



Review

Overview on polycyclic aromatic hydrocarbons: Occurrence, legislation and innovative determination in foods

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ABSTRACT

Polycyclic aromatic hydrocarbons are ubiquitous compounds, well-known to be carcinogenic, which can reach the food in different ways. Thus the analysis of such compounds has always been of great importance. The aim of the present review, is not only to give an overview of the most recent sample preparation and analytical approaches (such as pressurized liquid extraction, solid-phase microextraction, supercritical fluid extraction, etc.), but also to introduce such a topic to researchers who want to approach it for the first time; therefore, the most significant references related to general aspects, such as formation, toxicity, risk assessment, occurrence in food, are reported and briefly discussed.

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Abbreviations: A, anthracene; Ac, acenaphthene; ACN, acetonitrile; Ap, acenaphthylene; APCI, atmospheric pressure chemical ionization; APPI, atmospheric pressure photoionization; BaA, benz[a]anthracene; BaP, benzo[a]pyrene; BbF, benzo[b]fluoranthene; BcF, benzo[c]fluorene; BeP, benzo[e]pyrene; BghiP, benzo[ghi]perylene; Bjf, benzo[j]fluoranthene; BkF, benzo[k]fluoranthene; Car, carbone; Ch, chrysene; CNTs, carbon nanotubes; CPP, cyclopenta[*c,d*]pyrene; DBaEP, dibenzo[*a,e*]pyrene; DBaHA, dibenz[*a,h*]anthracene; DBaHP, dibenzo[*a,h*]pyrene; DBaIP, dibenzo[*a,e*]pyrene; DBaLP, dibenzo[*a,l*]pyrene; DVB, divinylbenzene; EC, European Commission; EFSA, European Food Safety Authority; EPA, Environmental Protection Agency; ESI, electrospray ionization; F, fluorene; FID, flame ionization detector; FI, fluoranthene; FLD, fluorimetric detector; GC, gas chromatography; GC × GC, comprehensive GC; HPLC, high performance liquid chromatography; HS, head-space; IP, indeno[1,2,3-*cd*]pyrene; JECFA, Joint FAO/WHO Expert Committee on Food Additives; JRC, Joint Research Centre; LC × LC, comprehensive LC; LOD, limit of detection; LOQ, limit of quantification; LVI, large-volume injection; MAE, microwave assisted extraction; MeOH, methanol; MEPS, micro-extraction in packed syringe; MIPs, molecular imprinted polymers; mMWNTs, magnetic multiwalled carbon nanotubes; MoE, Margin of Exposure; MS, mass spectrometer; MSPD, matrix solid-phase dispersion; MSPE, magnetic solid-phase extraction; Na, naphthalene; NaOAc, sodium acetate; NH₂, amino; P, pyrene; Pa, phenanthrene; PAHs, polycyclic aromatic hydrocarbons; PDMS, polydimethylsiloxane; PLE, pressurized liquid extraction; PS-DVB, styrene-divinylbenzene; PSA, primary secondary amine; PTV, programmed-temperature vaporization; SCF, Scientific Committee on Food; SPE, solid-phase extraction; SPME, solid-phase microextraction; TEF, toxic equivalent factor; UHPLC, ultra-high performance liquid chromatography; WHO, World Health Organization

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1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a large class of ubiquitous and toxic environmental and food processing contaminants. PAHs contain two or more fused aromatic rings, produced through incomplete combustion or pyrolysis of organic matter and geological processes [1]. They can be classified according to the number of condensed aromatic rings as light (2–3 rings) or heavy (4–6 rings) PAHs, the latter being more stable and toxic than the light ones.

Due to their proved carcinogenic activity, they have been largely investigated, both to fully understand the toxicity mechanism and to elucidate the main sources and occurrence in foods. A great effort has also been devoted to the improvement of the analytical method to determine such compounds in complex samples, such as food. Furthermore, legislation has tried to follow the new evidences by introducing, modifying and updating the related regulations, which have evolved significantly over the last decades.

Many reviews [2–9] related to PAH analysis in food and in environmental samples have been published over the years. The aim of the present review is to give a useful tool to scientists who want to approach the topic of PAHs contamination in foods. A particular attention has been devoted to a concise but complete overview of the general PAHs aspect, such as toxicity, sources and occurrence, giving the most important and complete references to deepen the specific topics. The development of the European legislation on the matter has been largely discussed. Finally, a review of the analytical methods for PAHs determination has been reported, with a focus on more innovative and less-solvent and time-consuming techniques developed over recent years.

