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## Reflection paper on Polycyclic Aromatic Hydrocarbons in herbal medicinal products/traditional herbal medicinal products

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Keywords	Herbal medicinal products; traditional herbal medicinal products; herbal substances; herbal preparations; polycyclic aromatic hydrocarbons; safety; HMPC.
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# 1 Introduction

Polycyclic aromatic hydrocarbons, also known as arenes, are a large family of non-functionalised aromatic compounds containing either two or more fused aromatic rings made up of carbon and hydrogen atoms (see Figure 1). The most abundant polycyclic aromatic hydrocarbons (PAHs) in the environment contain between two and seven rings and can be derived from both natural and anthropogenic sources, like combustion and pyrolysis processes. They have been extensively studied since it was discovered that some of them, for example, benzo[a]pyrene, are both genotoxic and carcinogenic.

Humans are exposed to PAHs through different pathways, where the major route for non-smokers is consumption of food (Eur. Com. SCF 2002; EFSA 2008). Food can be contaminated from environmental sources (natural and mostly anthropogenic), from industrial food processing (drying, smoking, roasting) and from some domestic cooking practices (grilling, frying, barbecuing) (Harvey 1997; Howsam & Jones 1998).

Recent studies have shown that some medicinal plants can be contaminated with PAHs. This can arise due to contamination by environmental sources, as the lipophilic properties of these compounds allow their adsorption onto atmospheric particles and direct deposition in sediments, soils and plants, or during the post-harvest processing.

Existing food regulations have recently been updated to set maximum levels for PAHs in cocoa fibre, banana chips, food supplements, dried herbs and dried spices; the updated EU Regulation 2015/1933 will apply from 1st April 2016.

This reflection paper concerns the implications of potential PAH contamination of herbal substances and/or herbal preparations used as active substances of herbal medicinal products (HMPs) or traditional herbal medicinal products (THMPs), the safety issues that arise and, where appropriate, the need for controls to be implemented.

The purpose of this reflection paper is to promote discussion about the presence of PAHs in herbal substances, herbal preparations and herbal medicines and to invite all interested parties including suppliers and manufacturers of herbal substances/herbal preparations, manufacturers of HMPs/THMPs, pharmaceutical industry associations, health care professional groups, learned societies, consumers and patients' associations, governmental institutions as well as EU and EEA-EFTA Member States to submit any scientific data or documented information (new, published or unpublished) and comments relevant to the evaluation of this problem.

## 2 Discussion

### ***2.1 Sources of Polycyclic Aromatic Hydrocarbons (PAHs)***

PAHs and their derivatives are widespread in the environment from both natural and anthropogenic sources. They are created when substances such as coal, oil, gas and organic waste are burned incompletely. At high temperatures organic compounds are partially cracked to smaller unstable fragments, mostly radicals that recombine to give relatively stable PAHs (pyrosynthesis). Another source of PAHs is aromatisation that occurs at lower temperatures (100–150°C), but this requires much more time and produces large quantities of alkylated PAHs (Moret and Conte, 2000).

PAHs are ubiquitous, being present in the atmosphere, surface water, sediments and soil, food and lipid tissues. For the general population, exposure to PAHs is mainly from food and inhaled air, although there have been cases of high level occupational exposure (European Commission, 2002).

PAHs of lower molecular mass (generally those with three or fewer aromatic rings) are numerous in the atmosphere and those of higher molecular mass (the majority) enter the environment adsorbed onto particulate matter. The hydrosphere and geosphere are affected by dry and wet deposition of PAHs. Solubility in water is a crucial characteristic for determining distribution patterns of PAHs in food (Lerario *et al.*, 2003). Phenanthrene, for example, has a relatively high water solubility when compared with other PAHs, and is therefore likely to have a higher environmental mobility; in fact, phenanthrene is the PAH found in highest concentrations in aquatic samples. As PAHs are lipophilic compounds, uptake by absorption by plants from contaminated soils is low. The waxy surfaces of vegetables and fruits can concentrate low molecular mass PAHs through surface adsorption (European Commission SCF, 2002).

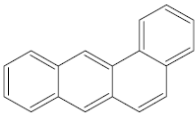
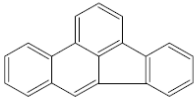
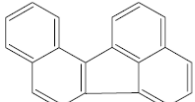
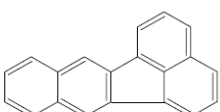
PAHs are known to be present in foodstuffs due to environmental exposure, but processing of food (such as smoking, industrial drying, barbecuing and other cooking methods) is thought to be the major source of PAHs in food. Charcoal grilling can generate high levels of PAHs, quantities produced being related to factors such as fat content, temperature and cooking time.

Vegetable oils, such as coconut, sunflower, olive and grape-seed oils and fats contribute significantly to the presence of PAHs in the diet. Their contamination is mainly due to the industrial drying processes to which the seeds are subjected when the seeds come into contact with combustion gases. It is a common practice to use open fires to heat seeds in industrial processes used to extract oils.

Fortunately, refining can reduce the amount of these contaminants. Deodorisation methods remove the most volatile of these PAHs whereas charcoal treatment can remove higher PAHs (as a general rule, those with more than four rings) (Moret and Conte, 2000). In coffee and tea, the roasting procedure can contribute to additional production of PAHs (European Commission SCF, 2002).

