

THEORETICAL APPROACH FOR ESTIMATION OF POTENTIAL EXPOSURE TO POLYCYCLIC AROMATIC HYDROCARBONS (PAHs) BY INTAKE OF FOOD SUPPLEMENTS WITH HERBAL INGREDIENTS

Angelika Georgieva¹, Stanislava Georgieva¹, Zlatina Peteva¹, Djeni Cherneva²,
Temenuža Trifonova³, Dilyana Dimova¹

¹Department of Chemistry, Faculty of Pharmacy, Medical University of Varna

²Department of Biology, Faculty of Pharmacy, Medical University of Varna

³Department Pharmaceutical Technologies, Faculty of Pharmacy,
Medical University of Varna

ABSTRACT

INTRODUCTION: Medicinal plants may be contaminated with polycyclic aromatic hydrocarbons (PAHs), particularly if they grow near industrial areas and urban regions. Benz[a]anthracene, chrysene, benzo[b]fluoranthene and benzo[a]pyrene are the four priority pollutants pointed out by European Food Safety Authority.

AIM: The aim of the study was to assess the potential exposure to PAHs for five medicinal plants mostly used as ingredients in food supplements.

MATERIALS AND METHODS: The potential exposure to PAHs was assessed as theoretical evaluation of daily intake (DI), hazard quotient (HQ), and margin of exposure (MOE) for carcinogenic and genotoxic chemicals. The PAH concentrations were determined in the plant species: *Matricaria chamomilla* L., *Thymus serpyllum* L., *Tilia tomentosa* Moench, *Sambucus nigra* L. and *Achillea millefolium* L. The levels of 13 PAHs in the samples were measured by gas chromatography using mass spectrometry detection. For evaluation of potential cancer risk, MOE was calculated as a ratio of the benchmark dose lower confidence limit (BMDL₁₀) and the DI for the sum of the 4 PAHs.

RESULTS AND DISCUSSION: The highest sum of the 4 PAHs was found in *Tilia tomentosa* Moench (4.13 µg/kg dry weight) and was below permissible limit of 50 µg/kg set by EU legislation. The most toxic compound benzo[a]pyrene was not found in plant species investigated. The highest value of HQ was evaluated for fluoranthene 0.0000869 (for food supplement with *Tilia tomentosa* Moench) and was much lower than 1, suggesting that intake of supplements would not pose any non-cancer risk. The MOE values were in the range from 3 129 520 to 29 824 561 (for food supplements with *Achillea millefolium* L. and *Matricaria chamomilla* L., respectively).

CONCLUSION: The theoretical safety assessment of medicinal plants showed that the intake of supplements with herbs would not pose any health risk.

Keywords: polycyclic aromatic hydrocarbons (PAHs), food supplements, risk assessment, margin of exposure (MOE)

Address for correspondence:

Angelika Georgieva
Faculty of Pharmacy
Medical University of Varna
84 Tzar Osvoboditel Blvd
9002 Varna
e-mail: angelika.georgieva@mu-varna.bg

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INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are a group of organic compounds that are composed of two or more fused aromatic rings (1). Polycyclic aromatic hydrocarbons are formed by the natural incomplete combustion processes of organic matter or from automobile emissions. Processing of food, such as smoking, drying and cooking, is a major source of contamination by PAHs (2).

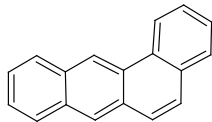
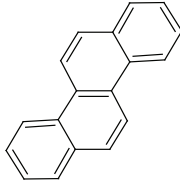
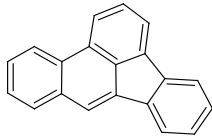
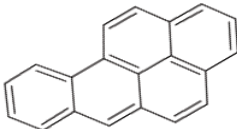
Polycyclic aromatic hydrocarbons are included in priority pollution list of the US Environmental Protection Agency (US EPA) (3), because they represent the largest group of compounds that have mutagenic, carcinogenic and teratogenic properties (4).

