The Occurrence of 16 EPA PAHs in Food – A Review

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Occurrence and toxicity of polycyclic aromatic hydrocarbons (PAHs) have been extensively studied in countries all over the world. PAHs generally occur in complex mixtures which may consist of hundreds of compounds. The U.S. Environmental Protection Agency (EPA) proposed in the 1970s to monitor a set of 16 PAHs which are frequently found in environmental samples. This article reviews the suitability of the 16 EPA PAHs for the assessment of potential health threats to humans stemming from the exposure to PAHs by food ingestion. It presents details on analysis methods, the occurrence of PAHs in food, regulatory aspects, and related risk management approaches. In addition, consideration is given to newer evaluations of the toxicity of PAHs and the requirements for risk assessment and management stemming from them.

Key Words: 16 EPA PAHs, analysis, food, legislation, polycyclic aromatic hydrocarbons

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAH) form a large class of diverse organic compounds, each of them containing two or more aromatic rings. PAH generally occur in complex mixtures, which may consist of hundreds of compounds. Humans are exposed to PAHs through different pathways. For non-smokers the major route of exposure is consumption of food (1,2); for smokers the contribution from smoking can be significant. Food can be contaminated from environmental sources (natural and mostly anthropogenic), from industrial
food processing, and from some domestic cooking practices. PAHs can enter the food chain by deposition from air, or by deposition and transfer from soil and water (3,4).

Occurrence and toxicity of PAHs have been evaluated by numerous organizations, e.g., the United States Environmental Protection Agency (U.S. EPA) (5), the International Agency for Research on Cancer (IARC) (6), the Scientific Committee on Food (SCF) (7), the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (8), the International Programme on Chemical Safety (IPCS) (9), and the European Food Safety Authority (EFSA) (10).

The U.S. Agency for Toxic Substances and Disease Registry (ATSDR) and EPA were required to prepare a list, in order of priority, of substances that are most commonly found at facilities on the National Priorities List (NPL) and which are the most significant potential threat to human health due to their known or suspected toxicity and potential for human exposure. This substances priority list is revised and published on a 2-year basis, with a yearly informal review and revision. The priority list is not a list of “most toxic” substances, but rather a prioritization of substances based on a combination of their frequency of occurrence, toxicity, and potential for human exposure. Thus, substances with low toxicity but high frequency of occurrence and exposure could be added to the priority list (11). EPA selected 16 PAHs, which are frequently found in environmental monitoring samples, namely acenaphthene, acenaphthylene, anthracene, fluorene, naphthalene, phenanthrene, pyrene, benz[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[g,h,i]perylene, benzo[a]pyrene, chrysene, dibenz[a,h]anthracene, and indeno[1,2,3-cd]pyrene. These 16 EPA PAHs were identified in the 1970s (12).

In the EU, SCF assessed the toxicity of PAHs. For most of the PAHs their carcinogenic and genotoxic potential constitute the critical factor for the hazard and risk characterizations. In its assessments of PAHs in food, the SCF took note of the evaluations performed by various international expert groups and prioritized compounds based on the health risk rather than on occurrence in food. The SCF recommended the monitoring of 15 PAHs in 2002, including 8 high molecular weight PAHs that are also part of the U.S. EPA list. SCF concluded that 15 out of 33 assessed PAHs, namely benz[a]anthracene, benzo[b]fluoranthene, benzo[j]fluoranthene, benzo[k]fluoranthene, benzo[ghi]perylene, benzo[a]pyrene, chrysene, cyclopenta[c,d]pyrene, dibenzo[a,h]anthracene, dibenzo[a,e]pyrene, dibenzo[a,h]pyrene, dibenzo[a,i]pyrene, dibenzo[a,l]pyrene, indeno[1,2,3-cd]pyrene, and 5-methylchrysene show clear evidence of mutagenicity/genotoxicity and, with exception of benzo[ghi]perylene, have also shown clear carcinogenic effects in various types of bioassays in experimental animals (1). It suggested to use benzo[a]pyrene as a marker of occurrence of carcinogenic PAHs in food, based on examinations of PAH profiles in food and on evaluation of a
carcinogenicity study of coal tar mixtures in mice (13). SCF also stressed the need to continue collecting data on other PAHs in order to be able to evaluate the contamination of food commodities and any possible changes in the PAH profile. Of the 15 EU priority PAHs, 12 were identical to those that were identified by IARC to be human carcinogens based on sufficient evidence of carcinogenicity in experimental animals in 1973–1987. The IARC has identified those PAHs as carcinogenic to humans (group 1), and probable (group 2A) or possible (group 2B) human carcinogens (Table 1). Other PAHs not defined as carcinogens may act as synergists (6).

Taking into account newer studies, JECFA re-evaluated PAHs in 2005 and concluded that 13 PAHs are clearly genotoxic and carcinogenic. The compounds are the same as those stated by SCF except benzo[ghi]perylene and cyclopenta[cd]pyrene. It was recommended to collect data on occurrence of those PAHs in the major food groups. In addition, JECFA suggested to include also benzo[c]fluorene as a further compound into the analysis as data on its occurrence in food were still insufficient (2).

Following the suggestion of SCF, the European Commission issued a recommendation on further investigation of PAH levels in certain foods (14). An evaluation of the data gathered by the Member States by EFSA in 2008 showed that certain PAHs such as chrysene were found in some food samples, which were negative for benzo[a]pyrene. In these cases benzo[a]pyrene cannot be an indicator of PAH contamination in food. As the most suitable indicators of occurrence of PAHs in food, eight compounds (PAH8), i.e., benz[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[ghi]perylene, benzo[a]pyrene, chrysene, dibenz[a,h]anthracene, and indeno[1,2,3-cd]pyrene were selected. However, in comparison with a selection of four compounds (PAH4), i.e., benz[a]anthracene, benzo[b]fluoranthene, benzo[a]pyrene, chrysene, PAH8 does not provide much added value (2).

**CONTAMINATION OF FOOD BY PAHS**

Food can be contaminated by PAHs that are present in air, soil, or water, or during food processing and cooking. PAHs are ubiquitous environmental contaminants which are widespread in air bound to particulate matter. In spite of their hydrophobic properties (especially heavy PAHs), they are found in water as well. These compounds are produced during combustion and pyrolysis processes of anthropogenic and natural origin. A high amount of PAHs are emitted from processing coal, during incomplete combustion of organic matter (e.g., wood and fossil fuels), from motor vehicle exhaust and cigarettes. Forest fires, volcanoes and hydrothermal processes are natural emission sources of PAHs (4). The contamination of vegetation in the neighborhood of industrial areas or along highways is higher than in rural areas. The levels of PAHs found in unprocessed foods in rural areas reflect the background contamination that could
Table 1: Molecular structure of PAHs assessed by the authorities

| Compound              | Structure | Molecular weight | US EPA PAHs | EU priority PAHs | IARC group<sup>a</sup> |
|-----------------------|-----------|------------------|-------------|------------------|-------------------------|
| Acenaphthene          | ![Structure](image1) | 154              | x           | —                | 3                       |
| Acenaphthylene        | ![Structure](image2) | 152              | x           | —                | Not assessed            |
| Anthracene            | ![Structure](image3) | 178              | x           | —                | 3                       |
| Fluoranthene          | ![Structure](image4) | 202              | x           | —                | 3                       |
| Fluorene              | ![Structure](image5) | 166              | x           | —                | 3                       |
| Naphthalene           | ![Structure](image6) | 128              | x           | —                | 2B                      |
| Phenanthrene          | ![Structure](image7) | 178              | x           | —                | 3                       |
| Pyrene                | ![Structure](image8) | 202              | x           | —                | 3                       |
| Benz(a)anthracene     | ![Structure](image9) | 228              | x           | x                | 2B                      |
| Benzo(b)fluoranthene  | ![Structure](image10) | 252              | x           | x                | 2B                      |
| Benzo(j)fluoranthene  | ![Structure](image11) | 252              | —           | x                | 2B                      |
| Benzo(k)fluoranthene  | ![Structure](image12) | 252              | x           | x                | 2B                      |
| Benzo(ghi)perylene    | ![Structure](image13) | 276              | x           | x                | 3                       |
| Benzo(a)pyrene        | ![Structure](image14) | 252              | x           | x                | 1                       |
| Chrysene              | ![Structure](image15) | 228              | x           | x                | 2B                      |
| Cyclopenta(cd)pyrene  | ![Structure](image16) | 226              | —           | x                | 3                       |
| Dibenz(a,h)anthracene | ![Structure](image17) | 278              | x           | x                | 2A                      |
| Dibenzo(a,e)pyrene    | ![Structure](image18) | 302              | —           | x                | 3                       |
| Dibenzo(a,h)pyrene    | ![Structure](image19) | 302              | —           | x                | 2B                      |

