

Analytical methods for polycyclic aromatic hydrocarbons (PAHs) in food and the environment needed for new food legislation in the European Union

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This article gives an overview on current European Union (EU) legislation on polycyclic aromatic hydrocarbons (PAHs) and analytical methods for official control of food and environmental samples. It aims to highlight the discrepancy in the approaches for legislation and analysis and the need for harmonization between these fields as both are linked. It describes the actions taken within the EU in setting up an on-line monitoring database for food products, proficiency tests and method-validation studies.

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1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a group of about 10,000 compounds, a few of which occur in considerable amounts in the environment and food. PAHs comprise fused aromatic rings and do not contain hetero-atoms or carry substituents. PAHs containing up to four fused benzene rings are known as light PAHs and those containing more than four benzene rings are called heavy PAHs. Heavy PAHs are more stable and more toxic than light ones. PAHs are lipophilic in nature; nevertheless, some of them can dissolve quite well in water [1].

Most PAHs in the environment derive from incomplete burning of carbon-containing materials, such as oil, wood, garbage or coal. A maximum amount of PAHs is formed when materials burn at temperatures in the range 500–700°C, as in wood fires or cigarettes [2]. Consider-

able amounts of PAHs are emitted during production of coke or aluminum [3,4]. High-temperature furnaces produce lower amounts of these compounds. PAHs from fires can bind to ashes and move long distances through the air [5]. As some PAHs (especially the lighter ones) are water-soluble, they can also be found in rivers and groundwater [6].

PAHs comprise the largest group of chemical compounds known to be cancer-causing agents. Some PAHs have been demonstrated to be carcinogenic and mutagenic. However, those PAHs that have not been found to be carcinogenic may act as synergists. Exposure of humans to single PAHs does not occur because PAHs are always encountered as complex mixtures. The fact that exposure to PAHs is always due to a mixture, which is not always of constant composition, makes the assessment of health consequences difficult. Nevertheless, some studies have shown correlations between exposure to individual PAHs and occurrence of human cancer. Such substances, regardless of their carcinogenic potential, serve as markers for exposure to the entire PAH mixture [1,7].

Human beings are exposed to PAHs via air and drinking water, but mostly by intake of food. Food contaminated with PAHs largely arises from production practices, although environmental contamination is also an issue. This holds

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especially true for vegetables and grains that do not take up significant PAHs from the soil; nevertheless, there could be other sources of contamination, such as particles from the air (especially when whole-grain products are produced (e.g., bread, and breakfast cereals) [8]). Grains and raw products for oil production (e.g., pumpkin seeds) 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 is applied regularly for production of some vegetable oils or when climatic conditions (rain during harvesting) are responsible for high moisture contents in the harvest. To avoid formation of mould and therefore secondary toxic metabolites during storage, the products have to fulfill the requirements for safe water activity. Drying of seeds and kernels is thought to be one of the prominent sources for the contamination of edible oils with PAHs. Food from animal production is also mainly contaminated through processing, as the carry over effect from feed to food has been shown to be insignificant. Heat processing of meat and dairy products, such as charcoal grilling [9,10], roasting [11], and smoking [12,13], are the main sources for contamination with PAHs. In addition, PAHs could arrive in food products through contaminated packaging material [14,15].

New EU legislation was introduced in early 2005 in response to food-contamination problems, based on data collected by the European Member States and assessment by the Scientific Committee on Food (SCF) in 2002. The SCF assessed 33 PAHs and identified 15 PAHs that possess both genotoxic and carcinogenic properties (Table 1). As measures are presently widely focused on benzo[*a*]pyrene (BaP), the SCF concluded that BaP could be used as a marker [8]. However, BaP constitutes only 1–20% of the total concentration of carcinogenic PAHs, so the SCF also recommended monitoring benz[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*j*]fluoranthene, benzo[*k*]fluoranthene, benzo[*ghi*]perylene, chrysene, cyclopenta[*cd*]pyrene, dibenz[*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 in food and the environment to enable long-term exposure assessments and to verify whether use of BaP is justified [8].