The European Food Safety Authority (EFSA) has identified sixteen PAHs as priority substances due to their potential genotoxicity or carcinogenicity to humans. Benzo[a]pyrene and the sum of benzo[a]pyrene, benz[a]anthracene, benzo[b]fluoranthene, and chrysene (4 PAHs) were determined as markers for the presence and the effect of carcinogenic polycyclic aromatic hydrocarbons in food (5,6). The In-

ternational Agency for Research on Cancer classified the 4 PAHs as carcinogenic or possible carcinogenic for humans (Table 1) (7). E.D. van Asselt et al. (8) classified PAHs as probable severe hazards and pointed a lack of scientific data on the presence of these compounds in spices and herbs.

Major routes of human exposure to PAHs are from food and inhaled air. Food can be contaminated by environmental PAHs and by food processing methods (e.g., heating, drying and smoking processes) (5). Medicinal plants and herbal extracts are used in the manufacture of food supplements, pharmaceuticals, and cosmetics. They are also used for preparing teas and water/alcohol extracts. Many plant species, including thyme, mint, basil, etc., are used as spices in cooking because of their taste and antibacterial properties (9). Herbs are rich in beneficial bioactive substances, but they may be contaminated with toxic compounds from the environment, such as polycyclic aromatic hydrocarbons, throughout their growth, gathering, storage or drying (1,10).

Table 1. IARC classification of 4 PAHs

PAHs	IARC Classification*	Structural Formula
Benz[a]anthracene	group 2B	
Chrysene	group 2B	
Benzo[b]fluoranthene	group 2B	
Benzo[a]pyrene	group 1	

*IARC—International Agency for Research on Cancer; group 1—carcinogenic to humans; group 2B—possibly carcinogenic to humans

In 2005, the Food Standard Agency conducted a survey on PAHs in herbal supplements and herbal teas and high levels of PAHs were found (11). Current occurrence data on PAH in dry herbs, medicinal plants, and herbal food supplements are limited (12). The EU Commission set maximum levels for benzo[a]pyrene and for the sum of the 4 PAHs in dried herbs and food supplements containing botanicals and their preparations (12).

In Bulgaria, there is a national tradition of harvesting and using herbs for medicinal purposes and as beverages. The most commonly used medicinal plants in Bulgaria are *Matricaria chamomilla* L., *Thymus serpyllum* L., *Tilia tomentosa* Moench, *Sambucus nigra* L., and *Achillea millefolium* L. (13,14,15). It is important for human health to assess dietary exposure to PAHs by using herbs as teas and intake of food supplements with herbal ingredients.

AIM

The aim of the study was to assess the potential exposure to PAHs for five medicinal plants mostly used as ingredients in food supplements.

MATERIALS AND METHODS

The levels of PAHs were determined in five plant species: *Matricaria chamomilla* L. (chamomile), *Thymus serpyllum* L. (thyme), *Tilia tomentosa* Moench (linden), *Sambucus nigra* L. (elder), and *Achillea millefolium* L. (white yarrow). The results were obtained from our previous research (16).

The concentrations of 13 compounds: acenaphthylene (ACL), anthracene (AN), benz[a]anthracene (BaA), benzo[b]fluoranthene (BbFA), benzo[k]fluoranthene (BkFA), benzo[ghi]perylene (BghiP), benzo[a]pyrene (BaP), chrysene (CHR), dibenz[a,h]anthracene (DbahA), fluorene (FL), indeno[1,2,3-cd]pyrene (IP), phenanthrene (PHE), and pyrene (PY), were analyzed by gas chromatography with mass spectrometry (GC FOCUS Polaris Q, Thermo Electron Corporation).

The results were summarized and used to assess the potential exposure to PAHs by intake of food supplements with herbal ingredients. Theoretical approach was used for assessing human exposure by calculation of daily intake (DI), hazard quo-

tient (HQ) and margin of exposure (MOE) for carcinogenic and genotoxic chemicals.

Assessment of Exposure to PAHs

The long-term dietary exposure assessment is based on the levels of PAHs and consumption of the corresponding food categories. Polycyclic aromatic hydrocarbons have the potential for chronic effects, therefore all the calculations below are based on the mean concentrations to represent the central tendency.

The potential exposure was evaluated as DI for individual PAHs (fluorene, anthracene, and pyrene) and for the sum of the 4 PAHs for assessment of non-cancer risk of PAHs.