(Continued on next page)
originates from long distance airborne transportation of contaminated particles (15). The contamination of food with environmental PAHs depends on a number of physical and chemical properties of PAHs such as their solubility in water and fats/oils, volatility, chemical reactivity, and biotic and abiotic degradability (16,17). The waxy surface of vegetables and fruits can concentrate low molecular mass PAHs, mainly through surface adsorption. The concentration of PAHs is generally greater on the plant surface (outer leaves, peel) than in internal tissue (18). Seafood and fish can be exposed to PAHs present in water and sediments due to atmospheric pollution or oil spills. The PAH content greatly depends on the ability of the aquatic organisms to metabolize them. Bivalve mollusks such as mussels and oysters can accumulate high molecular mass PAHs because they filter large volumes of water and they are not capable to metabolize efficiently all PAHs (3,19).

Processing of food (drying, smoking) and cooking of food at high temperatures (grilling, frying, roasting, baking) are commonly considered to be the major sources of food contamination by PAHs (20–22). Seeds and raw products for oil production may be contaminated with PAHs through artificial drying and heating during processing, if precautionary measures are not taken (e.g., indirect drying and good temperature control). Drying of seeds and kernels is thought to be one of the prominent sources for the contamination of edible oils with PAHs (23). Certain preservation techniques such as industrial smoking of meat and dairy products under inappropriate conditions could also lead to food contamination with PAHs (24). The PAH contamination of smoked foods

### Table 1: Molecular structure of PAHs assessed by the authorities (Continued)

| Compound | Structure | Molecular weight | US EPA PAHs | EU priority PAHs | IARC group<sup>a</sup> |
|----------|-----------|------------------|-------------|------------------|------------------------|
| Dibenzo(a,i)pyrene | ![Structure](image1) | 302 | — | x | 2B |
| Dibenzo(a,l)pyrene | ![Structure](image2) | 302 | — | x | 2A |
| Indeno(1,2,3-cd)pyrene | ![Structure](image3) | 276 | x | x | 2B |
| 5-Methylchrysene | ![Structure](image4) | 242 | — | x | 2B |
| Benzo(c)fluorene | ![Structure](image5) | 216 | — | x | 3 |

IARC (International Agency for Research on Cancer) classification: group 1 = carcinogenic to humans, group 2A = probably carcinogenic to humans, group 2B = possibly carcinogenic to humans, group 3 = not classifiable as to carcinogenicity to humans.
can be significantly reduced by replacing direct smoking with indirect smoking obtained by an external smoke generator. As PAHs are bound to particles, a filter may be used to remove particulate material from the smoke. This will reduce contamination with PAHs (25). Roasting and drying of coffee beans, cocoa beans and tea leaves may increase the PAH content (26,27). Currently, significant levels of PAHs were reported in certain food supplements, mainly those which undergo an inappropriate drying process (28). Furthermore, liquid smoke flavoring products are used by food industry to improve organoleptic characteristics. They are added to various foods either to replace the smoking process or to impart smoke flavor to foods which are not traditionally smoked. Smoke flavoring products are produced from smoke condensates, which might be a source of PAHs as well; however, the levels are in general lower than in products made with freshly generated smoke (29,30).

PAHs are also formed as a result of some domestic food preparation methods. When food, particularly meat and fish, are cooked over an open flame, PAHs are formed. If the grilled food is in direct contact with the flame, pyrolysis of the drippings from meat or fish generates PAHs that can be deposited on its surface. Even if not in direct contact, fat dripping onto the flame or hot coals generates these compounds that are carried back onto the surface of the food (31). PAH formation during charcoal grilling was shown to be dependent upon the fat content of the meat, duration of cooking and the temperature used. Smoked and grilled food may contribute significantly to the intake of PAHs if such foods are a large part of the usual diet. Simple practices such as selecting preferentially lean meat and fish and avoiding contact of foods with flames for barbecuing, using less fat for grilling, and cooking at lower temperature for longer time, results in a significant reduction of food contamination by PAHs (32).

Despite the low levels found, cereals and cereal products were identified as a major contributor to the intake of PAHs, owing to their high consumption. Another major contributor is vegetable fats and oils owing to the higher concentration of PAHs in this food group. Even though their concentration of PAHs can be high, smoked fish and meats and barbecued foods do not contribute significantly to exposure, as they are usually a small component of the diet. However, they do make larger contributions leading to higher PAH intakes when these foods make up a large part of the diet (2,33).

**REGULATORY LIMITS IN FOOD**

Although the 16 EPA PAHs are highly relevant for environmental monitoring programs, they did not enter food legislation. This is demonstrated in the following based on examples for legislation on PAHs in food for countries from all
over the world. However, it will be stressed that the provided details are not exhaustive.

**U.S. Legislation**

The U.S. federal government set regulatory standards and guidelines to protect people from the possible health effects caused by eating, drinking, or breathing PAHs. Under the Safe Drinking Water Act, EPA sets legal maximum limits on the level of benzo[a]pyrene in drinking water (0.2 μg/L). Another limit was set by the National Institute for Occupational Safety and Health (NIOSH) and the Occupational Safety and Health Administration (OSHA) based on the evaluation of occupational exposure to coal products. The OSHA has established a legally enforceable exposure limit of 0.2 mg/m³ air averaged over an 8-h period of work place exposure. An exposure limit was set also for mineral oil mist by OSHA and NIOSH at a level of 5 mg/m³ averaged over an 8-h exposure period and 5 mg/m³ for a 10-h work day, respectively (34,35). As far as we know, no maximum limits for PAH contents of foodstuffs have been introduced into U.S. legislation.

**EU Legislation**

The European Commission set maximum levels for benzo[a]pyrene for the first time in 2005 by Regulation (EC) No 208/2005 amending Regulation (EC) No 466/2001 (36). Later, the maximum levels for benzo[a]pyrene were laid down in Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs (37). A maximum level for the sum of the four PAHs (benzo[a]pyrene, benzo[a]anthracene, benzo[b]fluoranthene, and chrysene) was included by Regulation (EU) No 835/2011 amending Regulation (EC) No 1881/2006 (38). New maximum levels for the sum of PAH4 were introduced, whilst maintaining separate maximum levels for benzo[a]pyrene (Table 2). This approach aims at ensuring that PAH levels in food are kept at levels that do not cause health concerns and that foods in which benzo[a]pyrene is not detectable, but where other PAH are present are prevented from entering the market. The separate maximum level for benzo[a]pyrene was maintained to ensure comparability of previous and future data. However some of the maximum levels will be lowered as from 2014/2015.

Maximum levels are especially set for foodstuffs containing fats and oils and foods where smoking and drying processes or environmental pollution might cause high levels of contamination. The lowest maximum levels are set for food for infants and young children. In some cases the new PAH occurrence data show that background levels of PAHs are lower than previously thought in some food commodities (2,38,39). Benzo[a]pyrene maximum levels have therefore been adapted to reflect lower background levels in fresh and
Table 2: Maximum levels for benzo(a)pyrene and the sum of benzo(a)pyrene, benz(a)anthracene, benzo(b)fluoranthene, and chrysene as laid down in Regulation (EU) No 835/2011