BaP is also regarded and recommended as a marker in Air Quality Standards. However, the uncertainties surrounding the use of BaP as marker for carcinogenic PAHs has also been a subject of concern regarding the occurrence of carcinogenic PAHs in ambient air. The UK Expert Panel on Air Quality Standards has therefore also recommended increasing the number of PAH-monitoring sites in the UK and also covering more PAHs than BaP in the analyses [16].

Of the 15 EU priority PAHs, 12 are identical with those that were reasonably anticipated by the International Agency of Cancer (IARC) to be human carcino-

gens based on sufficient EU evidence of carcinogenicity in experimental animals in 1973–1987 [17]. In 2005, the European Commission (EC) asked the EU Member States for further investigations on the 15 EU priority PAHs together with one PAH (benzo[*c*]fluorene) highlighted by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 2005 (Table 1) [18]. Areas for investigation are outlined in Commission Recommendation 2005/108/EC [19]. For this reason, analysis of multiple PAHs (especially the 15 + 1 EU-priority PAHs) is necessary to respond to European legislation and to get a clearer picture of levels of different PAHs in food.

So far, due to the well-known toxicity of PAHs, there have already been many investigations throughout the world. However, analysis and monitoring have generally focused on BaP, or some selected or all 16 PAHs highlighted by the US Environmental Protection Agency (EPA). These 16 EPA-PAHs were identified in the 1970s [20]. For this reason, analytical methods and standard (calibration) materials are widely available and applied for various food and environmental matrices for these 16 analytes. Eight of these PAHs are known to be mutagenic or carcinogenic and comprise part of the 15 EU priority PAHs (Table 1), for which, so far, only one method for analysis in smoke flavorings has been recently developed and validated in a collaborative trial [21–23].

This article aims to give a brief overview on the current legislative situation of PAH analysis in the EU – in food as well as in the environment – and to highlight the needs for further investigations and research, especially in the area of method development and standardization to produce sound data for PAH-monitoring databases as basis for further risk assessments.

2. State-of-the-art and development of legislation on PAH levels

As already described, PAHs have been known to be environmental contaminants for decades, so several monitoring programs have been conducted to estimate the pollution of soil, water and air by PAHs. Since emissions are not linked to national territories, international standards and guidelines have been set in order to gain relevant, comparable data as a basis for sound decision-making. As a first internationally binding document, the Convention on Long-range Transboundary Air Pollution was set up in 1979 and extended in 1998 by the Aarhus Protocol on Persistent Organic Pollutants (POPs) that came into force in 2003 [24]. The latter recommends that four PAHs (BaP, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene and indeno[*1,2,3-cd*]pyrene) should be used as indicators for establishing emission inventories from stationary sources. In addition, it sets a timeframe within which the emissions of stationary

Table 1. Molecular structures of PAHs of concern				US-EPA	SCF	JECFA
Acenaphthene	ACP		154 AMU	x		
Acenaphthylene	ACY		152 AMU	x		
Anthracene	ANT		178 AMU	x		
Fluoranthene	FLT		202 AMU	x		
Fluorene	FLR		166 AMU	x		
Naphthalene	NAP		128 AMU	x		
Phenanthrene	PHE		178 AMU	x		
Pyrene	PYR		202 AMU	x		
Benz[a]anthracene	BaA		228 AMU	x	x	x
Benzo[b]fluoranthene	BbF		252 AMU	x	x	x
Benzo[j]fluoranthene	BjF		252 AMU		x	x
Benzo[k]fluoranthene	BkF		252 AMU	x	x	x
Benzo[ghi]perylene	BgP		276 AMU	x	x	
Benzo[a]pyrene	BaP		252 AMU	x	x	x
Chrysene	CHR		228 AMU	x	x	x
Cyclopenta[cd]pyrene	CPP		226 AMU		x	
Dibenz[a,h]anthracene	DhA		278 AMU	x	x	x
Dibenzo[a,e]pyrene	DeP		302 AMU		x	x
Dibenzo[a,h]pyrene	DhP		302 AMU		x	x
Dibenzo[a,i]pyrene	DiP		302 AMU		x	x
Dibenzo[a,l]pyrene	DlP		302 AMU		x	x
Indeno[1,2,3-cd]pyrene	IcP		276 AMU	x	x	x
5-Methylchrysene	5MC		242 AMU		x	x
Benzo[c]fluorene	BcL		216 AMU			x

sources have to be reduced to specified levels. The EU signed this Protocol in 1998 and approved it in 2004. Monitoring provisions for PAHs that were in line with the Aarhus Protocol were set by Regulation (EC) 850/2004 [25]. In Annex 1 of Directive 96/62/EC on ambient air-quality assessment and management, those PAHs (pollutants) are listed for monitoring and later on regulated by specific thresholds [26].