The daily intake (DI, ng/kg bw per day) was calculated by the equation (17):

$$DI = \frac{C \cdot Intake}{BW}$$

where:

C—concentration of PAHs in medicinal plant (ng/g).

Intake—equivalent amount of medicine plant in daily dose food supplement, recommended by the manufacturer (g/day) in Supplementary material №1.

BW—average body weight (kg bw), 70 kg for an adult person.

The information in the package leaflets of nine different food supplements with medicinal plants was investigated. The intake was calculated, using the equivalent content of medicinal plant in every tablet, capsule or tincture from the food supplement and the recommended daily dose (presented by the manufacturer) in Supplementary material №1.

The contamination risk of individual PAHs can be expressed as HQ, defined as the ratio of an estimate exposure (DI) and the reference dose (RfD) (17, 18):

$$HQ = \frac{DI}{RfD}$$

where:

DI—daily intake for the individual PAHs (ng/kg bw per day)

RfD—reference dose for chronic oral exposure (ng/kg bw per day).

A reference dose is defined as an estimate of a daily exposure to the human population that is likely to be without an appreciable risk of deleterious effects during a lifetime (19). The US Environmental Protection Agency has determined the reference doses of chronic oral exposure (RfDs) based on the non-carcinogenic potential of anthracene, fluorene, and pyrene (20, 21, 22). The hazard quotient suggests that there is a certain level of exposure (RfD) below which it is unlikely to have adverse health effects even in sensitive populations (17).

An appropriate approach for assessment of genotoxic and carcinogenic substances is evaluation of the MOE, recommended by EFSA (23). The Scientific Committee of EFSA has recommended the MOE values as the ratio of the benchmark dose lower limit (BMDL₁₀) to the estimated daily intake of the sum of the 4 PAHs (23):

$$MOE_{4PAHs} = \frac{BMDL_{10}}{DI_{4PAHs}}$$

where:

MOE_{4PAHs} is a margin of exposure for the sum of the 4 PAHs

BMDL₁₀—benchmark dose with less than 10% confidence limit (340 000 ng/kg bw per day),

DI_{4PAHs} is the sum of the daily intake for the 4 PAHs (ng/kg bw per day).

The margin of exposure was calculated for the sum of the four marker PAHs (4 PAHs) by using benchmark dose lower confidence limit 10% (BMDL₁₀). The BMDL₁₀ is the lower bound of a 95% confidence interval on the benchmark dose. BMDL₁₀ corresponding to a 10% tumor incidence was chosen as a reference point in the dose-response curve (23). The value of BMDL₁₀ has been determined from the analysis of the ratio dose-answer for the differ-

Table 2. PAH levels (ng/g dw) in medicinal plants, mean values

PAH	<i>Matricaria chamomilla</i> L. (n = 5)	<i>Thymus serpyllum</i> L. (n = 4)	<i>Sambucus nigra</i> L. (n = 6)	<i>Achillea millefolium</i> L. (n = 5)	<i>Tilia tomentosa</i> Moench (n = 6)
	Chamomile	Thyme	Elder	White yarrow	Linden
ACL	< LOD	< LOD	< LOD	< LOD	< LOD
FL	83.18 ± 9.15	198.99 ± 21.89	168.52 ± 18.54	141.21 ± 15.53	233.84 ± 25.72
PHE	209.67 ± 23.06	601.77 ± 66.19	480.16 ± 52.82	472.55 ± 51.98	619.81 ± 68.18
AN	2.10 ± 0.23	37.02 ± 4.07	43.00 ± 4.73	42.15 ± 4.64	32.38 ± 3.56
PY	3.60 ± 0.40	10.30 ± 1.13	9.91 ± 1.09	18.83 ± 2.07	6.98 ± 0.77
BaA*	< LOD	< LOD	< LOD	< LOD	0.26 ± 0.03
CHR*	1.33 ± 0.15	2.39 ± 0.26	2.86 ± 0.31	3.90 ± 0.43	3.87 ± 0.43
BbFA*	< LOD	< LOD	< LOD	< LOD	< LOD
BkFA	< LOD	< LOD	< LOD	< LOD	< LOD
BaP*	< LOD	< LOD	< LOD	< LOD	< LOD
IP	< LOD	< LOD	< LOD	< LOD	< LOD
DBahA	< LOD	< LOD	< LOD	< LOD	< LOD
BghiP	0.30 ± 0.03	< LOD	< LOD	< LOD	0.65 ± 0.07
Sum 13PAHs	300.18	850.45	704.42	678.65	897.80