| Foodstuffs                                                                 | Benzo(a)pyrene | Sum of benzo(a)pyrene, benz(a)anthracene, benzo(b)fluoranthene and chrysene |
|---------------------------------------------------------------------------|----------------|--------------------------------------------------------------------------------|
| 6.1.1 Oils and fats (excluding cocoa butter and coconut oil) intended for| 2.0            | 10.0                                                                            |
| direct human consumption or use as an ingredient in food                  |                |                                                                                 |
| 6.1.2 Cocoa beans and derived products                                    | 5.0 μg/kg fat  | 35.0 μg/kg fat until 31.3.2015                                                   |
|                                                                            |                | 30.0 μg/kg fat from 1.4.2015                                                     |
| 6.1.3 Coconut oil intended for direct human consumption or use as an     | 2.0            | 20.0                                                                            |
| ingredient in food                                                        |                |                                                                                 |
| 6.1.4 Smoked meat and smoked meat products                               | 5.0 until 31.8.2014 | 30.0 until 31.8.2014                                                            |
|                                                                            | 2.0 as from 1.9.2014  | 12.0 as from 1.9.2014                                                            |
| 6.1.5 Muscle meat of smoked fish and smoked fishery products, excluding  | 5.0 until 31.8.2014 | 30.0 until 31.8.2014                                                            |
| fishery products listed in points 6.1.6 and 6.1.7. The maximum level for | 2.0 as from 1.9.2014  | 12.0 as from 1.9.2014                                                            |
| smoked crustaceans applies to muscle meat from appendages and abdomen.   |                |                                                                                 |
| In case of smoked crabs and crab-like crustaceans it applies to muscle    |                |                                                                                 |
| meat from appendages.                                                     |                |                                                                                 |
| 6.1.6 Smoked sprats and canned smoked sprats; bivalve molluscs (fresh,    | 5.0            | 30.0                                                                            |
| chilled or frozen); heat treated meat and heat treated meat products     |                |                                                                                 |
| sold to the final consumer                                               |                |                                                                                 |
| 6.1.7 Bivalve molluscs (smoked)                                           | 6.0            | 35.0                                                                            |
| 6.1.8 Processed cereal-based foods and baby foods for infants and young   | 1.0            | 1.0                                                                             |
| children                                                                 |                |                                                                                 |
| 6.1.9 Infant formulae and follow-on formulae, including infant milk and    | 1.0            | 1.0                                                                             |
| follow-on milk                                                            |                |                                                                                 |

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**Table 2:** Maximum levels for benzo(a)pyrene and the sum of benzo(a)pyrene, benz(a)anthracene, benzo(b)fluoranthene, and chrysene as laid down in Regulation (EU) No 835/2011 (Continued)

| Foodstuffs | Maximum levels (μg/kg) |
|------------|------------------------|
|            | Benzo(a)pyrene | Sum of benzo(a)pyrene, benz(a)anthracene, benzo(b)fluoranthene and chrysene |
| 6.1.10     |               | |
| Dietary foods for special medical purposes intended specifically for infants | 1.0 | 1.0 |

Smoked bivalve mollusks. Data for smoked fish and smoked meat have also shown that lower maximum levels are achievable. Smoked sprats and canned smoked sprats have been found to contain higher levels of PAH than other smoked fish. Specific maximum levels should be established for these commodities. High levels of PAHs have been found in some types of heat treated meat and meat products sold to the final consumer. Maximum levels for PAHs were set in meat and meat products that have undergone a heat treatment process known to potentially result in formation of PAHs, i.e., grilling and barbecuing. Previously, a maximum level for benzo[a]pyrene in muscle meat of fish other than smoked fish was established as an indicator for potential environmental pollution. It has been shown that PAHs are quickly metabolized in fresh fish and do not accumulate in fish muscle (40,41). Therefore, maintaining a maximum level for PAHs in fresh fish is no longer appropriate. Cocoa butter was also included into the regulation as it contains higher levels of PAHs than other oils and fats. It was temporarily derogated in 2005 (until 2007) and from 2011 onwards levels are regulated. Cocoa butter is present in chocolate and other cocoa products often consumed by children. Current occurrence data on PAHs in cereals and vegetables are limited and indicate rather low levels of PAHs, which do not justify the immediate setting of maximum levels. Nevertheless, EFSA identified cereals and vegetables as being important contributors to human exposure due to their high consumption (2). Therefore, PAH levels in these two product groups should be further monitored. High levels of PAHs have been found in some food supplements. However, the levels are variable and depend on the specific type of food supplements. Further data on food supplement are still needed and should be collected.

Regulation (EC) No 2065/2003 on smoke flavorings used or intended for use in or on foods requires that the precursors to commercial smoke flavorings have to be approved for the marketing of the products in the EU. The application for
registration has to be accompanied by a dossier which must contain data on the chemical composition of the respective products. This includes a group of 15 EU PAHs. For benzo[a]pyrene and benz[a]anthracene, maximum permitted concentrations of 10 and 20 \( \mu \text{g/kg} \) have been set (42).

Council Directive 98/83/EC on the quality of water intended for human consumption established a maximum limit for benzo[a]pyrene of 0.010 \( \mu \text{g/L} \) and for the sum of benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[ghi]perylene, and indeno[1,2,3-cd]pyrene of 0.10 \( \mu \text{g/L} \) (43).

Regulation (EC) No 333/2007 lays down provisions for the methods of sampling and analytical performance criteria only for benzo[a]pyrene (44). Upon amending Regulation (EC) 1881/2006 by Regulation (EU) 835/2011, it was necessary to specify method performance criteria also for the other three compounds. For this reason Regulation (EU) No 836/2011 amending Regulation (EC) No 333/2007 laying down the methods of sampling and analysis for the official control was introduced (45). It contains strict requirements for methods of analysis in order to ensure that control laboratories use procedures with comparable levels of performance. The requirements for sampling methods differ especially depending on the type and weight of a lot, in order to obtain samples that are representative for the respective lot. As a specific and detailed requirement it is laid down that the analyst shall ensure that samples do not become contaminated during sample preparation. Sampling equipment packaging material coming into contact with the sample shall be made of inert materials such as aluminium, glass, or polished stainless steel. Plastics such as polypropylene or polytetrafluoroethylene shall be avoided because PAHs can adsorb onto these materials (44). Containers shall be rinsed with high purity acetone or \( n \)-hexane to minimize the risk of contamination. Losses of PAHs may also occur if the sample is exposed to light and high temperatures during sample collection and storage (2,45).

Regarding analytical methods, EU legislation on contaminants in food follows a “criteria approach”. This means that the legislator does not prescribe specific analysis procedures, but allows laboratories to use different methods of analysis, provided it can be demonstrated in an objective manner that they fulfil the analytical requirements laid down in the respective legislation. Preference is given to fully validated methods (i.e., methods validated by collaborative trial for the respective matrix). However, in their absence other suitable validated methods (e.g. in-house validated methods for the respective matrix) may be applied. Where possible, the validation of in-house validated methods shall include a certified reference material (2). Different parameters are set for the performance criteria such as applicability, specificity, repeatability, reproducibility, limits of detection (LOD) and quantification (LOQ), and recovery. LOD and LOQ are defined as less than 0.30 \( \mu \text{g/kg} \) and less than 0.90 \( \mu \text{g/kg} \) for each of the four substances, respectively (45). Recovery shall be in the range between 50% and 120% (45). Specificity is defined as “free from matrix or
spectral interferences, verification of positive detection”. HORRAT_r and HORRAT_R values of less than 2 are specified for the precision of the analysis methods (45).

If only in-house validated methods are available a “fitness-for-purpose” approach may be used to assess their suitability for official control. Suitable methods must produce results with a combined standard measurement uncertainty less than the maximum tolerated standard measurement uncertainty calculated using the formula:

\[
U_f = \sqrt{\left(\frac{\text{LOD}}{2}\right)^2 + (\alpha C)^2}
\]

where \(U_f\) is the maximum tolerated standard measurement uncertainty, LOD is the limit of detection, \(C\) is the concentration of interest, and \(\alpha\) is a numeric factor to be used depending on the value of \(C\) (e.g., for the concentration \(\leq 50 \mu g/kg\), \(\alpha\) is set to 0.2) (45).

EU legislation on contaminants such as PAHs, in food is applicable in the European Economic Area, which includes beyond the 28 EU Member States also Iceland, Lichtenstein, and Norway.

**Legislation in Other Countries**

Health Canada’s Food Directorate is responsible for the assessment of risks to human health from the exposure to hazardous substances in food. Maximum levels are enforced by the Canadian Food Inspection Agency (CFIA) and are established in the Canadian Food and Drug Regulations. Levels of chemicals in food are monitored through continued surveillance activities by both Health Canada and CFIA. Regarding PAHs in food, a maximum level was set solely for the benzo[a]pyrene content of olive-pomace oil (3 \(\mu g/kg\)). However, the general provision in the Canadian legislation that no person shall sell an article of food that has in or on it any poisonous or harmful substances, regardless of whether or not there exists a maximum level is also applicable to PAHs (46). Besides olive pomace oil, a maximum acceptable concentration for benzo[a]pyrene is also established for drinking water (0.01 \(\mu g/L\)) (47).

