. When absorbed through lipid membrane of cells of a tissue section of meat products, it remains adhered to cells and tissues. The polycyclic aromatic hydrocarbons (PAHs) have been explored a lot in reference to its atmospheric level and human health effects like genotoxicities, carcinogenic and mutagenic potentials (10). However, there is a information available about the tissue deposition in humans and dietary intake of food substances. Recently few studies reported to have PAHs in different food products. However direct consumption of food products, tissue depositions and risk of causing cancer has never been reported (11). This study highlights risk of consuming popular food products like poultry meat that has been exposed and contaminated with PAHs due to fuel emission. The total PAH emission factor for diesel vehicles was found to be 1181.14 $\mu\text{g/Kg}$. In gasoline and petrol, the concentration of PAHs may go up to 2600 mg/liter of fuel. Any increased exposure of PAHs to human health might be devastating in terms of causing all type of cancers (12). The mechanism of carcinogenicity of most of the PAHs are similar, that involves absorption of PAH molecules, toxicities to cell structure, nucleolar translocation, intercalation of DNA. However, the important point to understand is exposure level, concentration and lipid solubility of PAHs.

Figure-1



Fig. 1 Image highlighting the fuel emission from a vehicle.

Figure-2 Structures of polycyclic aromatic hydrocarbons (PAHs) considered as toxic pollutants as per environmental protection agency (EPA) and world health organization (WHO) present in fuel emission.

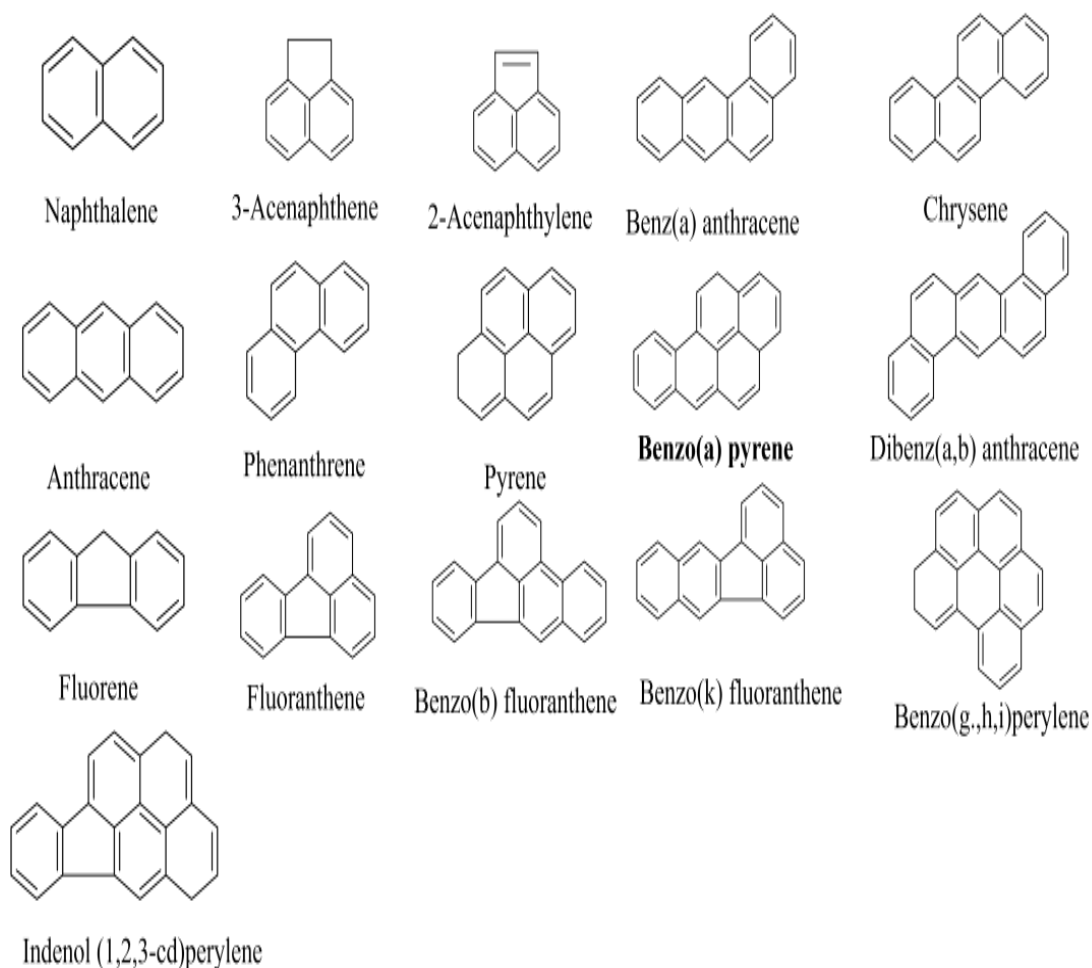


Figure-3

A) Control

Freshly slaughtered tissue section from a poultry chicken previously not exposed to fuel exhaust