Legend: LOD—limit of detection; * - group of the 4PAHs; PAHs: polycyclic aromatic carbons; ACL: acenaphthylene, AN: anthracene, BaA: benz[a]anthracene, BbFA: benzo[b]fluoranthene, BkFA: benzo[k]fluoranthene, BghiP: benzo[ghi]perylene, BaP: benzo[a]pyrene, CHR: chrysene, DbahA: dibenz[a,h]anthracene, FL: fluorene, IP: indeno[1,2,3-cd]pyrene, PHE: phenanthrene, and PY: pyrene

ent types of tumors, caused by the equivalent impact of the pollutants. The EFSA Panel on Contaminants in the Food Chain accepted BMDL₁₀ 340 000 ng/kg bw per day for the sum of the 4 PAHs as a marker for the carcinogenic PAHs in food (4). The MOE has been used for providing a risk priority to risk managers (24). At MOE values close to or less than 10 000 health concerns associated with dietary exposure to PAHs can occur (EFSA, 2005).

RESULTS AND DISCUSSION

The levels of PAHs in medicinal plants were summarized and the results are present in Table 2.

The distribution pattern of individual PAHs showed similar profiles in all herb species investigated and was as follows: phenanthrene > fluorene > anthracene > pyrene > chrysene. Acenaphthylene, benzo[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[1,2,3-cd]pyrene, and dibenz[a,h]anthracene showed levels below the detection limit (LOD) of the method in all samples.

In our research, the highest values for phenanthrene and fluorene were detected in *Tilia tomentosa* Moench. The highest level of anthracene was found in *Sambucus nigra* L. and *Achillea millefolium* L. The concentration of pyrene was determined in the range from 3.60 to 18.83 ng/g dw in *Achillea millefolium* L. Among the group of 4 PAHs, chrysene was determined as the predominant compound and showed the highest values in *Achillea millefolium* L. and *Tilia tomentosa* Moench. Benzo[a]pyrene, classified as carcinogenic to humans by IARC, was measured in all analyzed samples below LOD.

Phenanthrene, fluorene, anthracene, and pyrene are not classified as carcinogenic by the International Agency for Research on Cancer (IARC). Phenanthrene is one of the most widespread PAHs in the environment due to its a stable structure and persistence (25). Phenanthrene, fluorene, and anthracene, as PAHs with low molecular weight, were found in the atmosphere in steam phase, which makes them easier to be distributed than PAHs with high molecular weight (26). Holland et al. reported results for 28 PAHs in 49 samples of food supplements with herbal ingredients and PAHs with lower molecular weight have been found in more than half of the food supplements (11).

The least contaminated samples were *Matricaria chamomilla* L., while *Thymus serpyllum* L. and *Tilia tomentosa* Moench were more polluted by PAHs. The highest sum of the 4 PAHs (4.13 ng/g) was determined in linden (*Tilia tomentosa* Moench) and was beneath the permissible limit of 50 ng/g in dry herbs set in EU (12). Regarding total contamination by average of sum of 13 PAHs, the dried herbs can be arranged in the following order: chamomile < white yarrow < elder < thyme < linden.

These findings may result from the environmental pollution where linden trees grow (along roads), so potential source of pollution is car traffic. Similar results were obtained in recent studies by other authors (27,28).

The main factors that can affect the adsorption of PAHs in medicinal plants are the big surface of the leafs and blossoms (28). Consequently, plants with a high surface area of leaves consequently can easily reach high level of PAH accumulation from air (29). Some authors pointed out that the epicuticular waxes of linden contain triterpenoids, which can affect the retention of the dust particles containing PAHs (30).

The results showed that the contribution of low molecular weight PAHs to total pollution is over 95% due to their widespread distribution in the environment. The presented PAH profile with dominant less-toxic PAHs (PHE, FL, AN, and PY) was in accordance with results from other studies (27,28). It is important to evaluate exposure to low molecular weight PAHs because of their priority transfer in water herb infusion and alcohol extracts, mostly used in food supplements.