PAHs can also contaminate foodstuffs by diffusion from some packaging materials such as recycled polyethylene film (Moret and Conte, 2000; Tamakawa, 2004).

**Figure 1** - Polycyclic aromatic hydrocarbons (EFSA, 2008)

Compound	Abbr.	MW	CAS	Structure
<u>Benz[a]anthracene</u>	BaA	228.3	56-55-3	
<u>Benzo[b]fluoranthene</u>	BbFA	252.3	205-99-2	
<u>Benzo[j]fluoranthene</u>	BjFA	252.3	205-82-3	
<u>Benzo[k]fluoranthene</u>	BkFA	252.3	207-08-9	

**Figure 1** (continued) - Polycyclic aromatic hydrocarbons (EFSA, 2008)

<u>Benzo[ghi]perylene</u>	BghiP	276.3	191-24-2	
<u>Benzo[a]pyrene</u>	BaP	252.3	50-32-8	
<u>Chrysene</u>	CHR	228.3	218-01-9	
<u>Cyclopenta[cd]pyrene</u>	CPP	226.3	27208-37-3	
<u>Dibenzo[a,h]anthracene</u>	DBaH	278.3	53-70-3	
<u>Dibenzo[a,e]pyrene</u>	DBaeP	302.3	192-65-4	
<u>Dibenzo[a,h]pyrene</u>	DBaH	302.3	189-64-0	
<u>Dibenzo[a,i]pyrene</u>	DBaiP	302.3	189-55-9	
<u>Dibenzo[a,j]pyrene</u>	DBaJ	302.3	191-30-0	
<u>Indeno[1,2,3-cd]pyrene</u>	IP	276.3	193-39-5	
<u>5-methylchrysene</u>	MCH	242.3	3697-24-3	
<u>Benzo[c]fluorene</u>	BcFL	216.3	205-12-9	

Abbr.: Abbreviation, MW: Molecular weight, CAS: Chemical Abstract Service Number

## 2.2 Health Effects

There are several reviews about the assessment of risks of PAHs to human health (Domingo and Nadal, 2015). A comprehensive review 'Opinion of the Scientific Committee on Food on the risks to human health of Polycyclic Aromatic Hydrocarbons in food' deals with the key aspects involving PAHs risks and effects on humans (European Commission, 2002).

PAHs are toxic, many of the compounds in this class are both genotoxic and carcinogenic. They are actively involved in enzyme induction, immunosuppression and teratogenicity. Many PAHs are complete carcinogens as well as initiators, thus, in addition to their capacity of inducing mutations (via genotoxic mechanisms) they can also promote tumour formation.

Research on PAH-induced carcinogenesis began with the isolation by Cook *et al.* of benzo[a]pyrene (B[a]P) from coal tar in 1930 and the demonstration that the compound initiates tumours when repeatedly painted on mouse skin. A review article published by Nature covers the first 50 years since the isolation of B[a]P and the subsequent progress in unravelling the metabolic fate and mechanisms of chemical carcinogenesis (Phillips, 1983). Because of the knowledge accumulated on B[a]P toxicity, it has by default become the reference chemical for PAH mixtures.

The PAH-induced carcinogenesis is a multi-step process lasting decades (in humans). The binding of PAHs to DNA (oncogenic targets) is the initial step in the process. The binding capacity was thought to be responsible for the potency of the PAHs in inducing cancer. However, recent results have shown that relative to B[a]P, a PAH mixture forms lower levels of DNA adducts, yet is highly tumourigenic in mouse skin (Baird *et al.*, 2005). This is a clear indication of other factors contributing to PAH-induced carcinogenesis beyond DNA binding ability.

Evidence that PAHs are carcinogenic to humans comes primarily from occupational studies of workers following inhalation and dermal exposure. No data are available for humans for other routes of exposure. However, there is a vast collection of data for animals such as mice (Rizova *et al.*, 2005). Route of exposure is of importance in determining specific toxicities and target tissues of at least B[a]P, but also those of other PAHs (Nebert *et al.*, 2013).

Human exposure to PAHs occurs principally by direct inhalation, ingestion or dermal contact, as a result of their widespread presence and persistence in the environment (mostly urban ones). This exposure to ambient PAHs is usually a combination of diverse PAHs and other carcinogenic substances. These other substances may account for a more significant portion of the carcinogenicity of some mixtures, such as cigarette smoke, diesel emissions and urban aerosol.

There is an excess risk of lung, breast and urinary bladder cancer in workers at coke ovens, coal gasification plants, petroleum refineries, aluminium smelters, iron and steel foundries and those working with bitumen, diesel and asphalt. Risks of chronic respiratory abnormalities are also higher for people exposed to PAHs; these abnormalities include silicosis, asthma-like symptoms, lung function abnormalities and chronic bronchitis, especially, in aluminium plant workers (Mastrangelo *et al.*, 1996).

## 2.3 Methods of Analysis

Due to the ubiquity of PAHs and their potentially deleterious effects on human health, these compounds are often analysed in a variety of matrices such as foods, oils, waters, soils etc. Being such a large and diverse family, standard methods, based on field sampling and laboratory analysis, are often used.