B) Sample from a butcher shop across the road with meat products exposed to fuel exhaust



Fig. 3 The photographic images were taken by using a digital camera. A) Control sample imaged from freshly slaughtered meat sample without exposure of emission fuel, B) Sample from a butcher shop exposed to emission fuel of transport vehicles across the road.

Comparative imagery studies of meat products sold at the roadside butcher's shop and meat products slaughtered freshly

An insightful observational study was conducted and explained mechanism was partially supported by the studies. We consulted a series of butcher shops in local area and discussed their way of selling meat products. As per butchers and sellers of meat products, they purchase number of poultry chickens (hens) and process them with removing feathers, heat treatment and making pieces afterwards to sell. The process of selling begins on demand of the customers of the poultry chickens showcased on the

table across the transportation road. Prior to selling as per customers they cut the chicken in to number of pieces and give it to customers in 0.5-kilogram, 1 kilogram etc. However, once cut and the pieces are made, the cut down tissue section and biological fluids are exposed to environment near the transportation roads. To consider it to be safe, there is no regulation and cross validation of way of selling meat products. As explained in the discussion section the combustion fuel exhaust of the vehicles on the road is high. The air is high in amount of chemicals exhausted through vehicles. Based on the chemical data of chemicals exhausted by vehicles, the emission of toxic chemicals like polycyclic aromatic hydrocarbons (PAHs) was found to be high in concentration. Structurally, PAHs is heterogeneous in aromatic ring fusions that encompass a range of structural variations. The heterogeneous PAH molecules are formed after incomplete oxidative and pyrolytic combustion and degradation of organic matter containing carbons. This allows the structural variation in different PAHs formed through nucleation, fusion and substitution reactions (Fig 2). The combustion of organic materials in the exhausted fuel contains PAH molecules. PAHs have been known to cause inhalation-based toxicities and cancer (13). However, the lipophilicity of PAHs is high enough to contaminate and remain imbedded on the open and cut poultry meat products that are being sold. High hydrophobicity makes PAHs adsorbed efficiently through cell membrane of tissue section of open and cut meat products. To confirm, whether the exposure could be the main cause of chemical contamination, we took the samples being sold at the number of butcher shops across the roads and photographed it with a camera. The similar photographs were taken after purchasing a whole poultry chicken and was cut immediately to consume. In the set of photographs, the clear evidence of marked haziness and dull inner layers of surface of vital organs and muscles were visible in meat products purchased across the transportation road. However, the poultry chicken was clean, shining and contamination free when whole poultry chicken was purchased, freshly cut off from a butcher shop that sell whole poultry chicken (Fig 3 A and B). Another set of studies were conducted to correlate lipophilicities of PAHs and their preferential absorption to the cut and open biological fluids of consumable meat products. The lipophilicities of PAHs make them easily absorbed, imbedded and retained in the fat tissues, intercellular and interstitial fluids. To confirm high solubility and possible extraction of PAHs the solubility studies have been performed in organic solvents like benzene, hexane and toluene. It has been reported that PAHs are highly soluble in nonpolar and hydrophobic solvents like toluene. Therefore, an easy extraction and separation could be performed on meat samples obtained from exposed butcher shop and freshly slaughtered tissue section. The homogenization of tissue section after cutting in to small pieces of less than 5 cm could be performed using toluene 20 mL as a dissolving medium. The homogenized sample stirred at high speed for 30 minutes. The final homogeneous sample is centrifuged at 2000 rpm for 1 hour. The supernatant organic layer is removed carefully. The tissue pellet is discarded from centrifuge tubes. The supernatant that may contain range of chemicals could be analyzed further for chemical identification using chromatographic techniques like thin layer chromatography (TLC) and spectroscopic studies like mass spectroscopy and Fourier transformed infrared spectroscopy (FTIR) for confirmation of chemical signatures of polycyclic aromatic hydrocarbons (PAHs) (14). The comparative conclusion could be drawn on meat samples obtained from exposed butcher shop and freshly slaughtered tissue section.