Assessment of Non-Cancer Risk

Various non-carcinogenic effects, such as liver toxicity, hematological disorders, reproductive changes, have been reported for individual PAHs (acenaphthene, anthracene, fluorene, naphthalene, pyrene) in experimental studies with animals (31). The evaluation of the available data showed they are not genotoxic and carcinogenic compounds. The threshold approach for risk assessment would be appropriate for estimating exposure to these individual PAHs. The US EPA set RfDs for chronic oral exposure for anthracene, fluorene, and pyrene (21,22).

Phenanthrene (PHE) was found as a predominant compound in all plant species, but for this com-

pound, due to insufficient data for its toxicity, no RfD is set by US-EPA.

The potential exposure to individual PAHs was calculated using the recommended by manufacturers daily dose for food supplements and equivalent content of herbs in every supplement. Assessment of non-cancer health risks from exposure to PAHs was performed by calculation of daily intake of individual non-carcinogenic PAHs and HQ values, results are present in (Table 3). The highest DI values were estimated for FL by intake of food supplements—linden capsules and white yarrow tablets. The DI for AN is the highest through white yarrow tablets. The highest value of HQ was evaluated for fluorene (0.0000869 for food supplement with *Tilia tomentosa* Moench) but it is much lower than one, suggesting that potential exposure through supplements would not pose any non-cancer risk.

The MOE Approach to Carcinogenic and Genotoxic PAHs

The IARC has determined benzo[a]anthracene, chrysene, benzo[b]fluoranthene, and benzo[a]pyrene as carcinogenic and potentially carcinogenic to humans—Table 1.

The potential exposure to carcinogenic and genotoxic chemicals was assessed by MOE for the sum of the 4 PAHs, according to recommendation of EFSA (2005). Several authors have used the MOE approach based on BMDL₁₀ to assess a risk of PAHs to the human health (24,28,32).

The assessment of human cancer risk was estimated by DI_{4PAHs} of food supplements and an exposure limit (MOE_{4PAHs}) based on the sum of the 4 PAHs. The results are present in Fig. 1 and Table 4.

Table 3. Daily intake (DI, ng/kg bw per day) and HQ of individual PAH compounds in food supplements with herbal ingredients

Food supplements	Intake*, g/day	DI, ng/kg bw per day			
		FL	PHE	AN	PY
Reference Dose (RfD), (21,22), ng/kg bw per day		40 000	-	300 000	30 000
Chamomile tincture	0.600	0.713	1.797	0.018	0.031
Chamomile capsules	1.000	1.188	2.995	0.030	0.051
Average		0.951	2.396	0.024	0.041
HQ		0.0000238	-	0.0000001	0.0000014
Thyme tincture	0.750	2.132	6.447	0.397	0.110
HQ	-	0.0000533	-	0.0000013	0.0000037
Elder, fruit and flower, capsules	0.460	1.107	3.155	0.283	0.065
HQ		0.0000277	-	0.0000009	0.0000022
White yarrow tablets	1.950	3.934	13.164	1.174	0.525
White yarrow tincture	0.750	1.513	5.063	0.452	0.202
Average		2.723	9.114	0.813	0.363
HQ		0.0000681	-	0.0000027	0.0000121
Linden capsules	0.700	2.338	6.198	0.324	0.070
Linden capsules	1.380	4.610	12.219	0.638	0.138
Average		3.474	9.209	0.481	0.104
HQ		0.0000869	-	0.0000016	0.0000035

*- equivalent amount of medicine plant in daily dose food supplement, recommended by the manufacturer

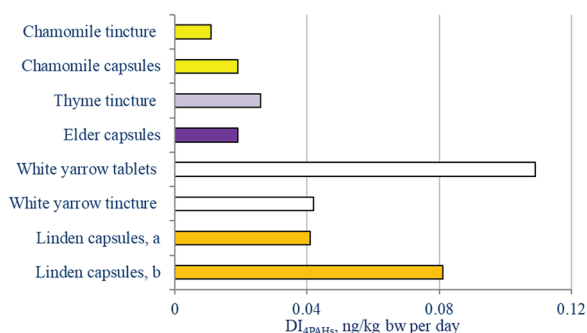


Fig. 1. Estimated daily intake for sum of the 4 PAHs in food supplements with herbal ingredients

ments based on data on PAHs in the herbs used without performing costly chemical analyses.