There are several well-established analytical procedures for the analysis of PAHs. Most of them involve pre-treatment of the sample, where the PAHs are extracted from the complex mixtures in which they

are present, into a new matrix by liquid/liquid, solid-phase and/or ultrasonic extractions (Wenzl *et al.*, 2006). Pre-treatment methods often serve as a pre-cleaning of interfering compounds and also a pre-concentration step (Mastral *et al.*, 2004). The application of ultrasound for accelerating or assisting PAH (and other inorganic and organic compounds), extraction from solid materials is sometimes used. Extracts are measured by either HPLC or GC (depending on the nature of the sample and its volatility) with the detectors being mass spectrometer (MS), ultraviolet (UV) or fluorescence spectrophotometers (Li *et al.*, 2003).

## **2.4 Legislative framework**

Due to the health effects and widespread occurrence of PAHs several pieces of legislation are in place in the European Union to limit the exposure to these compounds.

The Directive on Environmental Quality Standards, Directive 2008/105/EC, now amended by Directive 2013/39/EU establish limits to PAHs in surface waters. There are also specific maximum limits set at the EU level for PAHs in drinking water (Council Directive 98/83/EC).

Directive 96/62/EC on ambient air-quality and Regulation (EC) 850/2004 define monitoring provisions and specific thresholds for PAHs.

The Expert Opinion of BfR (2010) refers that besides from other hazardous properties, PAHs can be carcinogenic and currently 8 PAHs are classified as known carcinogens in Annex VI of Regulation (EC) No 1272/2008 of the European Parliament and the Council, of 16 December 2008, on classification, labelling and packaging of substances and mixtures.

There are also restrictions established in Annex XVII of Regulation (EC) 1907/2006 (REACH regulation) - substances or mixtures containing these eight PAHs above certain concentration limits have to be classified/labelled as carcinogenic themselves and may not be sold to the general public. Also, in the EU Toy Directive (Directive 2009/48/EC) the same concentration limits for known carcinogenic PAHs apply to toys.

Regarding food, in 2005 three Commission documents relating to PAHs were published. They include a Regulation limiting levels of B[a]P in foods [Commission Regulation (EC) No 208/2005, 'Amending Regulation (EC) No 466/2001 as regards polycyclic aromatic hydrocarbons', Official Journal of the European Union, L34/3, 8.2.2005], a Directive covering sampling and analysis [Commission Directive 2005/10/EC, 'Laying down the sampling methods and the methods of analysis for the official control of the levels of B[a]P in foodstuffs', Official Journal of the European Union, L34/15, 8.2.2005], and a Recommendation for further work to be carried out by Member States [Commission Recommendation, 'On the further investigation into the levels of polycyclic aromatic hydrocarbons in certain foods', Official Journal of the European Union, L34/43, 8.2.2005]. In 2011 the Commission Regulation (EU) No. 835/2011, of 18 August 2011, amending Regulation (EC) No 1881/2006 as regards maximum levels for polycyclic aromatic hydrocarbons in foodstuffs [Official Journal of the European Union, L215/4, 20.8.2011] was published. According to this Regulation, the maximum levels of PAHs are as follows:

<b>Polycyclic aromatic Hydrocarbons</b>			
<b>Foodstuffs</b>		<b>Maximum levels (µg/Kg)</b>	
6.1	Benzo(a)pyrene, benz(a)anthracene, benzo(b)fluoranthene and chrysene	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 direct human consumption or use as an ingredient in food	2.0	10.0
6.1.2	Cocoa beans and derived products	5.0 µg/kg fat as from 1.4.2013	35.0 µg/kg fat as from 1.4.2013 until 31.3.2015 30.0 µg/kg fat as from 1.4.2015
6.1.3	Coconut oil intended for direct human consumption or use as an ingredient in food	2.0	20.0
6.1.4	Smoked meat and smoked meat products	5.0 until 31.8.2014 2.0 as from 1.9.2014	30.0 as from 1.9.2012 until 31.8.2014 12.0 as from 1.9.2014
6.1.5	Muscle meat of smoked fish and smoked fishery products, excluding fishery products listed in points 6.1.6 and 6.1.7. The maximum level for smoked crustaceans applies to muscle meat from appendages and abdomen. In case of smoked crabs and crab-like crustaceans ( <i>Brachyura</i> and <i>Anomura</i> ) it applies to muscle meat from appendages.	5 until 31.8.2014 2.0 as from 1.9.2014	30 as from 1.9.2012 until 31.8.2014 12 as from 1.9.2014
6.1.6	Smoked sprats and canned smoked sprats ( <i>sprattus sprattus</i> ); bivalve molluscs (fresh, chilled or frozen); heat treated meat and heat treated meat products sold to the final consumer	5.0	30.0
6.1.7	Bivalve molluscs (smoked)	6.0	35.0
6.1.8	Processed cereal-based foods and baby foods for infants and young children	1.0	1.0
6.1.9	Infant formulae and follow-on formulae, including infant milk and follow-on milk	1.0	1.0
6.1.10	Dietary foods for special medical purposes intended specifically for infants	1.0	1.0

This Regulation also mentions that 'maximum levels for polycyclic aromatic hydrocarbons must be safe and as low as reasonably achievable (ALARA) based upon good manufacturing and agricultural/fishery practices' (Whereas No 11) and that 'High levels of PAH have been found in some food supplements. Nevertheless, the levels are variable and depend on the specific type of food supplements. Further



data on food supplements are needed and should be collected. Once these data become available, the need for setting maximum levels for PAH in food supplements will be evaluated' (Whereas No 21).