China National Standard, or GB (Guobiao) Standard, comprises a set of mandatory and recommended standards issued by the Standardisation Administration of China. On November 13, 2012, the Ministry of Health issued the food safety national standard GB 2762-2012 (Maximum Levels of contaminants in Foods) to replace the preceding 2005 edition (GB 2762-2005). GB2762-2012 came into force as of June 1, 2013. This GB Standard sets maximum levels of certain contaminants in foods including benzo[a]pyrene. Maximum limits for benzo[a]pyrene are set for four food categories (Table 3) (48).

In Brazil, regulations establish maximum levels for benzo[a]pyrene in smoke flavorings (30 \(\mu g/kg\), set by Resolução RDC n. 281/2003 (49)), olive oil
Table 3: Indicators of maximum levels of benzo(a)pyrene in foods regulated in China

| Food type/ Name                  | Maximum levels (μg/kg) |
|----------------------------------|------------------------|
| Paddy, wheat                    | 5                      |
| Smoked or baked meats            | 5                      |
| Smoked or baked aquatic products | 5                      |
| Fats and oils, and fat emulsions  | 10                     |

(2.0 μg/kg, set by Resolução RDC n. 2/2007 (50)), and drinking water (0.7 μg/L set by Portaria MS n. 518/2004 (51)).

**PAH ANALYSIS IN FOOD**

**Quality assurance tools supporting PAH analysis in food**

The determination of trace analytes in complex matrices is a serious challenge, and needs a comprehensive set of quality assurance tools. The reliability of analysis results can be supported by external quality control tools such as proficiency tests (PTs) and certified reference materials (CRMs), as well as the application of well characterized and standardized analysis methods. The validation of analytical methods is thus of extreme importance to demonstrate that they are fit for purpose. Fundamental guidance on the management of, among others, analytical chemistry laboratories and the demonstration of their competence is provided by EN ISO/IEC 17025:2005 (52). Currently, great emphasis is placed on food control laboratories to follow ISO 17025 practices and to obtain accreditation according to this standard for their analytical methods in order to ascertain a high level of reliability of the generated data (53,54).

Valuable information on the performance of a laboratory in the execution of certain tests comes from the feedback provided by PT programs. Regularly participating in PTs is an essential requirement for laboratory accreditation. Several national and supranational PT providers such as the Food Analysis Performance Assessment Scheme (FAPAS®) or the Deutsche Gesellschaft für Fettforschung (DGF) have offered PT rounds on the determination of PAHs in food for a number of years. Comprehensive information on offered proficiency tests can be found in the EPTIS database (55).

In the EU, the European Union reference laboratories (EU-RLs) in the area of food and feed safety are required to organize annually in their area of competence proficiency tests for the national reference laboratories of the EU Member States. The EU-RL for PAHs (EU-RL PAH) organizes one to two PT rounds
The advantage of the EU-RL PT scheme is that continuous feedback and training over time has led to outstanding measurement capabilities of the participants (56,57).

CRMs are useful quality control tools for laboratories and are used for method validation (trueness determination) and method performance verification to ensure that method performance criteria are met. CRMs provide a certified value, which represents the best possible estimate for the true value, and its uncertainty. In that respect, CRMs that represent real food matrices are especially important. However, one has to acknowledge that it is impossible to provide suitable CRMs for each possible analyte/matrix combination. The availability of CRMs for PAHs in food matrices is limited (Table 4) and thus PT test materials might be a suitable substitute for quality control purposes (58).

Standards Methods for Food Samples

The first official method for the analysis of benzo[a]pyrene was developed for food products such as smoked foods, meat, cheese, total diet composites and beverages. Collaborative studies of this method covering either only benzo[a]pyrene or PAHs in general in food products were conducted under the auspices of the Association of Official Analytical Chemists (AOAC) and International Union of Pure and Applied Chemistry (IUPAC). The procedure consisted of an initial saponification of the sample in ethanolic potassium hydroxide solution, followed by a partition step between iso-octane, water and alcohol and column chromatography on Florisil. Further purification is done by liquid-liquid extraction with dimethylsulphoxide and thin-layer chromatography on cellulose plates. For determination of individual PAHs, ultraviolet spectrophotometry, confirmed by the spectrophotofluorometry, was used. The method was adopted as an AOAC official first action method in 1972 and accepted as a recommended method by IUPAC in 1975. Statistical evaluation of the data obtained by interlaboratory tests, in which ham samples were fortified with benzo[a]pyrene, benzo[e]pyrene, benzo[a]anthracene, and benzo[ghi]perylene at a level of 10 μg/kg and analyzed by the above mentioned method showed a repeatability relative standard deviation (RSD_r) between 7.4% and 12.7%. On this basis, the method has been adopted as the official method of AOAC (59).

A method for the determination of benzo[a]pyrene in animal and vegetable fats and oils was standardized by the International Organization for Standardization (ISO 15302) in 1998. This method is based on reverse phase HPLC-fluorescence detection (FLD) after clean-up on alumina. The range was from 0.1 μg/kg to 10 μg/kg. Later, the method was revised and the applicable range was extended to 50 μg/kg (60).
Another international standard, ISO 15753 (61), focuses on the determination of a whole set of 16 EPA PAHs in animal and vegetable fats and oils, although it does not provide quantitative results for the very volatile compounds such as naphthalene, acenaphthene, and fluorene. This international standard describes two methods, a general method and a method specific for coconut oil and vegetable oils with short chain fatty acids. PAHs are extracted, purified by solid phase extraction (SPE) and then are determined by reversed phase HPLC-FLD. Palm oil and olive pomace olive oil cannot be analyzed by this
method due to matrix interferences. The reported quantification limits for almost all analyzed compounds were 0.2 μg/kg (61).

The determination of PAHs in edible fats and oils of in total 17 PAHs (including 14 EPA PAHs) by ISO 22959 (62) is achieved by on-line coupling of donor acceptor complex chromatography to reversed phase HPLC-FLD. The oil samples are loaded onto a column with a stationary phase which acts as an electron acceptor. The column retains the PAHs (electron donors) by π-π interactions. After elution of oil the PAHs are on-line transferred to the analytical reversed phase column. The limits of quantification for individual PAHs are 0.1 μg/kg and the validated concentration ranges for each analyte from 0.1 μg/kg to 3.5 μg/kg (62).

A method based on gas chromatography coupled to mass spectrometry (GC-MS) was published in 2004 and currently is under further development (PD ISO/TR 24054). Also, this method focused on animal and vegetable fats and oils and is able to determine up to 27 PAHs (among them also the 16 EPA PAHs). Test portions are fortified with appropriate 13C internal standards and subjected to saponification followed by liquid-liquid extraction. After the purification step on a silica gel column instrumental analysis is undertaken by GC-MS (63).

After the oil spill in the Gulf of Mexico in 2010 AOAC International invited method developers to submit methods for measurement of PAHs in seafood for consideration and possible evaluation through the AOAC Official Methods program. The selected method was based on the extraction with ethyl acetate, salting-out with NaCl and MgSO₄ followed by silica SPE clean-up and instrumental analysis by GC/MS (64). In total, 32 PAHs were determined including 16 EPA PAHs and several of their methylated homologues with LOQ ranging from 0.05–0.25 μg/kg, recoveries between 73–97%, and repeatability (RSD) between 2–13%. The method was subjected to collaborative study and later accepted as the official method (65).

A European Standard (EN) for the determination of four PAHs (benz[a]anthracene, benzo[a]pyrene, benzo[b]fluoranthene, and chrysene) based on GC/MS is under development. PAHs are extracted from food by pressurized liquid extraction (PLE) and purified by size exclusion chromatography (SEC) followed by the solid-phase extraction (SPE) on silica. The release of the EN by the European Committee for Standardization (CEN) is expected by end of 2014.

**Analysis Methods for the Determination of the 16 EPA PAHs in Food**

Depending on the target analytes, the methods published in the scientific literature for the determination of PAH in food can be divided into two groups. The first group focuses on the 16 PAHs specified by EPA and the second on
the 15+1 EU priority PAHs specified by SCF/JECFA. As this special edition of the PAC journal targets the 16 EPA PAHs, methods dealing solely with the determination of the 15+1 EU priority PAHs were not considered in this review and only those for the EPA PAH analysis were reviewed. Nevertheless, sample preparation for the determination of both of these PAH groups is usually based on the same principles.

