EU marker polycyclic aromatic hydrocarbons in food supplements: analytical approach and occurrence

Zuzana Zelinkova and Thomas Wenzl*

European Commission, Joint Research Centre, Institute for Reference Materials and Measurements, Standards for Food Bioscience Unit, Geel, Belgium

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Several food supplements comprising botanical, oil and bee products collected from retail markets in different countries were tested for the occurrence of 4 EU marker Polycyclic aromatic hydrocarbons (PAHs; benzo[a]anthracene, chrysene, benzo[b]fluoranthene and benzo[a]pyrene). A robust GC/MS-based stable-isotope dilution method was used taking into account the differences in the type of matrices. The accuracy of the results was assessed by implementing several quality control tools. Sixty-eight samples of 94 analysed products exceeded the level of 0.5 μg/kg for the sum of the four EU marker PAHs (ΣPAH4). Benzo[a]pyrene exceeded the limit of quantification in 49 samples. The PAH with the highest abundance in all products was chrysene. On average, propolis extracts and other bee products showed relatively high levels of ΣPAH4 (mean 188.2 μg/kg), whereas the contamination levels of fish oil supplements were very low or mostly undetectable. Considerably high ΣPAH4 amounts found in some samples could remarkably increase the daily exposure of consumers to PAHs, demonstrating the need for continuous monitoring of ΣPAH4 in food supplements.

Keywords: polycyclic aromatic hydrocarbons; PAHs; food supplements; GC/MS; fish oil; propolis

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a large group of chemical contaminants, generally occurring in complex mixtures consisting of hundreds of compounds. They are produced by natural and anthropogenic processes, mainly by incomplete combustion of organic matter. Humans are exposed to PAHs through different pathways, where the major route for non-smokers is consumption of food (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).

In 2002 the European Commission’s Scientific Committee on Food (SCF) identified 15 individual PAHs as being of major concern for human health (SCF 2002). The toxicological importance of these compounds was confirmed by the International Agency for Research on Cancer (IARC 2014). These 15 EU priority PAHs were monitored in food to enable exposure assessments and to verify the validity of the use of benzo[a]pyrene as a marker for a “total-PAH content”. The European Commission set maximum levels for benzo[a]pyrene for the first time in 2005 by Regulation (EC) No. 208/2005, amending Regulation (EC) No. 466/2001 (EC 2005).

Later, the maximum levels for benzo[a]pyrene were laid down in Regulation (EC) No. 1881/2006, setting maximum levels for certain contaminants in foodstuffs (EC 2006). In 2008 the European Food Safety Authority (EFSA) reviewed the previous risk assessment and evaluated the suitability of benzo[a]pyrene as a marker for the occurrence and toxicity of PAHs in food. The EFSA concluded that benzo[a]pyrene was not a suitable indicator and selected the sum of four (ΣPAH4) or eight PAHs (ΣPAH8) as the most suitable indicators. However, PAH8 does not provide much added value compared to ΣPAH4 (EFSA 2008). Following these conclusions, the Standing Committee on the Food Chain and Animal Health agreed to base risk management measures on ΣPAH4 – benzo[a]anthracene, benzo[a]pyrene, benzo[b]fluoranthene and chrysene. Maximum levels for benzo[a]pyrene were maintained to ensure comparability with historical data. Currently, maximum levels in food are specified in Commission Regulation (EU) No. 835/2011 of 19 August 2011 for benzo[a]pyrene and ΣPAH4 (EU 2011a). Coherently, also Commission Regulation (EC) No. 333/2007, which lays down minimum method performance criteria, was revised by Commission Regulation (EU) No. 836/2011 (EU 2011b). Maximum levels are set for foodstuffs containing fats and oils and foods where smoking (smoked meat, smoked fish and fishery products) and
drying processes (cocoa beans and derived products) or environmental pollution (bivalve molluscs) might cause high levels of contamination. The lowest maximum levels are set for food for infants and young children (EU 2011a).

Commission Regulation (EU) No. 835/2011 states that high levels of PAHs have been found in some food supplements often containing botanical ingredients such as ginkgo, ginseng, green tea, spirulina, or bee products such as propolis. Nevertheless, the levels are variable and depend on the specific products and method of production. Further data on food supplements are needed and should be collected (EU 2011a).

There are two classes of food supplements – vitamins and minerals – defined by Commission Regulation (EC) No. 1170/2009 (EC 2009). However, the European Commission recognised that about half of the commercial turnover is generated with food supplements containing other substances than vitamins and minerals. Among them are different amino acids, enzymes, pre- and probiotics, essential fatty acids, botanicals and other substances (COM 2008).