Recently, another Commission Regulation (Commission Regulation (EU) 2015/1933, of 27 October 2015, amending Regulation (EC) No 1881/2006 as regards maximum levels for polycyclic aromatic hydrocarbons in cocoa fibre, banana chips, food supplements, dried herbs and dried spices) was published. The previous limits applicable for cocoa were changed and limits applicable for banana chips, food supplements, dried herbs and dried spices were introduced (see Table below)

Section 6: 'Polycyclic aromatic hydrocarbons' of the Annex to Regulation (EC) No 1881/2006 is amended as follows:

(1) point 6.1.2 is replaced by the following:

'6.1.2	Cocoa beans and derived products with the exception of the products referred to in point 6.1.11	5,0 µg/kg fat as from 1.4.2013	35,0 µg/kg fat as from 1.4.2013 until 31.3.2015 30,0 µg/kg fat as from 1.4.2015'
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(2) the following points 6.1.11, 6.1.12, 6.1.13, 6.1.14 and 6.1.15 are added:

'6.1.11	Cocoa fibre and products derived from cocoa fibre, intended for use as an ingredient in food	3,0	15,0
6.1.12	Banana chips	2,0	20,0
6.1.13	Food supplements containing botanicals and their preparations <sup>(*)</sup> <sup>(**)</sup> Food supplements containing propolis, royal jelly, spirulina or their preparations <sup>(*)</sup>	10,0	50,0
6.1.14	Dried herbs	10,0	50,0
6.1.15	Dried spices with the exception of cardamon and smoked <i>Capsicum</i> spp.	10,0	50,0

(\*) Botanical preparations are preparations obtained from botanicals (e.g. whole, plant parts, fragmented or cut plants) by various processes (e.g. pressing, squeezing, extraction, fractionation, distillation, concentration, drying up and fermentation). This definition includes comminuted or powdered plants, plant parts, algae, fungi, lichen, tinctures, extracts, essential oils (other than the vegetable oils referred to in point 6.1.1), expressed juices and processed exudates.

(\*\*) The maximum level does not apply to food supplements containing vegetable oils. Vegetable oils used as an ingredient in food supplements should comply with the maximum level established in point 6.1.1.'

It is important to note that some European countries have national regulations. Martena *et al.* (2011) noted that, for example, the Dutch Commodities Act has included, since January 2006, a maximum level of 10 µg kg<sup>-1</sup> B[a]P for food supplements with botanical ingredients and a maximum of 2 µg kg<sup>-1</sup> B[a]P for supplements without these ingredients. According to them, these limits were introduced as a result of a survey by the Voedsel en Waren Autoriteit (VWA – the Dutch Food and Consumer Product Safety Authority) into the presence of B[a]P in food supplements.

Also the ESCO Working Group on Botanicals and Botanical Preparations (EFSA, 2009a) noted that the composition of a botanical or botanical preparation may vary significantly due to factors that cannot be easily controlled. The Working Group considered it appropriate to define requirements on pesticide residues, mycotoxins, heavy metal and polycyclic aromatic hydrocarbon residues in the specifications. Although this may refer to quality control, the Working Group considered this an important issue in the field of botanicals and botanical preparations that cannot be ignored. It was noted that some contaminants (e.g. PAHs in dried preparations) may arise from the manufacturing process and need to be kept within safety limits. Taking this all together the Working Group proposes to extend the section

on specifications with the following text: "The specifications should include concentrations of major groups of constituents present in the botanical preparation including, for example: amino acids, lipids, polysaccharides, volatile oil, inorganic ions, polyphenols, alkaloids, terpenes, alkenylbenzenes, lignin, saponins etc. as well as the major constituents within these classes. In addition, maximum levels for possible contaminants including e.g. heavy metals, mycotoxins, pesticide residues, and polycyclic aromatic hydrocarbon (PAH) residues should be proposed in line with existing guidelines."

In the European Pharmacopoeia testing for PAHs has been introduced into monographs of certain substances (e.g., Liquid paraffin, Light liquid paraffin, Hard paraffin, Soft paraffin white, Soft paraffin yellow) and in the general monograph "Vegetable fatty oils". This is a limit test based on a spectrophotometric method involving, after extraction of the PAHs, measuring under defined experimental conditions, the absorbance of the test solution between 260 and 420 nm, and then comparing this with the absorbance of a naphthalene solution at 270 nm. At no wavelength in the range 260 to 430 nm does the absorbance of the test solution exceed one-third of the reference solution at 275 nm.