When analyzing complex samples such as foodstuffs, many other matrix components are unavoidably co-extracted together with the target analytes. Oils, waxes, essential oils, and natural pigments such as carotenoids and chlorophylls are the most typical matrix components occurring in extracts obtained from samples of plant origin. In animal tissues, lipids represent the major class of co-extracts. Effective separation of these substances, which can adversely affect identification and/or quantification of PAHs, is a prerequisite for obtaining reliable results. However, not only good separation of target analytes from co-extracts, but also the overall throughput of the clean-up step (which is largely dependent on the possibility of its automatization) is an important criterion to consider when choosing an analysis procedure (66). The required sample clean-up of food samples depends on the selectivity of the applied extraction technique and on the chromatographic method to be applied. This becomes more difficult if one aim is to eliminate or minimize the use of hazardous substances such as organic solvents in the analytical extraction step. New trends focus on utilizing more benign extraction solvents as well as micro-extraction procedures.

The methods used for extraction of PAHs from food greatly depend on the nature of the food matrix. The common approach for the isolation of PAHs from fatty foods, mainly edible oil samples, involves saponification of lipids by methanolic or ethanolic KOH or NaOH solution followed by the isolation of the PAHs containing unsaponifiable fraction by liquid-liquid extraction (LLE) with cyclohexane (21,67) or n-hexane (68). Several other different procedures are described for PAHs extraction, namely solid-liquid extraction (SLE) with toluene (69), n-hexane (70), or a mixture of cyclohexane and ethyl acetate (71), LLE with n-hexane (72), mixture of n-hexane and acetone (73) or, more effectively, salting-out-assisted LLE in which a relatively high salt concentration and high ionic strength may improve analyte extraction with cyclohexane (74) or n-hexane (75). Also, a traditional Soxhlet extraction was applied for PAH analysis using dichloromethane (76) or its mixture with n-hexane (77). Nowadays, automated and more efficient extraction techniques such as PLE (78–83) and microwave-assisted extraction (MAE) (70,84,85) are more widely used. The extractants used in PLE and MAE are the same as for classical extraction techniques (Soxhlet extraction), therefore PLE and MAE are essentially non-selective extraction methods. An effective clean-up procedure for removing co-extracted interfering substances is thus crucial for the determination of PAHs that are present at trace levels in the crude extracts.
Gel-permeation chromatography (GPC), providing separation according to differences in the molecular size (or more exactly effective molecular volume), together with adsorption chromatography and solid phase extraction (SPE) constitutes the dominating purification technique for the processing of primary food extracts (66). The GPC procedure may employ several types of gels for the purification of extracts and the elution of sample components is usually carried out by different elution solvents, e.g., dichloromethane, \textit{n}-hexane, cyclohexane, ethyl acetate, chloroform, and their mixtures (73,75–77,79). As regards adsorption chromatography and SPE, the use of silica (72,74,81,85,86), alumina (70,71,78), Florisil (68,80), aminopropyl (21), C18 (21), or styrene-divinylbenzene co-polymers (83,87) are reported in the literature. The combination of GPC and further SPE clean-up resulted in relatively clean extracts from food matrices, avoiding problems with chromatographic interferences for most compounds (66,86).

However, multi-stage sample preparation usually results in high consumption of hazardous organic solvents, high costs, long analysis time, and might increase the risk of analyte losses, low precision of analysis and bias. A one-step PLE method can be achieved by adding a stationary phase (e.g., silica or alumina) directly to the PLE extraction cell. This method has previously been used in the analysis of polychlorinated biphenyls in fish samples. The use of activated silica for extraction of PAHs from lard yielded both fat-free extracts and high recoveries for all PAHs (82). Sulphuric acid-impregnated silica gave fat-free extracts, but led to low recoveries of acenaphthylene, acenaphthene, and anthracene. Neutral, basic, and acidic alumina also provided effective fat retention, but the recoveries of high-molecular weight PAHs were generally low in case of sausages. PLE efficiency of PAHs increases with temperature, while fat retention capacity is reduced. Hence, the extraction temperature has to be chosen with care to obtain both high recoveries of PAHs and fat-free extracts (82). This aspect was also described for fish and fish oil samples that were extracted and simultaneously cleaned-up in the presence of aluminium oxide and silica gel in the PLE step; the process ensured a sufficiently purified sample (79).

Removal of lipids from PLE extracts was tested by treatment with sulphuric acid. The extracts of fortified fish tissues obtained by PLE were treated with sulphuric acid (18 M) to remove lipids, but unfortunately several PAHs were affected as well. Notably, acenaphthylene, anthracene, and benzo[\textit{a}]pyrene were completely eliminated, whereas the peak intensities of other PAHs were reduced. This may be attributed to the strong oxidizing nature of 18 M H\textsubscript{2}SO\textsubscript{4}, resulting in the decomposition of some PAHs. When the concentration of sulphuric acid was reduced to 9 M during the clean-up process, no significant loss of PAHs was observed (80).

Headspace solid-phase microextraction (HS-SPME) is an extraction technique that fulfils well the requirements for a rapid and automated extraction
of complex liquid matrices. Fully automated workflows can be developed, being thus a reliable alternative to conventional solvent-based extraction techniques. The main parameters of the HS-SPME technique that need to be carefully studied are the fiber composition, sample volume or weight, temperature and incubation, and desorption time of the sample. In addition, some modifiers may be added to the samples in order to achieve better sensitivity. The addition of salt increases the ionic strength of the sample so that the solubility of the analytes decreases, facilitating their transfer to the gas phase. Applied to the analysis of soft drinks and also vegetable samples, this showed sufficient recoveries and repeatability with low detection limits (88,89).

The QuEChERS (quick, easy, cheap, effective, rugged, safe) method was developed for the determination of pesticides in vegetable and fruit samples. It is characterized by short extraction and purification times, as well as low solvent consumption. Recently, several modifications of the QuEChERS method were proposed for the analysis of PAHs in seafood such as shrimp, scallops, mussel, and fish, and in meat. Because of the differences in the matrix effect exerted by different food commodities, the applicability of QuEChERS methods has to be evaluated carefully (90–96).

In recent years, magnetic solid-phase extraction (MPSE), a promising technique for sample preparation, has attracted much interest. It is a new mode of extraction based on the use of magnetic or magnetizable adsorbents, which can be readily isolated from sample matrix with an external magnet. Hence, two of the significant advantages of MSPE are simplicity and convenience. Furthermore, in MSPE, the adsorbents can be uniformly dispersed into the sample solution by vortexing or shaking, which makes the contact area between the adsorbents and the analytes large enough to ensure a fast mass transfer. It leads to high extraction efficiency in a short time, which is desirable in high throughput sample preparations. Magnetic multiwalled carbon nanotubes (mMWCNTs) have been employed as a sorbent for MSPE of PAHs from edible oils. They are adsorbed onto mMWCNTs through $\pi$-$\pi$ interaction after vigorous vortexing. The PAHs are then desorbed with toluene by ultrasonic agitation and directly analyzed (67).

The two main instrumental analysis techniques for determination of PAHs in food are HPLC-FLD and GC-MS. Earlier methods based on HPLC with ultraviolet (UV) or photo diode array detection (PDA) or GC with flame ionization detection (FID) are outdated due to their poor selectivity and sensitivity. Mass spectrometric methods have become popular because of the high selectivity of this detector enabling the confirmation of analyte identity based on the mass spectrum and the possibility to use stable isotope labelled PAHs as internal standards. Single quadrupole MS is the main detection technique applied but the use of tandem mass spectrometry (MS/MS) and high resolution mass spectrometry increased in recent years (97).