Discussion

The source of energy to live a life is essential for every living being on the planet earth. However, animals and plants have different ways to obtain energy. The animal kingdom relies both on animal and plant foods to get energy through different metabolic processes. Among, animals, human beings are civilized animal that acquired the capability to produce foods through cultivation of plants and domestication of animals. The domestic animals are part of human life over the course of thousands of years of civilization. Among domestic animals, the animals that are being utilized as a food to get energy are poultry chickens, fish, goats, lambs and few cattle. When it comes to consuming meat as energy source the contamination and hygiene are the most

important parameters to consider in getting healthy, nourishing and adequate calories in safest possible way. Most of the societies and communities prefer getting healthy meat products through freshly slaughtered animals either through themselves or through butchers.

Although time gap between slaughtering the animals and surrounding environment is the main cause of most of the contamination issues. The contamination could be microbial, dust, submicron particles and chemicals (15-16). If the contamination is from air borne chemicals, which in developing countries are high in concentration in per cubic milliliter of air as pollution, the chances of toxic effect will be high. In developing countries like India, people sell slaughtered meat products across the transportation roads. This, in turn makes the consumable meat products exposed to high amount of combustion product of fuel from transport vehicle. However, the people who live under poverty line do not have enough options to earn money and live. In such case, a concrete policy guideline under the umbrella of state or central government must be adopted and enacted in every city to regulate its distribution. In this study with simple understanding of observation and already available chemical toxicity data of few chemicals present in the combustion fuel, their effects on ingestion with meat products contaminated with PAHs are highlighted as potential toxic agents. These chemicals are routinely exposed to open and cut meat products for selling across the roads. Based on air pollution data we examined such chemicals are highly toxic to human lungs through inhalation. However, if ingested through consumable meat products as an exposed deposited source, such chemicals might cause tissue deposition, carcinogenicity and genotoxicity in human host and their off springs. The polycyclic aromatic hydrocarbons (PAHs) group of chemicals that are highly hydrophobic and lipophilic in nature. Their polycyclic aromatic ring structure gives rise to lipophilicity. The resonance and largest number of aromatic disjoint pi sextets present in PAHs enable these molecules to easily settle down and remain imbedded on the slaughtered and open tissue section (17). The emission factor of most of the PAHs (benzopyrene derivatives) from heavy duty vehicles (HDV) fall in the range of 1-9. Thus, preferential absorption through cell surface of an open tissue section is high due to high emission factor and lipophilicity of PAH molecules. In emitted fuel, PAHs are semi-volatile in nature. PAH molecules can be partitioned between gas phase and particle phase. The gas phase PAHs are easily participated in the dry and wet deposition on the surface from aerosol. The reported solubility of PAHs, as per Henry's law have been found to be in the range of $3.1 \times 10^{-5} \text{ m}^3 \text{ atm}^{-1} \text{ mole}^{-1}$ (12). All the above discussed factors potentiate the preferential deposition of PAH molecules from atmosphere to open and cut flesh that kept open for long period of time to sell. The closer a butcher shop is nearby to the transportation road, the higher the possibility of deposition of PAH molecules on to the cut and open tissue section. If ingested through meat consumption, the digestive tract does not have any mechanism to degrade such molecules except transportation to liver. In animals, PAHs are metabolized by the microsomal cytochrome P450 enzymes. Most of the metabolic products in liver is generated by the phase 1 metabolic oxidative reactions. The oxidation of benzo[a]pyrene and other PAHs yield a range of metabolites through oxidation and peroxidation reactions by liver cytochrome P450 enzymes. Other than benzo[a]pyrene the number of other metabolites produced are quinones, diol-epoxides, 3-hydroxy benzo[a]pyrene and 7 β , 8 α -dihydroxy-9 α ,10 α -epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene (BPDE) (18). Apart from toxicities to other tissues through blood distribution and plasma accumulation, it easily facilitates the cell entry of such lipophilic molecules. The distribution of PAH metabolites is the determining factor in causing toxicities and carcinogenicity (19). Inside the human cell, the PAH metabolites is translocated into the nucleus. Although, concentration is a crucial consideration in reaching the nucleus, the structure of PAHs play an important role in interaction (20). The structure of PAH metabolites encompasses the resemblance with the purines and pyrimidine bases present in deoxyribose nucleic acids (DNA). The open portion of DNA molecule for transcription and translation from condensed chromosome structure, become the target for PAH metabolites. The intercalations between PAHs and DNA are governed by the equilibrium state of unstable and stable aromatic rings present in the PAH metabolites (21). The reaction between PAH