## **2.5 Occurrence of PAHs in medicinal plants**

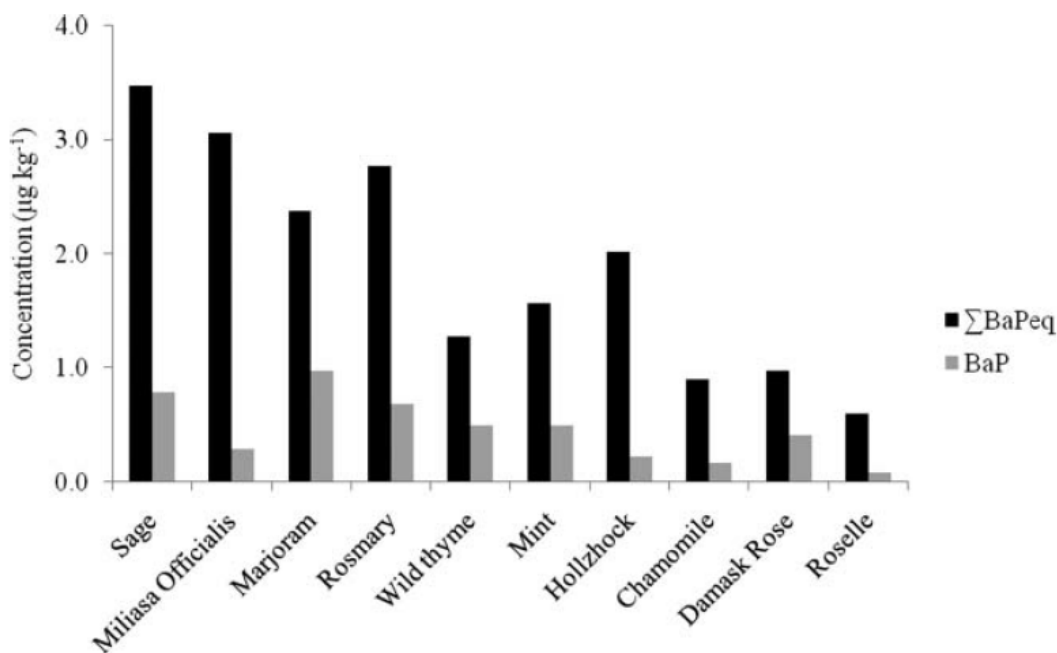
It is well known that plants may be contaminated with environmental PAHs, particularly if they grow close to urban centres and industrial areas but also during drying processes. Grains and raw products for the production of vegetable oils may be highly contaminated with PAHs during the drying process (Danyi, 2009). Also, very high concentrations of carcinogenic PAHs were found in yerba mate (*Ilex paraguariensis*) leaves and in hot and cold mate infusions, supporting the hypothesis that the carcinogenicity of mate (drinking mate has been associated with cancers of the oesophagus, oropharynx, larynx, lung, kidney, and bladder) may be related to its PAH content (Kamangar *et al.*, 2008).

There are a few articles dealing with the occurrence of PAHs in medicinal plants. Bakker *et al.* (2001), for instance, studied the localisation of deposited PAHs in leaves of *Plantago major* and *Plantago media*. They divided the leaves of these 2 plants into three fractions. First, the leaves were washed (wash-off fraction), then cuticular wax was extracted (wax fraction). Finally, the remaining leaf material was extracted (interior fraction). The presence of PAHs could be demonstrated in all three fractions. For both plants, the distribution of PAHs over the three fractions changed with molecular weight of the PAHs. The wash-off fraction increased with increasing molecular weight, likely because high molecular-weight PAHs occur predominantly bound to particles, which can be readily washed off from the leaves. In contrast, the amount of PAHs detected in the interior of the leaves decreased with increasing molecular weight. This can be explained by a slow desorption of the PAHs from the particles and a low diffusion rate of the larger molecules. This study shows that washing reduces the amount of high molecular-weight PAHs on plant surfaces. Therefore, washing of leafy vegetables is important to minimise human dietary intake of PAHs.

Krajian and Odeh (2013), for example, report the determination of 16 US Environmental Protection Agency polycyclic aromatic hydrocarbons (EPA PAHs) in 10 medicinal plants from Syria, in different used plant parts (leaves or flowers). They also mentioned other published studies, namely, the study of Ishizaki, Sito, and Kataoka, in which 29 crude drugs (one sample from each kind) were analysed. In another study, Ciecierska and Obiedzinski determined the levels of 19 PAHs in lime (*Tilia cordata*), pansy (*Viola wittrockiana*), mint (*Mentha longifolia*), and lemon balm (*Melissa officinalis*). They also made reference to another study, by Yu *et al.*, that reported the content of 16 PAHs as representative contaminants in nine Chinese medicinal herbs (Panax, Fructus liquidambaris, liquorice root, mulberry twig, cassia seed, eucommia bark, rose flower, indigowoad leaf and fleecflower root).

Figure 2 shows a comparison of the results of these authors for B[a]P and  $\Sigma$ B[a]P<sub>eq</sub> (sum of level in B[a]P equivalents) in the studied medicinal plants.