HPLC/FLD has been extensively applied for the determination of PAHs in foodstuffs and beverages, since it is rather cheap and simple, in comparison
with other detection systems (97). Low concentration of anthracene and perylene are best measured by use of HPLC/FLD because of their selective and sensitive fluorescence-detection characteristics (98). The reported limits of detection are frequently at the sub-ppb level (0.01–1 μg/kg). However, low LODs might not be achieved for all PAHs due to selectivity issues as well as due to the inherent fluorescence properties. GC/MS provides more accurate results for the determination of benzo[ghi]perylene because of its low fluorescence (98). Moreover, acenaphthylene is not detected by FLD because it does not emit any fluorescence. Another disadvantage is the impossibility of internal standardization with isotopically labeled compounds as FLD cannot distinguish them from the native PAHs; benzo[b]chrysene can be used alternatively as an internal standard (99,100). A large improvement of the analysis of PAHs in complex matrices can be achieved by using HPLC/MS, although sufficient sensitivity was achieved only in the atmospheric pressure photoionization (APPI) mode (101–103). For HPLC separation specially designed reversed-phase columns are commercially available (see the paper by S.A. Wise, L.C. Sander and M.M. Schantz in this issue).

The most critical aspect when developing a GC method is the selection of an appropriate stationary phase for the separation of target PAHs (104). Methyl- and phenyl-substituted polysiloxanes are the most widely used stationary phases for PAH separation, typically 5% phenyl-95% methylpolysiloxane substitution (HP-5, HP-5ms). Columns prepared with polysiloxane stationary phases give relatively low background from column bleed, even at high temperatures (> 300°C) (105). Considering the 16 EPA PAHs, there are several groups which might co-elute, also with others PAHs presented in the sample. Five groups of PAHs represent this type of resolution problems: (i) chrysene and triphenylene; (ii) cyclopenta[cd]pyrene, benzo[a]anthracene and chrysene; (iii) benzo[b]fluoranthene, benzo[j]fluoranthene and benzo[k] fluoranthene; (iv) dibenz[a,c]anthracene and dibenz[a,h]anthracene; and (v) dibenzo[a,h]anthracene and indeno[1,2,3-cd]pyrene (100, 104). In case of co-elution, a sum of grouped PAHs is usually reported, which could affect the correct implementation of food legislation. A general problem in the GC analysis of PAHs is the high discrimination observed for high molecular-weight compounds in comparison to other compounds with low and medium molecular weight. This discrimination is usually caused by different behavior of the analytes in the injection port, which causes unequal transfer of the analytes into the column. Discrimination may also be due to the strong interaction with the stationary phase, resulting in broad peaks. In order to reduce discrimination in the injection port, injection techniques such as programmed temperature vaporization (PTV) and on-column injection can be applied (106). The combination of PTV and large volume injection has been successfully applied for the analysis of both light and heavy PAHs in food samples. The optimized PTV in solvent vent mode always provided higher sensitivity than
the PTV process used in splitless mode, and improved the signal-to-noise ratio for the heaviest PAHs (97).

The performance of different stationary phases, including typical 5%-phenyl columns and other more polar ones, such as 50%-phenyl columns (e.g., DB-17ms®) and mid-polar to polar column (Optima® δ-6) were thoroughly evaluated. The utilization of a 50%-phenyl column (mid-polar phase) solved the resolution problems of three groups of co-eluted PAHs (Figure 1) (104). Benzo[b]fluoranthene, benzo[j]fluoranthene, and benzo[k]fluoranthene data were reported as their sum, because of substantial co-elution. Although the use of comprehensive GC × GC may allow separation PAHs from matrix interferences in vegetable oil or fish samples, it did not succeed in improving the separation of critical pairs of PAHs (107,108). Insufficient resolution was reported also for cyclopenta[cd]pyrene, which interfered with benz[a]anthracene, and for indeno[1,2,3-cd]pyrene and dibenz[a,h]anthracene (Figure 2). Currently, commercially available Select PAH® columns were designed specifically for the PAH analysis, and provide satisfactory separation of all groups of PAHs (both EPA and EU priority PAHs) by one-dimensional GC (109). However, baseline separation of chrysene from the isobaric triphenylene is not possible with this column (110).

Detection of PAHs following GC separation is most commonly accomplished by use of quadrupole electron ionization (EI) MS. In contrast with many other organic contaminants, most PAHs yield intense molecular ions with little fragmentation in this ionization mode. The number of fragments produced is extremely low, mainly the [M-H]+ or [M-2H]++ ions are observed (83). GC/MS operating in the selective ion monitoring (SIM) mode has advantages over full-scan mode, for example lower detection limits and specific monitoring capabilities can be achieved. In general, ease of operation and reasonable purchase and operating costs have enabled GC/MS to become a popular method for the determination of PAHs in laboratories worldwide (105). The application of tandem MS and other advanced analyzers provides an increase in selectivity. GC/MS/MS compared to GC/MS shows a five times increase in sensitivity in fish matrix. PAHs are highly condensed and stable, so the M++ → M+++ pseudo-transition can be monitored. Their stability increases with the number of condensed rings. For this reason, the energy in the collision cell is increased from 25 to 35 eV for dibenz[a,h]anthracene, indeno[1,2,3-cd]pyrene, and benzo[ghi]perylene to cause a loss of two protons and to generate the [M-2H]++ ion. This second ionization causes a decrease in noise by fragmenting the co-extracted substances co-eluted with PAHs, whereas PAHs are only weakly affected. Because the noise caused by co-extracted lipophilic substances is reduced, the signal-to-noise ratio of PAHs increased simultaneously (87). The application of GC with atmospheric pressure laser ionization (APLI) MS detection, as used for the determination of PAHs in environmental samples, has not been reported yet in food analysis (111).
Figure 1: Gas chromatographic separation of critical pairs/triplets on different columns stationary phases. (A) DB-17MS column, 60 m length, 0.25 mm i.d., 0.25 μm film thickness; (B) DB-5MS column, 60 m length, 0.25 mm i.d., 0.25 μm film thickness; (C) Optima® δ-6 column, 30 m length, 0.25 mm i.d., 0.25 μm film thickness (102); (D) Select PAH, 15 m length, 0.25 mm i.d., 0.1 μm film thickness (chromatogram was taken from authors experimental results). (BaA = benz(a)anthracene, CPP = cyclopenta(cd)pyrene, CHR = chrysene, Trp = triphenylene, BbF = benzo(b)fluoranthene, BkF = benzo(k)fluoranthene, BjF = benzo(j)fluoranthene, DhA = dibenz(a)anthracene, IcP = indeno(1,2,3-cd)pyrene.) © Springer Science and Business Media. Reproduced by permission of Springer Science and Business Media. Permission to reuse must be obtained from the rightsholder.

In recent years, time-of-flight (TOF) MS with GC and LC has become increasingly prominent in food analysis. The unique aspect of this detection method is that accurate mass measurements and full-scan data can be acquired. Because of its high mass resolving power, TOF MS can enable
better structural conformation and better signal-to-noise ratios than single quadrupole analyzers, especially with complex samples (112).

In conclusion, the popularity of capillary GC for the determination of PAHs is based on a favorable combination of better selectivity and sensitivity compared to that achieved by HPLC. The presence of co-extracted compounds in samples can interfere with the identification and quantification of PAHs by HPLC. The ease of use and compatibility with MS detection are additional reasons why many analysts prefer this technique. On the other hand, HPLC is better able to separate individual isomers such as benzo[b] fluoranthene, benzo[j]fluoranthene, benzo[k]fluoranthene, and chrysene/triphenylene. Standard deviations indicated that repeatability of both methods is sufficient, being usually within 10%. The 16 EPA PAHs can be simultaneously separated by HPLC using gradient elution and detected by FLD with seven settings of programmable wavelengths. The retention times by HPLC were shorter than those by GC (113,114).

For most of the PAHs with three, four, and five rings, the recoveries and repeatability of measurements were in a satisfactory range. On the other hand, lower recoveries and poor RSDs were observed in some reported methods for the more volatile PAHs such as naphthalene, acenaphthene, acenaphthylene, and fluorene (71,72,77,84–86). This phenomenon was neither detection
technique-dependent nor linked to a particular sample extraction or purification technique. Therefore, special precaution should be taken in the determination of PAHs with higher volatility, including gentle treatment of the sample during the extraction and purification. Some laboratories also experienced high blank levels of low mass weight PAHs, which could originate from solvent impurities (81,82,96).

As a part of a collaborative study on the analysis of PAHs in edible oils, results obtained by either using HPLC/FLD or GC/MS were evaluated. Differences related to chromatographic methods were not found and stressed the suitability of both techniques for PAH analysis with satisfactory selectivity and sensitivity. About 95% of the laboratories were able to quantify benzo[a]pyrene together with five other PAHs included in the commonly known list of 16 EPA PAHs (100).