metabolites and DNA produces enough DNA-adducts that the transcriptional regulation of number of genes affected by the intercalation process, The PAH metabolites also affect metabolic reprogramming of translocated cells. The effect of altering metabolic reprogramming in turn effects the cell survival and generates the metastatic cancer cells. The effect is called as Warburg effect (22). The other cytotoxic effects of PAHs are membrane hyper polarization, mitochondrial dysfunction, alteration of cell gyration and imbalance of transport of Na^+ and H^+ ions (23). Above discussed molecular changes at the cellular level imparts the transformation of normal cells into cancerous states. However, histopathological changes are like the cells transformed to cancerous states by other factors. Since, major enzymes involved in the degradation of PAHs are dehydrogenase, peroxidase and oxygenase. At molecular level, metabolic enzymes facilitate the redox and substitution reactions. The substitution reactions yield unstable active metabolites. The unstable metabolites have aromatic disjoint pi of aromatic rings common among PAH molecules. Few aromatic rings in the PAH metabolites flip between aromatic and non-aromatic structures (24). This in turn allow the PAH molecules to participate in intercalation process with the negatively charged DNA molecule in the nucleus and causes the intercalation. At molecular level, this exerts its effect on hyperpolarization of plasma membrane. Later process decreases the gyration of cell membrane, and a cell becomes cancerous in nature. The lipophilicities of polycyclic aromatic hydrocarbons (PAHs) play an important role in preferential absorption through biological fluids and tissue mass exposed for long period of time. Therefore, consumption of such meat product containing PAHs and a range of generated toxic metabolites through liver metabolism could be of great interest for biochemist and pharmacokinetic scientists in correlating it with cancer and genotoxicities if explored extensively through studie

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Conflict of interest-

The authors declare no competing financial interest.

References

- H. Shen, S. Tao, R. Wang, B. Wang, G. Shen, W. Li, S. Su, Y. Huang, X. Wang, W. Liu, B. Li, K. Sun, Global time trends in PAH emissions from motor vehicles, *Atmos. Environ.* 2011, 1, 45, 12, 1-14.
- C. H. Whaley, E. Galarneau, P. A. Makar, M. D. Moran, J. Zhang, How much does traffic contribute to benzene and polycyclic aromatic hydrocarbon air pollution? results from a high-resolution North American air quality model centred on Toronto, Canada, *Atmos. Chem. Phys.*, 2020, 20, 2911-2925.
- L. C. Marr, T. W. Kirchsteller, R. A. Harley, A. H. Miguel, S. V. Hering, S. K. Hammond, Characterization of polycyclic aromatic hydrocarbons in motor vehicle fuels and exhaust emissions, *Environ. Sci. Technol.*, 1999, 33, 18, 3091-3099.
- J. M. Kelly, P. D. Ivatt, M. J. Evans, J. H. Kroll, A. I. H. Hrdina, I. N. Kohale, F. M. White, B. P. Engelward, N. E. Selin, Global cancer risk from unregulated polycyclic aromatic hydrocarbons, *GeoHealth*, 2021, 10, 1029, 1-19.
- L. Plant, R. D. Knapp, L. C. Smith, Mechanism and rate of permeation of cells by polycyclic aromatic hydrocarbon, *J. Biol. Chem.*, 1987, 262, 6, 2514-2519.