Generally, PAH might be expected in food supplements that underwent improper processing (thermal treatment, improper drying process), or in which PAHs accumulated from the environment (e.g. propolis extracts). Analytical challenges were highlighted by the industry for herbal food supplements. Industry representatives pointed out the difficulties with measurement of PAHs in food supplements and stated that the accuracy of the data on the PAH contents of herbal food supplements could be low, due to the application of improper analytical methods.

The European Union Reference Laboratory for PAHs (EURL PAHs), which is operated by the European Commission’s Joint Research Centre, committed to perform a survey of the four EU marker PAHs in a broad set of food supplements, with a particular focus given to the accuracy of the analyses in order to demonstrate the reliability of the data provided. The data produced shall strengthen the occurrence database which is used for risk assessment.

**Materials and methods**

**Food supplements samples**

A broad set of herbal and botanical food supplements, fish and edible oil food supplements and propolis and bee products was acquired in 2013 in retail markets of Belgium, the Netherlands, Austria, Czech Republic, Poland, Ireland and France. The total number of collected samples was 94; 69 samples were of the botanical origin and sold as a powder in gelatin capsules or solid pills, 15 samples of fish and edible oils sold in a liquid form or liquid in gelatin capsules and 10 samples of propolis and bee products in a liquid or semi-liquid form (Table 1). Additionally, 11 food supplement products were purchased from India, comprising 8 products of multi-component botanical origin and three products containing

| Sample code | Name                        | Country of purchase | Comments                                      | Type of pills | Group* |
|-------------|-----------------------------|---------------------|-----------------------------------------------|---------------|--------|
| 1           | Aloe vera                   | NL                  | Concentrated extract                          | Solid pills   | 1      |
| 2           | Green tea extract           | NL                  | Standardised extract                          | Solid pills   | 1      |
| 3           | Siberian ginseng            | NL                  | Extract                                       | Solid pills   | 1      |
| 4           | Ginger                      | NL                  | Standardised extract                          | Powder in gelatine | 1    |
| 5           | Green coffee                | NL                  | Green coffee extract (Robusta)                | Powder in gelatine | 1    |
| 6           | Cat’s claw                  | NL                  | Cat’s claw powder                            | Powder in gelatine | 1    |
| 7           | Super green tea             | NL                  | Green tea extract, caffeine                   | Solid pills   | 1      |
| 8           | Valerian                    | NL                  | Root extract                                  | Powder in gelatine | 1    |
| 9           | St. John’s wort             | NL                  | Standardised extract                          | Powder in gelatine | 1    |
| 10          | Kelp                        | NL                  | Standardised extract                          | Solid pills   | 1      |
| 11          | Cha-de-burge                | NL                  | Brazilian tree (Cordia salicifolia), foliage extract | Powder in gelatine | 1    |
| 12          | Pu-erh tea                  | NL                  | Dark Chinese tea extract                      | Powder in gelatine | 1    |
| 13          | Cod liver oil               | NL                  | –                                              | Oil in gelatine | 2      |
| 14          | Green tiger                 | NL                  | Green tea extract, choline, taurine           | Powder        | 1      |
| 15          | Fenugreek powder            | NL                  | Tigonella foenum-graecum                      | Powder        | 1      |
| 16          | Silybum marianum            | NL                  | Milk thistle extract                          | Powder in gelatine | 1    |
| 17          | Ginkgo biloba               | AT                  | Dried extract of ginkgo foliage              | Solid pills   | 1      |
| 18          | Ginseng                     | AT                  | Root extract                                  | Solid pills   | 1      |
| 19          | Pumpkin                     | AT                  | Seed extract                                  | Solid pills   | 1      |
| 20          | Green tea                   | AT                  | Extract                                       | Powder in gelatine | 1    |
| 21          | Propolis                    | AT                  | Propolis from the beehive                    | Liquid        | 3      |

(continued)
Table 1. Continued.