This Directive was revised in 2001 by Decision 2001/752/EC, and BaP was specified as the marker substance for all PAHs [27]. In parallel, a working group on ambient air pollution, appointed by the EC, also suggested in its position paper measuring benz[a]anthracene, benzo[b]fluoranthene, benzo[j]fluoranthene, benzo[k]fluoranthene, indeno[1,2,3-*cd*]pyrene, dibenz[*a,h*]anthracene and fluoranthene [28]. PAH emission data from stationary sources are fed into the European Pollution Emission Register (EPER) by the EU Member States, together with data for about 50 other contaminants [29]. The EPER contains monitoring data for six individual PAHs. However, a specific maximum limit has been set for only BaP in ambient air in Directive 2004/107/EC [30]. An overview on the diversity of monitoring requirements is given in Table 2.

Council Directive 96/61/EC focuses on integrated pollution prevention and control [31]. It lays down measures designed to prevent, or where this is not practicable, to reduce emissions in the air, water, and land from a number of installations that are listed in its Annex 1. Although PAHs are not explicitly mentioned, the relevant authorities are enabled to set maximum emission levels for them, since they fall under the category of substances that “have been proved to possess carcinogenic or mutagenic properties”.

The Water Framework Directive (2000/60/EC) sets out a strategy against pollution of water, also demanding the establishment of a list of priority pollutants [32]. Subsequently, Decision 2455/2001/EC names 33 substances or classes of substances, such as PAHs, which have to be monitored by the EU Member States in surface waters, groundwaters and coastal waters [33]. For

PAHs, the focus is on five representative substances (i.e. BaP, benzo[b]fluoranthene, benzo[k]fluoranthene, indeno[1,2,3-*cd*]pyrene, and benzo[*ghi*]perylene). In addition naphthalene, anthracene, and fluoranthene are listed separately as pollutants, but, in contrast to the five just mentioned, they have not yet been identified as priority hazardous substances. However, the EC will review the classification of naphthalene and anthracene.

Specific maximum limits were set at the EU level for PAHs in drinking water (Council Directive 98/83/EC) [34] and in different kinds of food (Commission Regulation (EC) 208/2005) [35] for the protection of human health while simultaneously not restricting competition, although the latter was likely since legislation differed very much within the different EU Member States.

While the Drinking Water Directive sets a maximum level for BaP (0.010 µg/l) and for the sum of benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[*ghi*]perylene and indeno[1,2,3-*cd*]pyrene (0.10 µg/l), Regulation (EC) 208/2005 on food contaminants follows a recommendation by the SCF to use BaP as a marker [8]. A brief overview of the situation in several EU Member States is given in Table 3 for the time before Regulation (EC) 208/2005 came into force. Information on food groups covered by Regulation (EC) 208/2005 and the respective maximum levels are given in Table 4. Provisions were set for sampling and the minimum performance of analytical methods.

3. EU PAH-monitoring database to assess suitable markers

Regulation (EC) No. 466/2001, as amended by Regulation 208/2005 (EC 2005) [35], sets maximum levels for PAHs, specifically for BaP, in certain food products. In view of uncertainties remaining about the levels of carcinogenic PAHs in food, especially about those PAHs possessing both genotoxic and carcinogenic properties identified by the former EC SCF [8], the Regulation

Table 2. Monitoring of PAHs depending on background document

	Aarhus protocol	Decision 2001/752/EC	Position paper on PAHs, 2001 [5]	EPER	Regulation (EC) 850/2004	WFD
Benzo[a]pyrene	x	x	x	x	x	x
Benzo[b]fluoranthene	x		x	x	x	x
Benzo[k]fluoranthene	x		x	x	x	x
Indeno[1,2,3- <i>cd</i>]pyrene	x		x	x	x	x
Benz[a]anthracene			x			
Benzo[j]fluoranthene			x			
Dibenz[<i>a,h</i>]anthracene			x			
Fluoranthene			x	x		
Benzo[<i>ghi</i>]perylene				x		x

EPER: European Pollution Emission Register; WFD: Water Framework Directive.