CONCLUSION

A theoretical approach was used for estimation of potential exposure to PAHs by taking herbal supplements. Estimated PAH uptake pointed to low health risks associated with medicinal plants, used as ingredients in food supplements. The MOE approach indicates a very low concern for human health by intake of food supplements at the mean estimated dietary exposures. This approach can also be applied to assess the safety of other foodstuffs containing herbs and spices.

Table 4. Margin of exposure (MOE_{4PAHs}) by the DI for the sum of the 4 PAHs in food supplements with herbal ingredients

Plant Species	Food Supplements	Sum of 4 PAHs, ng/g dw	DI _{4PAHs} , ng/kg bw per day	MOE _{4PAHs}
		Maximum level - 50 ng/g dw (12)	-	> 10 000 (23)
<i>Matricaria chamomilla</i> L.	Chamomile	1.33	0.011 ÷ 0.019	17 894 737 ÷ 29 824 561
<i>Thymus serpyllum</i> L.	Thyme	2.39	0.026	13 277 545
<i>Sambucus nigra</i> L.	Elder	2.86	0.019	18 090 605
<i>Achillea millefolium</i> L.	White yarrow	3.90	0.042 ÷ 0.109	3 129 520 ÷ 8 136 752
<i>Tilia tomentosa</i> Moench	Linden	4.13	0.041 ÷ 0.081	4 180 940 ÷ 8 242 424

The DI_{4PAHs} were calculated in the range from 0.011 to 0.109 ng/kg bw per day (for camomile and yarrow supplements, respectively) (Fig. 1).

According to EFSA, health concerns associated with dietary exposure to PAHs can occur at MOE values close to or less than 10 000 (23). The lowest MOE_{4PAHs} values were calculated for food supplements with white yarrow and linden. However, these values were far above the EFSA limit. These results were considered of low concern for public health.

The MOEs establish in our study were higher than MOE evaluated for Polish tea, fruit and herbal teas, reported by Ciemniak et al. and Roszko et al. (28,33), and comparable to those reported by other authors for herbal teas (34,35).

Determination of PAHs in food supplements is difficult due to the high content of interfering substances, such as alkaloids, sugars, organic acids, and pigments. The theoretical MOE approach provides an opportunity to assess the safety of food supple-

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Supplementary material 1. Equivalent amount of medicine plant in daily dose food supplement

Food Supplements with Herbal Ingredients, Package	Equivalent Herb Content	Daily dose, Recommended by the Manufacturer	Intake Equivalent Amount of Medicine Plant in Daily Dose Food Supplement
Chamomile tincture, 50 mL	1 mL = 0.100 g chamomile	4 x 30 drops (1.5 mL) = 6 mL	0.600 g chamomile
Chamomile capsules, 100 capsules x 250 mg	1 capsule = 0.250 g chamomile	2 x 2 capsules	1.000 g chamomile
Thyme tincture, 50 mL	1 mL = 0.250 g thyme	3 x 25 drops (1 mL) = 3 mL	0.750 g thyme
Elder capsules, 100 capsules x 575 mg	Contents in 2 capsules: <i>Sambucus nigra</i> (flowers powder)—0.230 g	2 x 2 capsules	0.460 g elder (flowers)
Elder capsules, 60 capsules x 1150 mg	Contents in 1 capsule: <i>Sambucus nigra</i> (flowers powder)—0.230 g	2 x 1 capsules	0.460 g elder (flowers)
White yarrow tablets	1 tablet = 0.325 g white yarrow	6 tablets	1.950 g white yarrow
White yarrow tincture, 50 mL	1 mL = 0.250 g white yarrow	3 x 25 drops (1 mL) = 3 mL	0.750 g white yarrow
Linden capsules, 100 capsules x 350 mg	1 capsule = 0.350 g linden (flowers)	2 x 1 capsule	0.700 g linden
Linden capsules, 120 capsules	1 capsule = 0.230 g linden	6 capsules	1.380 g linden