**OCCURRENCE OF PAHS IN FOODS**

Food consumption is an important pathway for human exposure to environmental contaminants. The contribution by food ingestion to the total intake of PAHs was compared in Europe with the intake from drinking water and inhalation of air (1). Food consumption was shown to be the main source of PAH intake and thus highlighted the importance of research on PAHs in food and the development of mitigation strategies to reduce their contents in food (1).

PAHs might occur in fats and oils such as sunflower, olive, soybean, palm, and rapeseed oils. Vegetable oils, naturally free of PAHs, can be contaminated by PAHs during their production (heating processes or extraction with organic solvents) or through air or contact with smoke from fuel combustion (115). Some methods exist to remove PAHs during oil refining, for instance the use of active carbon or steam distillation processes (116, 117). The average sum of PAHs in virgin olive oil was found to be higher than for olive oils (a blend of refined and virgin oils) and olive pomace oils (86,118). For sunflower oils, 50% or more of the total concentration of 18 PAHs analyzed (15 EPA PAHs) belonged to the group of carcinogenic PAHs (3.7 μg/kg out of 7.4 μg/kg and 93 μg/kg out of 172 μg/kg), while for vegetable oils in general (olive oils, rapeseed oils, sesame oils) less than 25% of the total PAH content was constituted by the genotoxic compounds (86). The majority of PAHs (usually 90%) found in vegetable oils belong to the class of light PAHs; only sunflower (about 50% light PAHs) and rapeseed oils (70% light PAHs) contain an increased proportion of heavier PAHs. The content of benzo[a]pyrene in oils is usually below 2 μg/kg (86).

The average content of PAHs is lower in fish musculature than in the tissues of mollusks, since fish (in contrast to bivalves) have the ability to oxidize and further metabolize PAHs to water-soluble compounds that are excreted by the living organism. Mostly the edible part of fish from unpolluted seas not
contain detectable amounts of benzo[a]pyrene. Only four low molecular weight PAHs were reported in some fish species (sardine, horse mackerel, chub mackerel), namely naphthalene, acenaphthene, fluorene, and phenanthrene. Neither high molecular weight PAHs nor benzo[a]pyrene were detected (84). These results were confirmed by the study on the same fish species, where just three low molecular weight PAHs (acenaphthene, fluorene, and phenanthrene) were found. The sum of their contents ranged from 0.73 μg/kg to 6.6 μg/kg (95). A similar PAH profile was observed for catfish (94). The dominant PAH measured in tuna samples was naphthalene (119).

The average concentration of benzo[a]pyrene in bivalve mollusks is mostly much below 10 μg/kg. However, samples taken from polluted waters may contain higher amounts (113). Cultured and native mussels were used to evaluate the contamination by PAHs, ranging from uncontaminated aquaculture areas to low and moderately contaminated coastal regions up to a chronically contaminated bay region. Cultured mussels from the farms contained lower amounts of 16 EPA PAHs (20.31–23.90 μg/kg), with a predominance of naphthalene, phenanthrene, fluoranthene, chrysene, and benzo[b]fluoranthene. The levels found in the mussels from coastal areas located in a low urbanized region were slightly higher ranging from 21.85 to 49.60 μg/kg for the total 16 PAH content. The occurrence of 4–6 ring PAHs suggested the presence of PAHs derived from petrogenic and pyrogenic sources. The mussels from the urban bay area showed highest contamination levels with total amounts between 69.30 μg/kg and 238.93 μg/kg (76). In the edible parts of shellfish, the content of benzo[a]pyrene is below the detection limits or not higher than a few μg/kg (94).

Smoke is generated by thermal pyrolysis of hard wood when there is limited access to oxygen. The temperature of smoke generally plays an important role because the amount of PAHs in smoke formed during pyrolysis increases linearly with the smoking temperature within the interval 400–1000°C (120). Direct exposure of meat or fish products to smoke results in higher PAH contents compared to indirect smoking methods. Also, hot smoking resulted in higher PAH levels than cold smoking (120). Un-smoked salmon is usually of low contamination. PAHs of low molecular weight are predominant but in low concentrations (from 0.06 to 0.19 μg/kg). Salmon smoked by four different industrial cold-smoking processes (smoldering, thermostated plates, friction, and liquid smoke) were investigated for PAH occurrence. Whatever the setting of the parameters, all smoking processes led to higher levels of PAHs of low molecular weight. The concentrations of PAH from fluorene to fluoranthene varied between 1 μg/kg and 5 μg/kg. Smoldering gave the highest and liquid smoke the lowest levels of low molecular weight PAHs (87). Only three PAHs were detected in a commercial smoked salmon: naphthalene, phenanthrene, and fluoranthene with concentrations from 11 μg/kg to 27 μg/kg (80). Another study reported slightly higher levels of individual
PAHs in smoked salmon ranging between 5 μg/kg and 60 μg/kg with fluoranthene and benzo[ghi]perylene displaying the lowest levels and naphthalene and pyrene consistently accounting for more than 70% of total PAHs (91). Meat samples have been extensively monitored for PAHs, especially smoked meat products. The number of PAHs detected in smoked sausage and smoked pork sausages varied from 6 to 12 out of 16 EPA PAHs with levels from 3 μg/kg to 52 μg/kg (80). Commercial smoked meat products showed low PAH content with benzo[a]pyrene concentration not exceeding 0.1 μg/kg. Higher levels could be observed in products, where the smoking was carried out in a traditional way, putting the meat near the fireplace for several days (e.g., Italian smoked sausage Pitina) (85). The study on the occurrence of PAHs in cheese smoked by various processes also confirmed the influence of the smoking process on PAH levels. In general, commercial smoked cheese is of lower contamination (sum of PAHs 2.3–57 μg/kg) than the traditionally “home-made” cheese (sum of PAHs 73–114 μg/kg), while 70–90% of total PAHs were those with three aromatic rings (77). Higher contamination of this traditional cheese was obviously due to the deposition of PAH containing solid particles on its surface. Purification of the smoke generated in industrial smoking processes allows only flavoring compound to reach the products while most of the hazardous compounds of wood pyrolysis are removed (77).

The effects of frying, grilling, roasting, toasting, using domestic cooking appliances, and barbecuing were investigated by carrying out controlled cooking experiments for a range of foods including sausages, beef, burgers, chicken, and lamb. Overall, barbecued beef burgers contained the highest levels of PAHs, with benzo[a]pyrene concentrations of up to 29 μg/kg. Beef burgers cooked at a larger distance (7 cm above charcoal) contained higher PAH concentrations than those cooked at a smaller distance (4 cm above charcoal). However, sausages were found to contain highest concentration when cooked 4 cm above charcoal. In general, barbecuing with charcoal plus wood chips gave the highest levels of benzo[a]pyrene. Extended cooking time resulted in moderate increases of PAHs for some types of barbecued food. The changes in PAH levels are likely to be influenced by differences in the surface area, the surface texture and the manner in which fat is lost during cooking (121).

Among the 16 EPA PAHs studied in heat-treated milk only 8 PAHs were detected: phenanthrene, anthracene, and pyrene with the highest levels and benz[a]anthracene, chrysene, benzo[k]fluoranthene, benzo[a]pyrene, and benzo[ghi]pyrene with lower levels. The total PAH levels found in pasteurized (6.52 μg/kg) and UHT whole milk (7.75 μg/kg) were higher than in raw milk (5.43 μg/kg). The total PAH levels in UHT whole milk were higher (7.75 μg/kg) than in UHT semi-skimmed milk (5.94 μg/kg), which is caused by the differences in fat content (122). A national survey of toxic pollutants in the United States milk supply was carried out in 2000 and 2001. Eight PAHs were monitored in addition to other pollutants. Based on the grand composite mean
across the seasons, the national average PAH levels ranged from non-detect (< 40 ng/L) to 777 ng/L (123). Naphthalene and phenanthrene had the highest grand composite values in both winter and summer season. Acenaphthene and benzo[a]pyrene were not detected in any sample (123).