Table 3. Overview of maximum limits for PAHs in certain types of food, set in certain EU Member States before Commission Regulation (EC)208/2005 came into force (abbreviations according to Table 1; BeP: benzo[e]pyrene; DgP: dibenzo[g,h,i]pyrene)

Czech Republic	Meat products	BaA, BbF, BkF, DhA, DhP, DiP, IcP, CHR	3.0 µg/kg
	Roasted coffee, cereals, fruits, vegetables, fats and oils	BaA, BbF, BkF, DhA, DhP, DiP, IcP, CHR	2.0 µg/kg
	Fish, spirits, flavorings	BaA, BbF, BkF, DhA, DhP, DiP, IcP, CHR	1.0 µg/kg
	Beverages, beer, wine, tea	BaA, BbF, BkF, DhA, DhP, DiP, IcP, CHR	0.5 µg/kg
Slovak Republic	Smoked meat products and non-smoked fish products	BaP	1.0 µg/kg
	Smoked fish products		2.0 µg/kg
	Oils and fats of plant origin		5.0 µg/kg
	Other foodstuffs of plant origin		10.0 µg/kg
Italy	Olive pomace oil and refined olive pomace oil	BaP, BeP, BaA, BbF, BkF, DgP, IcP sum of above	2.0 µg/kg each 5.0 µg/kg
Germany	Cheese and cheese products, smoked or containing smoke flavorings. Cheese products manufactured by using smoked or smoke-flavored food ingredients	BaP	1.0 µg/kg
	Smoked meat and meat products. Meat products, partly containing smoked food ingredients	BaP	1.0 µg/kg
	Food ready for consumption containing food flavorings, with the exception of food treated with freshly produced smoke	BaP	Max. 0.03 µg/kg may be added by adding flavorings
Poland	Smoke flavorings	BaP	Max. 0.03 µg/kg may be added by adding flavorings
Belgium	Smoked meat and meat preparations	BaP	2.0 µg/kg
Spain	Olive pomace oil	BaA, BbF, BkF, DhA, IcP, BaP, BeP, BgP sum of above	2.0 µg/kg each 5.0 µg/kg
Greece	Olive pomace oil	BaA, BbF, BkF, DhA, IcP, BaP, BeP, BgP sum of above	2.0 µg/kg each 5.0 µg/kg
Sweden	Olive pomace oil and refined olive pomace oil	BaA, BbF, BkF, DhA, IcP, BaP, BeP, BgP sum of above	2.0 µg/kg each 5.0 µg/kg
United Kingdom France Ireland Cyprus Luxembourg Denmark Slovenia	No legal limits, but some countries have recommended maximum levels		

provides for a review of the measures by the EC by 1 April 2007. Information is required to support that revision, and the European Food Safety Authority (EFSA) was asked to initiate and to co-ordinate PAH-data collection in various food categories. The review should also reveal whether only BaP can be used as a marker of exposure from PAHs.

In response, EFSA set up an on-line analytical database in collaboration with experts from the EU Member States and the EC to investigate the following aspects:

- the respective levels and relative proportions of BaP and other carcinogenic PAHs, in particular the 15 EU priority PAHs [35] and the additional PAH identified by FAO/WHO Joint Expert Committee on Food Additives [18];
- the effects on PAH levels of different production and processing methods used (e.g., for edible oils and fats and for smoked and dried food); and,
- environmental and technical sources of PAH contamination.