- Y. Cao, L. Zhang, Y. Geng, Y. Li, Q. Zhao, J. Huang, P. Ning, S. Tiang, Evaluation of permeability and potential toxicity of polycyclic aromatic hydrocarbons to pulmonary surfactant membrane with parallel artificial membrane permeability model, *Chemosphere*, 2022, 290, 10, 132465.
- F. C. Obioha, J. A. Ogugua, J. A. Nwanta, A. O. Anaga, Determination of polycyclic aromatic hydrocarbons in edible cattle hides in Enugu State, Nigeria, *Public Health Toxicol.*, 2024, 4, 2, 8, 1-7.
- Strandberg, A. Julander, M. Sjoström, M. Lewné, H. K. Akdeva, C. Bigert, An improved method for determining dermal exposure to polycyclic aromatic hydrocarbons, *Chemosphere*, 2018, 198, 274-280.
- Bukowaska, K. Mokra, J. Michaliwicz, Benzo[a]pyrene-environmental occurrence, human exposure, and mechanisms of toxicity, *Int. J. Mol. Sci.*, 2022, 23, 6348, 1-30.
- Tuvikene, Responses of fish to polycyclic aromatic hydrocarbons, *Ann. Zool. Fennici.*, 1995, 32, 295-309.
- Y. Zhang, Q. Hu, J. Fu, X. Li, H. Mao, T. Wang, Influence of exposure pathways on tissue distribution and health impact of polycyclic aromatic hydrocarbon derivatives, *Environ. Health*, 2023, 1, 150-167.
- J. M. Kelly, P. D. Ivatt, M. J. Evans, J. H. Kroll, A. I. H. Hardina, I. N. Kohale, F. M. White, B. P. Engelward, N. E. Selin, Global cancer risk from unregulated polycyclic aromatic hydrocarbons, *GeoHealth*, 2021, 5, 1-19.
- Bukowska, K. Mokra, J. Michalowicz, Benzo[a]pyrene occurrence, human exposure, and mechanism of toxicity, *Int. J. of mol. Sci.*, 2022, 23, 6348, 1-30.
- P. Mottier, V. Parisod, R. J. Turesky, Quantitative determination of polycyclic aromatic hydrocarbons in barbecued meat sausages by gas chromatography coupled to mass spectrometry, *J. Agric. Food Chem.*, 2000, 48, 4, 1160-1166.
- H. Jiang, S. L. Gelhaus, D. Mangal, R. G. Harvey, I. A. Blair, T. M. Penning, Metabolism of benzo[a]pyrene in human bronchoalveolar-H358 cells using liquid chromatography-mass spectrometry, *Chem Res Toxicol.*, 2007, 20, 9, 1331-1341.
- K. Vahakangas, The distribution of benzo[a]pyrene and its metabolites in isolated perfused lung and liver, 1979, 4, 5, 413-418.
- Kleinpeter, A. Koch, Identification and quantification of local antiaromaticity in polycyclic aromatic hydrocarbons (PAHs) based on magnetic criterion, *Org. Biomol. Chem.*, 2024, 22, 3035-3044.
- Hall, M. R. Sarkar, S. G. Bell, Selective oxidation of substituted aromatic hydrocarbons and the observation of uncoupling via redox cycling during naphthalene oxidation by the CYP101B1 system, *Catalysis sci. tech.*, 2017, 7, 1-12.
- X. M. Chen, Y. J. Chu, C. G. Liu, Degradation mechanism of benzo[a]pyrene initiated by the OH radical and O₂: an insight from density function theory calculations, *ACS Omega*, 2020, 5, 25552-25560.
- Ramesh, S. A. Walker, D. B. Hood, M. D. Guillen, K. Schneider, E. H. Weyand, Bioavailability and risk assessment of orally ingested polycyclic aromatic hydrocarbons, *Int. J. Toxicol.*, 2004, 23, 301-333.
- K. L. Harris, L. D. Banks, J. A. Mantey, A. C. Huderson, A. Ramesh, Bioaccessibility of polycyclic aromatic hydrocarbons: relevance to toxicity and carcinogenesis, *Expert Opin. Drug. Metab. Toxicol.*, 2013, 9, 11, 1465-1480.
- K. Hardonniere, E. Saunier, A. Lamarie, M. Fernier, I. Gallais, C. H. Toussaint, B. Mograbi, S. Antonio, P. Benit, P. Rustin, M. Janin, F. Habarou, C. Ottolenghi, M. T. Lavault, C. Benelli, O. Sergeant, L. Huc, S. Bortoli, D. L. Gossmann, The environmental carcinogen benzo[a]pyrene induces a Warburg like metabolic reprogramming dependent on NHE1 and associated with cell survival, *Nat. sci. rept.*, 6, 30776, 1-013.

- W. E. Acree Jr., M. H. Abraham, Solubility predictions of crystalline polycyclic aromatic hydrocarbons (PAHs) dissolved in organic solvents based on the Abraham general solvation model, *Fluid Phase Equilibria*, 2002, 201, 245-258.
- W. Chen, H. Zong, Y. Xie, J. Xu, J. W. Cai, S. D. Wang, G. Zhou, Polycyclic aromatic hydrocarbons containing antiaromatic chalcogenopyrano [3,2-b]- chalcogenopyrans, *Org. Chem. Front.*, 2024, 11, 390-400.\