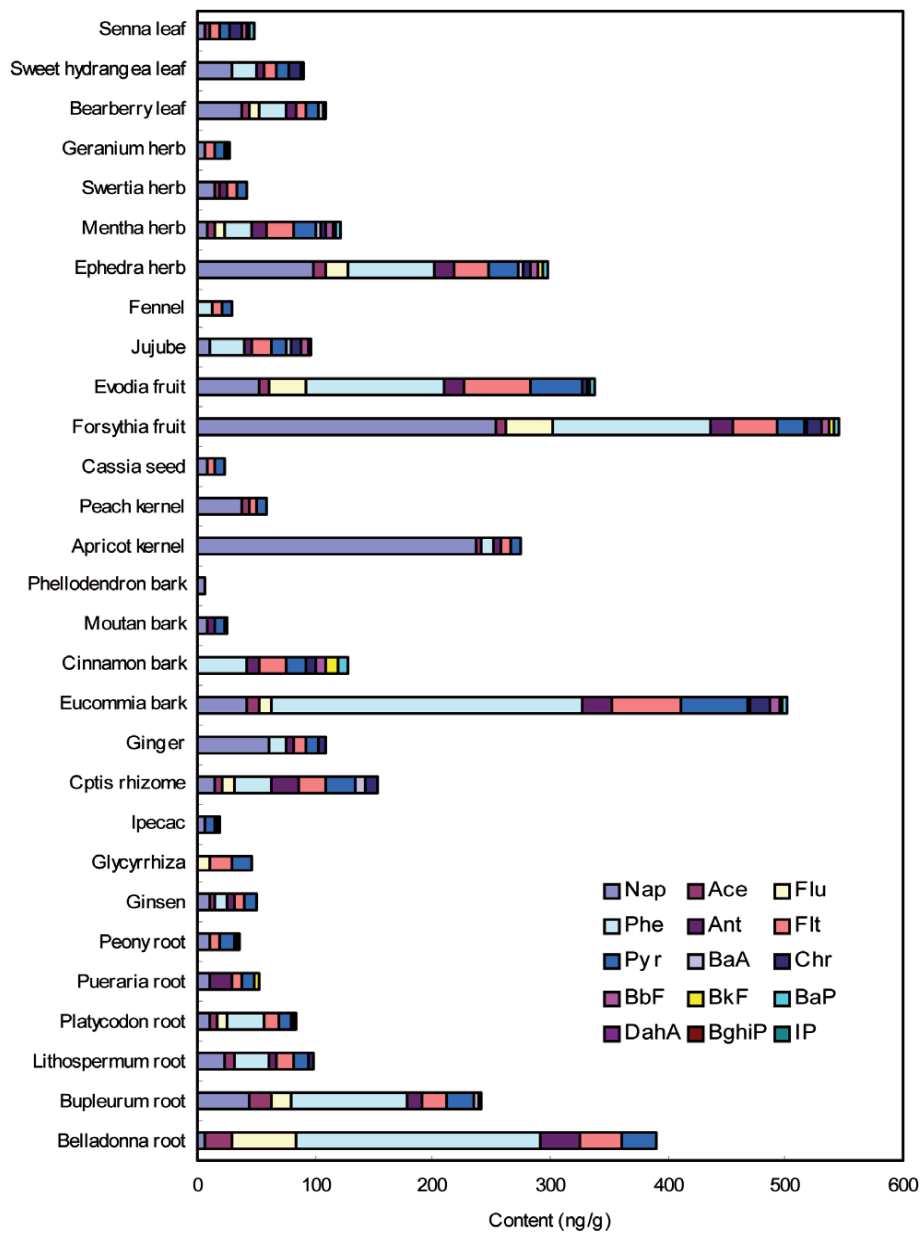
**Figure 2** from Krajian and Odeh (2013)



Krajian and Odeh (2013) concluded that the sum of the 16 PAHs ( $\Sigma$ PAHs) in the investigated medicinal plants ranged from 47 to 890  $\mu\text{g kg}^{-1}$  where the highest  $\Sigma$ PAHs was found in a Sage plant sample. Light PAHs were dominant in all studied medicinal plants. The sum of eight genotoxic PAHs ( $\Sigma$ PAH8) have shown a better indicator of the degree of contamination with carcinogenic PAHs compared to B[a]P in these products.

Kataoka *et al.* (2010) describe the development of an automated on-line method using a new sample preparation technique, in-tube solid-phase microextraction (SPME), coupled with high-performance liquid chromatography-fluorescence detection, to analyse PAH contamination in herbal medicines. Total PAH contents in 29 herbal medicines are shown in the Figure 3. They found that low-molecular weight PAHs were detected more frequently than high-molecular weight PAHs. Naphthol, flouranthene, and pyrene were especially prominent in most samples, but their concentrations were less than 254 ng/g each. In contrast, DahA, BghiP, and IP were not detected at all. Although growing medicinal plants may accumulate PAHs from air or soils, differences in PAH contents in different plant loci, such as leaf, fruit, seed, bark and root, were not observed. Herbal medicines may be sensitive to contamination with PAHs during various production steps, including heat-drying. In particular, a carcinogenic B[a]P, at concentrations of 3.8-8.6 ng/g, was detected in some herbs, fruits, and bark, though its threshold level for foods has been set by EU regulations at 2 ng/g. Taken together, these results suggest that herbal medicines may often contain PAHs, indicating the need to evaluate the safety of these contaminants.

Figure 3 from Kataoka *et al.* (2010)



Zongyan *et al.* (2014) report the analysis of seventy-nine Chinese herbal medicines that were collected from several markets for the content of 16 PAHs, using gas chromatography coupled to tandem mass spectrometry. The total PAHs concentrations were in the range of 21.1-2856.0  $\mu\text{g}\cdot\text{kg}^{-1}$ . Among the 16 PAHs, the predominant species detected were 2-4 ring PAHs, accounting for 85% of the total. For different sample types, the PAHs levels of flowers, leaves, roots and stems were higher than that of fruits and seeds. 24.1% of the samples exceeded the maximum residue limit for B[a]P (5  $\mu\text{g}\cdot\text{kg}^{-1}$ ), indicating a potential risk to human health.