Roasting is a crucial step for the production of coffee as it enables the development of color and flavor, which are essential for the characterization of the coffee quality. The presence of PAHs in coffee samples has also been reported and may be attributed to either contamination of the initial green beans or formation of these compounds during the roasting step. With regard to individual PAHs in roasted coffee, the most abundant compounds are phenanthrene, fluoranthene, and pyrene with concentrations in the range 3–50 μg/kg. Significant concentrations of benz[a]anthracene and chrysene were also found for strong roasted coffee with levels near 13 μg/kg for both compounds (26). Benzo[a]pyrene was reported in commercially ground and instant coffees in the range from <0.01 μg/kg to 1.2 μg/kg (124, 125). Results for coffee brew showed similarities with ground coffee; phenanthrene, fluoranthene, and pyrene were the main PAHs, with concentrations ranging from 0.078 μg/L to 0.79 μg/L (124). The measured values in coffee brew indicate that coffee does not significantly contribute to the daily human intake of carcinogenic PAHs (126).

Tea is, next to water, the most widely consumed beverage in the world. Recent studies showed that tea leaves may contain contaminants that can be released into infusions and might be harmful to human health. A wide variety of PAHs was detected in tea leaves, particularly in smoked and non-smoked leaves. Benzo[a]pyrene in non-smoked tea leaves ranged from 0.6 μg/kg to 5.6 μg/kg and in smoked from 2.8 μg/kg to 21.9 μg/kg (27). Significant levels were also published for benz[a]anthracene and chrysene. Even higher concentrations were measured in Mate tea leaves. Mate is a tea-like plant native in subtropical South America. It is dried over a wood fire, which might explain the high PAH contamination. Tea leaves are normally not consumed themselves but used for the preparation of infusions. Recent studies showed that the PAH concentrations in infusions are far lower than those measured in the corresponding tea leaves. The release of PAHs from tea increased with infusion time and therefore this time should be kept to a minimum for teas with high amounts of PAHs, like smoked tea and Mate tea (27,127).

Vegetables are important constituents of the human diet across the world both in terms of quantities consumed and nutritional value. PAHs have been detected in various vegetables. Although the reported levels are overall rather low compared with those in seeds, edible oils, and smoked meat, the frequent occurrence of PAHs contamination and the large vegetable consumption can also make them a significant source to human exposure. The principal pathway for contamination of vegetables with PAHs is gas and particle deposition, while in some cases a significant uptake of PAHs from soil was observed (128,129). The predominant PAHs in vegetables are those with two and three
rings, whereas the four-ring PAHs are usually absent. Considering the vegetable categories, the highest levels of total PAHs were found in lettuce (up to 96.98 μg/kg), suggesting a higher accumulation on the large surface area of vegetable leaves (89). PAH levels differed significantly between sampling locations. Samples growing in highly industrialized areas or near roadways, or irrigated with wastewater generally showed higher levels than those from rural areas. Vegetables collected from the vicinity of a power plant site in India were significantly more polluted with PAHs compared to vegetables from an environment that contained PAHs only at the general background level. The contents of PAHs in vegetables from the power plant site ranged from 0.07 μg/g to 1.1 μg/g. The variation of PAH levels between different vegetables was also significant with highest concentrations in spinach followed by radish and bottle gourd, which could be explained by the greater surface area of the leafy parts that facilitate atmospheric deposition. The most abundant PAH species in vegetables from the power plant site were acenaphthene, fluoranthene, fluorene, and pyrene. The percentage contributions of carcinogenic PAHs to total PAHs were 0.4% in cowpea, 5% in bottle gourd, 8% in spinach, and 11% in radish (69).

According to EFSA's findings, cereals and cereal based products are the main source of human exposure to PAHs, mainly due to the high amounts consumed (1,2). Contamination of bread by PAHs can be caused by both the contamination of bakery raw materials, primarily flour, and the baking process. Similar profiles of PAHs were observed in all analyzed kinds of bread. Low levels of PAHs were found primarily for the four low molecular weight PAHs (phenanthrene, anthracene, fluoranthene, and pyrene). They constituted around 90% of all PAHs determined in different variants of wheat-rye bread, rye bread, and wholemeal rye bread. However, detectable levels of benz[a]anthracene, chrysene, benzo[b]fluoranthene, and benzo[k]fluoranthene were also reported. Furthermore, benzo[a]pyrene was detected only in the crust of wholemeal rye bread at the highest temperature of baking (260°C). In general, for all kinds of bread, the highest total PAH contents were found in the crust (up to 13 μg/kg for the sum of 19 PAHs), and the lowest in the crumb (130).

Relatively high levels of PAHs were reported for various individual food supplements often containing botanical ingredients such as ginkgo, ginseng, green tea, spirulina, or bee products such as propolis. In samples analyzed in the period from 2003 up to and including 2007, benzo[a]pyrene was present in 44% of the food supplements with the lower-bound mean level of 3.37 μg/kg. In the survey from 2008 until 2009, 63% of the food supplements contained PAHs with a lower-bound mean value for benzo[a]pyrene of 5.33 μg/kg (28). Propolis samples were characterized by very different PAH contents, with benzo[a]pyrene ranging from 0.7 μg/kg to 1371 μg/kg. However, chrysene was always the most abundant PAH with amounts between 4.7 μg/kg and
3176 μg/kg. Lower levels of benzo[a]pyrene were found in the propolis extracts ranging from the limit of quantification to 41.8 μg/kg (131). It can be concluded that use at the maximum recommended dose levels of individual food supplements, especially those containing certain botanicals or propolis, can contribute significantly to the total dietary intake of this class of contaminants (e.g., for the propolis extracts the benzo[a]pyrene intake corresponding to about 15–30% of the EFSA European dietary exposure level (3.9–6.5 ng kg bw\(^{-1}\)day\(^{-1}\)) (2,131).

In conclusion, from the group of 16 EPA PAHs the substances with low molecular weight (up to molecular mass of 202 atom mass units) are primarily found in food. However, it has to be stressed that these compounds have a lower toxicity profile compared to the high molecular weight members of the EPA list. The relevance of the low molecular weight PAHs for dietary intake was also confirmed by the study on the dietary exposure to PAHs in Spain (39). The highest individual PAH levels corresponded to phenanthrene (26.66 μg/kg), naphthalene (25.87 μg/kg), and fluoranthene (13.66 μg/kg), while the lowest levels were reported for benzo[a]pyrene (1.28 μg/kg) and benzo[k]fluoranthene (1.31 μg/kg). With respect to food groups, the highest levels of total PAHs were detected in meat and meat products (25.56 μg/kg), oils and fats (23.48 μg/kg) and cereals (20.44 μg/kg). For the average male adult (70-kg body weight), the dietary intake of the sum of PAHs was higher (12.0 μg/day) in 2006 than that found in 2000 (8.4 μg/day). The reported results pointed out the need for monitoring programs for PAHs in food in order to map the changes in dietary intake of these pollutants (39).

CONCLUSIONS

The determination of PAHs in foodstuffs has already a long history. In the past, only benzo[a]pyrene was targeted. Later, a high number of publications dealt with the analysis of PAHs included in the U.S. EPA list. Since the evaluation of the toxicological effects of PAHs has resulted in the prioritization of a different set of compounds, which is reflected in EU legislation (15+1 EU priority PAHs), scientific literature on food is more focused on these substances.

Considering the legislation on PAHs in food, the most comprehensive regulations are given in the European Union for different food categories in which higher levels of PAHs could be expected. Maximum limits are set for a group of four PAHs including benzo[a]pyrene. Regarding other countries, maximum limits are rare. If maximum levels are set for PAHs, they usually comprise benzo[a]pyrene only in matrices such as drinking water and sporadically in olive oils.

There is still a lack of standardized methods for the analysis of PAHs; however, a number of in-house validated methods are reported in the literature.
Because the concentrations of PAHs in food are very low, generally in the range of ppb units, the methods usually consist of several extraction and sample purification steps to reach the low detection limits. Some researchers reported difficulties to achieve sufficient recovery rates and repeatability for the determination of the low molecular weight PAHs with two or three benzene rings. Another problem area is still the insufficient gas chromatographic separation of some of the higher molecular weight PAHs.

The occurrence of PAHs in food has been extensively studied in countries all over the world. Data from surveys indicate that the most abundant PAHs in foodstuffs are the low molecular weight PAHs from the EPA list (particularly those of two or three benzene rings). These PAHs are less relevant from the toxicological point of view and do not contribute to the genotoxic and carcinogenic potential of PAHs. High contents of genotoxic and carcinogenic compounds in food were in the past and are presently still linked to poor production practices. Surveillance schemes such as the ones implemented in the EU are deemed necessary to monitor human exposure to PAHs via food and to take necessary mitigation actions when and where necessary.

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