Table 4. Maximum levels of benzo[a]pyrene in food, specified in Commission Regulation (EC) 208/2005 (Numbering of food groups is identical with numbering in Regulation)

Product		Maximum level ($\mu\text{g}/\text{kg}$ wet weight)
7.1.1.	Oils and fats intended for direct human consumption or use as an ingredient in foods	2.0
7.1.2.	Foods for infants and young children	1.0
7.1.2.1.	Baby food and processed cereal-based foods for infants and young children	
7.1.2.2.	Infant formulae and follow-on formulae, including infant milk and follow-on milk	
7.1.2.3.	Dietary foods for special medical purposes intended specifically for infants	
7.1.3.	Smoked meats and smoked meat products	5.0
7.1.4.	Muscle meat of smoked fish and smoked fishery products excluding bivalve mollusks	5.0
7.1.5.	Muscle meat of fish, other than smoked fish	2.0
7.1.6.	Crustaceans, cephalopods, other than smoked	5.0
7.1.7.	Bivalve mollusks	10.0

The PAH on-line analytical database is available on the EFSA web site [36].

- Part A includes some general information that needs to be provided on the analytical laboratory (e.g., accreditation status or whether the laboratory participates regularly in proficiency testing (PT) schemes).
- Part B has a description of the analytical method required by food group, which should be specified according to the CODEX Food Categorization system (CODEX STAN 192 – Annex B) [37]. Special emphasis should be given to the following food products: edible fats and oils; dried fruit; meat and meat products; fish and fish products; and, foodstuffs intended for particular nutritional uses.
- Part C has to be filled in for each sample with a detailed sample description and information of type of processing applied. The latter information will be very useful to see whether alternative or optimized methods would lower the levels.

To maximize use of the database, monitoring data from not only EU Member States but also other laboratories are needed (e.g., official food control, research and food industry laboratories). Despite the fact that not all laboratories will be able to supply data on all 15 + 1 EU-priority PAHs, EFSA will accept analytical data on as many PAHs as possible.

According to the SCF [8], smoked and grilled food may contribute significantly to the intake of PAHs, if such food products are part of the usual diet. As already described, the highest PAH concentrations are normally found in charcoal-grilled, barbecued food, foods smoked by traditional techniques, mussels and other seafood from polluted waters.

Despite the fact that the SCF concluded that BaP may be used as marker of the occurrence and the effect of the carcinogenic PAHs in food, the analysis should encompass the whole range of PAHs in order to improve evaluation of the contamination of food commodities with the 15 + 1 EU-priority PAHs and to detect any future change in that range. For this reason, data ful-

filling the same criteria would be greatly welcomed from environment laboratories. The on-line analytical database will help to provide more insights into the respective levels and relative proportions of BaP and other carcinogenic PAHs, particularly the 15 + 1 EU-priority PAHs.

4. Analysis of PAHs in food samples

In general, the analysis of organic contaminants in food samples is hampered by interfering compounds present in the complex food matrices. The challenge for analysts is to maximize recovery of analyte and minimize the accompanying interferences by proper extraction and clean-up procedures. Since this article is targeting this situation and the need for the official control of PAHs within the EU, it is not intended to be a comprehensive review on analytical methods published in scientific journals.

The first official method for the analysis of BaP in food was based on ultraviolet absorption of an extract of the food in question purified by thin layer chromatography that was published in the 1970s [38]. Since then an international standard has been provided for only BaP in fats and oils [39]. The method is based on the measurement of BaP by high performance liquid chromatography with fluorescence detection (HPLC-FLD). The scientific community acknowledged the lack of official methods, so standards from the International Organization for Standardization (ISO) are currently in preparation for the determination of an enlarged set of PAHs in edible fats and oils. Two of the methods use HPLC-FLD for quantification and cover both BaP and the “carcinogenic PAHs” [39], while the third method includes the 15 SCF PAHs. For the latter, values in the range 0.1–1 μg of individual PAH per kg of oil were reported as limits of quantification [39]. However, all three draft ISO methods cover more or less only a subset of the 15 + 1 EU-priority PAHs.

Some countries have developed national standards. For example, Germany focused on smoked meat

products. In 1983, Germany published two official methods for the screening of BaP concentrations in non-smoked and smoked meat products [40]; they were then extended to general food in 2004. In 1989, a quantitative method for the determination of BaP in meat products was established and added to the German compendium of official analytical methods intended for food control [40].

Not only are there few national or international standard methods, but also little information about collaborative trials has been published so far. One of the few papers presents details on a collaborative trial for characterizing a European certified reference material covering five PAHs in coconut oil [41].