2. Sources, toxicity and occurrence.

All compounds containing carbon and hydrogen can act as PAH precursors at high temperature (500–700 °C). In fact, they are partially cracked to smaller unstable fragments (pyrolysis), mostly radicals, which recombine to give relatively stable PAHs (pyro-synthesis). Formation of PAHs can occur also at lower temperatures (100–150 °C) but it requires a period on the geological time-scale and a larger amount of alkylated PAHs are originated, as in the case of natural fossil fuel formation [10].

PAHs are present in the environment due to natural sources (forest fires, volcanoes, hydrothermal processes, oil seepage and carbonisation) and anthropogenic ones (mainly, the combustion of fossil fuels and the direct release of oil and oil products) [1,11].

A number of PAHs have been proven genotoxic and mutagenic, while other PAHs not defined as carcinogenic may act as synergists [12]. It is interesting to highlight that PAHs themselves are not toxic, but that they are activated by the attempt of the organism to eliminate these xenobiotics by increasing the polarity through the addition of polar groups. The most important metabolites of benzo[a]pyrene (BaP) is BaP-7,8-diol-9,10-epoxide, which presents the highest tumour inducing activity, because it forms adducts with proteins or DNA. A deeper and detailed discussion of activation mechanism can be found in several documents published by the World Health Organization (WHO) and the European Scientific Committee on Food (SCF) [12–16]. Very little systematic information is available on the effect of alkylation on the biological activity of PAHs, nevertheless it seems

strictly correlated with the position of substitution in the ring. In particular, methyl substitution might enhance the toxicity compared to the non-alkylated PAHs, while bulky substitutions would reduce the bio-activity of such compounds [17].

Humans can be exposed to PAHs through three main routes: inhalation, skin contact, and ingestion.

The rate of absorption by the lungs depends on the structure of the PAH, the size and the chemical nature of the particles where they are adsorbed [16–20]. Most of the toxicity studies on PAHs have been carried out on dermal, subcutaneous and inhalation exposure, mainly as an occupational issue. However, it has been demonstrated that ingestion through food and water is a major route of exposure [12,21]. The composition of the diet affects PAHs absorption in the intestinal mucosa. Indeed, absorption of ¹⁴C-BaP in Wistar rats is increased by lipophilic foods and is inhibited by high-fibre foods [22].

The occurrence of PAHs in food is mainly due to environmental contamination, technological processing, and contact with non-food-grade quality mineral oil and contaminated packaging. Considering the numerous sources of PAHs that release them into the environment, the atmospheric fallout is an important cause of contamination in plant and vegetables directly in the field [23,24]. In particular, only light PAHs are present in the gaseous phase, while both the light and the heavy ones may be adsorbed on the particulate.

Among the technological processes, heating (i.e., grilling and smoking) involving direct contact with combustion gases is an important source of contamination [25,26]. The contribution to contamination levels depends on: the time and temperature of processing (higher temperature and longer time increase the amount of PAHs); the distance from the heat source (the higher the distance, lower the contamination level in foods); the kind of process (grilling, roasting, smoking, drying), in particular, if food is directly in contact with the combustion products; the type of fuel used (e.g., burning of carbon produce less PAHs than wood), and the amount of fat in the processed food (fat is the major precursor of PAHs). Finally, contamination with non-food-grade mineral oil may occur, mainly related to lubricant oil, jute bags, recycled paper packaging, printing inks [27–31].

The most important contributors to dietary intake are vegetable oils and fats, both as a seasoning and indirectly as an ingredient in food formulation [25]. Very high levels of PAHs can also be found in dried fruits (when combustion products are allowed to come directly in contact with food or due to contamination during transport) [32], and in smoked meat and fish products [26] (related to the smoking method) [33]. Among fishery products, blue fish species (i.e., tuna, mackerel and salmon) generally present lower contamination levels than molluscs, even if they originated from polluted areas. Indeed, fish, in contrast with bivalves, oxidize and metabolise PAHs to water-soluble compounds, which are eventually excreted. Several reports discuss in depth the occurrence of PAHs in food [12,34,35].

PAHs occurring in foods are always present as a complex mixture. This has to be considered during the selection of the most suitable risk assessment approach to evaluate the PAH toxicity. The risk assessment process consists of several steps including hazard identification, hazard characterisation, exposure assessment and risk characterisation. Three of the most popular approaches are toxicity equivalent factor (TEF), comparative potency, and BaP as a surrogate (the detailed discussion of such topics are out of the aim of the

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