There are also several published articles dealing with the analysis of PAHs in food supplements, such as those from the UK Food Standards Agency (FSA 2005), EFSA (2008), Danyi *et al.* (2009), Martena *et al.* (2011) and Zelinkova and Wenzl (2015). These last authors reported that from 2003 up to and including 2007, B[a]P was present above the Limit of quantification (LoQ) in 553 (44%) of 1258 supplements and the lower-bound mean B[a]P level was 3.37  $\mu\text{g}\cdot\text{kg}^{-1}$ . In a 2008 and 2009 survey, 210

(63%) of 333 food supplements showed B[a]P levels above the LoQ and the lower-bound mean B[a]P level was 5.33  $\mu\text{g kg}^{-1}$ . In 2008 and 2009, besides B[a]P 12 other EFSA priority PAH were also determined and the lower-bound mean PAH2, PAH4 and PAH8(-IcP) levels were 20.8, 33.8 and 40.9  $\mu\text{g kg}^{-1}$ , respectively. In this period mono-ingredient supplements with resveratrol (a supplement only containing resveratrol from *Polygonum cuspidatum* root showed the highest B[a]P level (150  $\mu\text{g kg}^{-1}$ )), St. John's wort, *Ginkgo biloba* and propolis showed the highest mean PAH levels. Prior to 2008, the highest mean B[a]P levels were found in monoingredient supplements with dong quai, green tea, *Ginkgo biloba*, St. John's wort, valerian, propolis and pollen. Martena *et al.* (2011) also highlighted that the results they obtained were in agreement with a report of the FSA of 15 SCF (Scientific Committee on Food) priority PAH in a wide range of food supplements (n=207) sampled in 2004 (FSA 2005). The FSA data also showed relatively high mean PAH levels in *Ginkgo biloba* supplements and bee products (high levels were limited to propolis supplements).

There are also issues with PAHs in herbal products and ingredients based on seaweeds (such as *Ascophyllum nodosum* and *Fucus vesiculosus* and microalgae (such as *Chlorella* and *Spirulina*) (van der Spiegel *et al.*, 2013).

### 3 Conclusion

As described above, herbal substances and herbal preparations can contain PAHs as contaminants, resulting from environmental sources (e.g., plants growing in industrial areas) and also arising from post-harvest treatments.

There is therefore a need to address the implications of potential polycyclic aromatic hydrocarbon contamination of herbal substances and/or herbal preparations used as active substances of HMPs and THMPs, the safety issues that arise and, where appropriate, the need for appropriate controls to be implemented.

HMPC invites all interested parties to submit any scientific data or documented information (new, published or unpublished) and comments relating to the levels of PAHs and the possible exposure of patients to these compounds as a result of using HMPs/THMPs in order to explore the need for appropriate controls to be introduced. After evaluation of available data the HMPC will inform in its public meeting report on the follow-up and appropriate guidance to be developed.

## 4 References

Baird WM1, Hooven LA, Mahadevan B. Carcinogenic polycyclic aromatic hydrocarbon-DNA adducts and mechanism of action. *Environ Mol Mutagen.* 2005,; 45 (2-3): 106-114.

Bakker MI, Koerselman JW, Tolls J and Kollöffel C. Localization of deposited polycyclic aromatic hydrocarbons in leaves of *Plantago*. *Environmental Toxicology and Chemistry*, 2001, 20: 1112-1116.

BfR. Carcinogenic polycyclic aromatic hydrocarbons (PAHs) in consumer products to be regulated by the EU - risk assessment by BfR in the context of a restriction proposal under REACH. Expert Opinion Nr. 032/2010, 26 July 2010, Available under: [http://www.bfr.bund.de/cm/349/carcinogenic\\_polycyclic\\_aromatic\\_hydrocarbons\\_pahs\\_in\\_consumer\\_products\\_to\\_be\\_regulated\\_by\\_the\\_eu.pdf](http://www.bfr.bund.de/cm/349/carcinogenic_polycyclic_aromatic_hydrocarbons_pahs_in_consumer_products_to_be_regulated_by_the_eu.pdf)

Danyi S, Brose F, Brasseur C, Schneider YJ, *et al.* Analysis of EU priority polycyclic aromatic hydrocarbons in food supplements using high performance liquid chromatography coupled to an ultraviolet, diode array or fluorescence detector. *Anal Chim Acta*, 2009, 633: 293-299.

Domingo JL, Nadal M. Human dietary exposure to polycyclic aromatic hydrocarbons: A review of the scientific literature. *Food Chem Toxicol*, 2015, 86: 144-153.

EFSA (European Food Safety Authority), Scientific Opinion of the Panel on Contaminants in the Food Chain on a request from the European Commission on Polycyclic Aromatic Hydrocarbons in Food. *The EFSA Journal*, 2008, 724: 1-114.

EFSA (European Food Safety Authority). EFSA Scientific Cooperation (ESCO) Report: Advice on the EFSA guidance document for the safety assessment of botanicals and botanical preparations intended for use as food supplements, based on real case studies. *The EFSA Journal*, 2009a, 7(9): 280.

EFSA (European Food Safety Authority). EFSA Scientific Committee; Guidance on safety assessment of botanicals and botanical preparations intended for use as ingredients in food supplements, on request of EFSA. *The EFSA Journal*, 2009b, 7(9): 1249-1267.