Without going into detail, the majority of scientific papers concerning PAHs in food matrices have focused mainly on BaP. A considerable number tackled the set of the 16 US EPA PAHs, but only a small number went beyond these. Just one recent study for analysis of PAHs in smoke flavors focused on the 15 + 1 EU-priority PAHs [21,23].

However, it was stressed in 2000 that suitable analytical methods were still urgently needed for routine analytical purposes [42]. This conclusion had already been reached before the new set of EU priority analytes was specified. Since then, this demand has even increased.

Prior to validation and standardization of methods for the 15 + 1 EU-priority PAHs in various different food matrices, the analytical community felt [43] that there was a need to investigate the proficiency of laboratories. Information on performance of laboratories is very important for assessing the reliability of data intended for the on-line monitoring database mentioned above. Increasingly, proficiency tests are offered by various institutions. To ensure reliable data for official food control, the EC responded recently (in 2006) by assigning a Community Reference Laboratory (CRL) to PAHs. The CRL will work in close collaboration with the EU National Reference Laboratories (NRLs) from the EU Member States.

5. Analysis of PAHs in environmental samples

This section aims primarily to review official methods for the determination of PAHs in environmental samples. Some publications in scientific journals are cited, where appropriate, in order to show the broad diversity of applied techniques. To review all scientific papers in that field would go far beyond the scope of this article.

In the 1980s, the US EPA was very concerned with analyzing PAHs in different environmental matrices and setting up analytical methods, especially for the 16 EPA priority PAHs [44]. A number of methods were developed and standardized for the extraction of PAHs from solids, such as soils, sludge, and waste, applying Soxhlet

extraction (SE), automated SE, supercritical fluid extraction (SFE) and pressurized liquid extraction (PLE) [44]. Extraction of this set of EPA-PAHs from environmental solids was also targeted by investigating the comparability of results obtained by SE, PLE, SFE and sub-critical water extraction [45], as well as the applicability of fluidized bed extraction [46]. An extraction method for the determination of PAHs, polychlorinated biphenyls, and total petroleum hydrocarbons from soil samples applying SFE by using supercritical carbon dioxide as extractant was recently validated by collaborative trial and compared to SE [47]. The results obtained by those two techniques were comparable for most of the analytes. Even simpler extraction techniques, such as liquid extraction at room temperature on a shaking device, were shown to be suitable for extracting PAHs from soil [39].

The required sample clean-up depends very much on the selectivity of the applied extraction technique and of course on the chromatographic method to be applied. It was demonstrated that the SFE extracts of urban air particles contained fewer co-extractives than SE or PLE extracts [46]. As the latter two methods apply non-polar organic solvents for analyte extraction, humic acids and aliphatic hydrocarbons are also dissolved. However, those interfering compounds are frequently removed by clean-up procedures, such as adsorption chromatography with silica gel, alumina or florisil [39]. Such procedures were described in a US-EPA method [44].

The chromatographic separation of the PAHs is performed either by GC with mass spectrometric (MS) detection operated in selected ion monitoring (SIM) mode, or by HPLC-FLD. Besides the methods devised by the US EPA, standard procedures were also published by ISO. ISO standard 13877:1998 describes a method for the determination of PAHs in soil by HPLC, whereas the very recently published standard ISO 18287:2006 specifies a method for the determination of PAHs in soil by GC-MS [39].

Analytical protocols for the determination of PAHs in air and water have also been developed. Suitable GC and HPLC methods were published in 1989 in "The Compendium of Methods for the Determination of Toxic Organic Compounds in Ambient Air" and revised in 1996 [44]. For the analysis of PAHs in water, several methods exist, even focusing on different numbers of analytes [39]. The chromatographic methods do not differ very much from those applied for the analysis of soil or air. However, analyte enrichment is a prerequisite for the analysis of PAHs in water. Several techniques have been developed for that purpose, such as liquid-liquid extraction (LLE), solid-phase extraction (SPE), solid-phase microextraction (SPME) or stir-bar sorptive extraction (SBSE) [48–53]. Membrane extraction systems for the isolation of unpolar contaminants from aqueous samples have also come under investigation [54].

6. Requirements – conclusions and outlook

Analytical methods used by food analysts in general may serve various purposes (e.g., implementation of legislative limits, monitoring for exposure estimation within the risk-assessment process, and monitoring for detection of frauds or compliance with labeling). In the context of analysis of the 15 + 1 EU-priority PAHs, the first two reasons apply. For implementation of legislation, fast, precise methods are necessary. For risk assessment, the analytical results should be as precise as possible and cover a large range of concentrations. At the same time, it is important to have a high sample throughput to obtain sufficient data. This is necessary to detect even small trends with statistical significance and to maximize the soundness of analytical results.

In food analysis, assurance of high-quality data has a long history. This holds especially true in official food control, as comparability of data is a requirement for international trade and to avoid unnecessary court cases due to unfavorable results. Accreditation according to ISO 17025 for laboratories therefore became a basic requirement for official food control. A requirement for accredited laboratories is participation in proficiency tests, which offer a good information source on the quality of data produced by the laboratories, which, in turn, is important to guarantee high-quality results (e.g., for databases or court cases).

Certified reference materials play a vital role in verifying the accuracy and in establishing traceability of analytical measurements. In that respect, certified matrix reference materials that represent real food matrices are especially important. Currently, the production of individual pure certified reference materials covering all the 15 + 1 EU-priority PAHs is being completed, and that is an important step forward towards ensuring appropriate calibration of the analytical instruments. Nevertheless, is highly desirable to have food-matrix reference materials certified for their PAH content available.

Current EU legislation sets maximum allowed concentrations for BaP in various food products in the range 1–10 µg/kg [35] and for BaP and benzo[*a*]anthracene in liquid smoke flavoring primary products of 10 µg/kg and 20 µg/kg, respectively [55]. Legislation describes methods of sampling, sample preparation, and criteria for methods of analysis for BaP in food, whereas, for all other EU priority PAHs, there is a recommendation to measure them in food products as widely as possible in order to obtain data on the occurrence and the specific concentration pattern of PAHs in the various matrices so as to assess whether BaP or other PAHs may serve as markers for all priority PAHs. In the case of smoke flavorings, criteria for all priority PAHs will be listed in the respective legislation (in preparation), as a method has already been collaboratively validated for this purpose [21]. Regarding other food commodities, validated (collaborative trial)

methods have been established for only the determination of BaP. The ISO Technical Committee on food products has three methods in preparation, but none covers all the 15 + 1 EU-priority compounds.

To implement EU legislation, analytical methods must be appropriately validated and laboratories must be assessed in inter-laboratory studies in order to evaluate to which (lower) level of concentration the set of EU's 15 + 1 priority PAHs can be reliably determined. Since this depends very much on the food matrix studied, various test materials (e.g., edible oils, meat products, grains and dairy products) containing various levels of BaP and the other PAHs must be produced. The concentration levels should ideally be close to the maximum permitted concentration, as the reliability of data in that range is essential, since they impact on compliance/non-compliance decisions.

Current PT-scheme providers also need to adapt their programmes to the full range of priority PAHs. This is a key element to ensure the quality of data to be introduced into the EU PAH database. Proficiency tests organized so far have mainly covered BaP, benzo[*a*]anthracene, benzo[*b*]fluoranthene, and indeno[1,2,3-*cd*]pyrene. Depending on the study, fluorene, fluoranthene, chrysene and benzo[*ghi*]perylene have also been investigated. However, the PAH levels of some of the food matrices tested were far above the maximum limits. A very first proficiency test covering all the EU's priority PAHs was organized recently [56]. The results showed that there is still a great need for improvement as very few of the laboratories were able to analyze all EU's 15 + 1 priority PAHs satisfactorily.

It must be stressed that the requirements listed above on validated methods, reference materials and PT have also to hold true for the analysis of environmental samples (e.g., soil and water) in order to assess the impact of contamination from the environment on the pattern of PAHs found in food products. It is therefore of utmost importance to get into a dialogue with the respective organizations dealing with environmental matters, to raise awareness of the recently introduced food legislation, and, preferably, to harmonize monitoring campaigns. This would increase not only the impact of the studies within the area of food safety, but also go beyond to the more general area of human health, and that would lead to a faster process with regard to methods of analysis and impact studies especially with the aim of obtaining one or more prominent marker(s) (ideally to be rapidly and easily analyzed).

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