European Commission. Opinion of the Scientific Committee on Food on the Risks to Human Health of Polycyclic Aromatic Hydrocarbons in Food, 2002, SCF/CS/CNTM/PAH/29.

FSA. PAHs in dietary supplements; Food Survey Information Sheet 86/05. Food Standards Agency. London 2005. Available under: <http://tna.europarchive.org/20110116113217/http://www.food.gov.uk/multimedia/pdfs/fsis8605.pdf>

Harvey, R. G. Polycyclic aromatic hydrocarbons. Wiley-VCH, New York, 1997, 667 pp.

Howsam, M. & Jones, K. Sources of PAHs in the environment. In: Neilson A.H. (ed.) PAHs and Related Compounds, Springer Verlag, Berlin, 1998, 137–174.

Kamangar F, Schantz MM, Abnet CC, Fagundes RB, Dawsey SM. High Levels of Carcinogenic Polycyclic Aromatic Hydrocarbons in Mate Drinks. *Cancer Epidemiology, Biomarkers & Prevention*, 2008, 17: 1262-1268.

Kataoka H, Ishizaki A, Saito K. On-line automated analysis of Polycyclic Aromatic Hydrocarbons – Applications to herbal medicines. *Chimica Oggi - Chemistry Today*, 2010, 28: 21-24.

Krajian H, Odeh A. Polycyclic aromatic hydrocarbons in medicinal plants from Syria, *Toxicological & Environmental Chemistry*, 2013, 95: 942-953.

- Lerario VL1, Giandomenico S, Lopez L, Cardellicchio N. Sources and distribution of polycyclic aromatic hydrocarbons (PAHs) in sediments from the Mar Piccolo of Taranto, Ionian Sea, southern Italy. *Ann Chim.* 2003, 93(4): 397-406.
- Li, S., Olegario, R.M., Banyasz, J.L. and Shafer, K.H. Gas chromatography-mass spectrometry analysis of polycyclic aromatic hydrocarbons in single puff of cigarette smoke. *Journal of Analytical and Applied Pyrolysis*, 2003, 66: 155–163.
- Martena MJ, Grutters MMP, De Groot HN, Konings EJM and Rietjens IMCM. Monitoring of polycyclic aromatic hydrocarbons (PAH) in food supplements containing botanicals and other ingredients on the Dutch market. *Food Additives & Contaminants: Part A*, 2011, 28: 925-942.
- Mastral, A.M., Garcia, T., Lopez, J.M., Murillo, R., Callen, M.S. and Navarro, M.V. Where are the limits of the gas-phase fluorescence on the polycyclic aromatic compound analysis? *Polycyclic Aromatic Compounds*, 2004, 24: 325–332.
- Mastrangelo, G., Fadda, E. and Marzia, V. Polycyclic aromatic hydrocarbons and cancer in man *Environmental Health Perspectives*, 1996, 104: 1166–1170.
- Moret S and Conte LS. Polycyclic aromatic hydrocarbons in edible fats and oils: Occurrence and analytical methods. *Journal of Chromatography A*, 2000, 882: 245–253.
- Nebert DW, Shi Z, Gálvez-Peralta M, Uno S, Dragin N. Oral benzo[a]pyrene: understanding pharmacokinetics, detoxication, and consequences--Cyp1 knockout mouse lines as a paradigm. *Mol Pharmacol*, 2013, 84(3): 304-313.
- Phillips, DH. Fifty years of benzo(a)pyrene. *Nature*. 1983; 303: 468–472.
- Rizova, V., Nikodinovski, M. and Kendrovski, V. PAHs – the sources of pollution of the environment and their toxicological impact on human's health. *Journal of Environmental Protection and Ecology*, 2005, 6: 1–7.
- Tamakawa, K. Polycyclic aromatic hydrocarbons in foods. *In*: Nollet, L. (ed.): Handbook of Food Analysis. Marcel Dekker, Inc., New York, 2004, 1449–1483.
- Tfouni SAV, Serrate CS, Leme FM, Camargo MCR, Teles CRA, Cipoli KMVAB, Furtani RPZ. Polycyclic aromatic hydrocarbons in coffee brew: Influence of roasting and brewing procedures in two Coffea cultivars. *LWT – Food Science and Technology*, 2013, 50: 526-530.
- van der Spiegel M, Noordam MY and van der Fels-Klerx HJ. Safety of Novel Protein Sources (Insects, Microalgae, Seaweed, Duckweed, and Rapeseed) and Legislative Aspects for Their Application in Food and Feed Production. *Comprehensive Reviews in Food Science and Food Safety*, 2013,12: 662-678.
- Wenzl T, Simon R, Kleiner J and Anklam E. Analytical methods for polycyclic aromatic hydrocarbons (PAHs) in food and the environment needed for new food legislation in the European Union. *Trends in Analytical Chemistry*, 2006, 25: 716-725.
- Zongyan C, Na G, Yanzhong C, Jinjie Z, Yongming L, Le Z. Investigation and assessment of polycyclic aromatic hydrocarbons contamination in Chinese herbal medicines. *Environmental Chemistry*, 2014, 33(5): 844-849.
- Zelinkova Z, Wenzl T. EU marker polycyclic aromatic hydrocarbons in food supplements: analytical approach and occurrence. *Food Additives & Contaminants: Part A*, 2015, 32 (11):1914-1926.