| Sample code | Name                                | Country of purchase | Comments                                      | Type of pills | Group* |
|-------------|-------------------------------------|---------------------|-----------------------------------------------|---------------|--------|
| 22          | Honey bee booster                   | AT                  | Propolis extract, royal jelly, pollen         | Liquid        | 3      |
| 23          | Valerian                            | AT                  | Root extract                                  | Powder in gelatine | 1      |
| 24          | Yam-root                            | AT                  | Root extract from Mexican Yam                 | Powder in gelatine | 1      |
| 25          | Day & Night                         | AT                  | Selected plant extracts for calorie controlled diet | Solid pills   | 1      |
| 26          | Salmon oil                          | AT                  | Omega 3 concentrate                           | Oil in gelatine | 2      |
| 27          | Omega-3 – see Fish oil              | AT                  | See fish oil 68.7%                            | Oil in gelatine | 2      |
| 28          | Dandelion juice                     | AT                  | Extract from dandelion leaves                 | Liquid        | 1      |
| 29          | Maca powder                         | CZ                  | Root extract from Peruvian Maca               | Powder        | 1      |
| 30          | Maqui berry Organic                 | CZ                  | Aristotelia chilensis – dried berry          | Powder        | 1      |
| 31          | Ginseng                             | CZ                  | Panax ginseng – dried root                    | Dried slice of root | 1      |
| 32          | Pangamin                            | CZ                  | Dried Saccharomyces cerevisiae and malt extract | solid pills   | 1      |
| 33          | Omega 3 & Omega 6                   | CZ                  | Fish oil, evening primrose oil                | Liquid        | 2      |
| 34          | Omega 3                             | CZ                  | Fish oil, orange aroma                        | Oil in gelatine | 2      |
| 35          | Oyster mushroom                     | CZ                  | Pleurotus ostreatus extract                   | Solid pills   | 1      |
| 36          | Apricarc                            | CZ                  | Powder and oil from apricot kernel            | Powder in gelatine | 1      |
| 37          | Ginkgo biloba                       | CZ                  | Ginkgo extract, soybean oil                   | Semi-solid in gelatine | 1      |
| 38          | Cranberries                         | CZ                  | Extract from berries                          | Powder in gelatine | 1      |
| 39          | St. John’s wort                     | CZ                  | Powder from haum                              | Solid pills   | 1      |
| 40          | Bovine colostrum                    | CZ                  | Kolostrum forte 500 mg                        | Powder in gelatine | 1      |
| 41          | Skeleton                            | CZ                  | Cuttle fish bone powder, fish oil             | Semi-solid in gelatine | 1      |
| 42          | Green tea                           | CZ                  | Green tea extract                              | Powder in gelatine | 1      |
| 43          | Chlorella organic                   | CZ                  | Chlorella vulgaris – freshwater algae         | Solid pills   | 1      |
| 44          | Spirulina organic                   | CZ                  | Spirulina – freshwater algae                  | Solid pills   | 1      |
| 45          | Evening primrose & borage           | CZ                  | Evening primrose oil + borage oil             | Oil in gelatine | 2      |
| 46          | Sea buckthorn oil                   | CZ                  | Hippophae rhamnoides oil (100% oil)           | Liquid        | 2      |
| 47          | Evening primrose oil                | CZ                  | 100% oil                                      | liquid        | 2      |
| 48          | Linseed oil                         | CZ                  | 100% oil                                      | Liquid        | 2      |
| 49          | Pangamin                            | CZ                  | Saccharomyces cerevisiae + algea 🌊           | Liquid        | 2      |
| 50          | Garlic super                         | CZ                  | Garlic oil, sunflower oil                     | Oil in gelatine | 2      |
| 51          | Vegemax                             | CZ                  | Freeze-dried vegetable                        | Solid pills   | 1      |
| 52          | Silybum marianum                    | CZ                  | 100% granule                                  | Powder        | 1      |
| 53          | Young barley BIO                    | CZ                  | Powder from dried sprout of barley            | Powder        | 1      |
| 54          | Garlic                              | CZ                  | Garlic extract, soybean oil                   | Oil in gelatine | 2      |
| 55          | Yucca                               | CZ                  | Extract                                       | Solid pills   | 1      |
| 56          | Acai berry                          | CZ                  | Acai berry + green tea extract + Garncia cambogia | Powder in gelatine | 1      |
| 57          | Garcinia                            | CZ                  | Garcinia cambogia                             | Powder in gelatine | 1      |
| 58          | Ganoderma lucidum                   | CZ                  | Spore powder of fungi                         | Powder in gelatine | 1      |
| 59          | Birch                               | CZ                  | Birch leaves powder                           | Solid pills   | 1      |
| 60          | Cat’s claw                          | CZ                  | Uncaria tomentosa dried inner bark            | Solid pills   | 1      |
| 61          | Propolis intense                    | FR                  | Tincture, propolis extract                    | Liquid        | 3      |
| 62          | Fish oil                            | CZ                  | Mixture of 3 species – salmon, mackerel, sardines | Oil in gelatine | 2      |
| 63          | Echinacea forte                     | BE                  | Echinacea extract                             | Powder in gelatine | 1      |
| 64          | St. John’s wort                     | BE                  | Standardised extract                          | Powder in gelatine | 1      |
| 65          | Ginkgo complex                      | BE                  | Ginkgo extract, rice powder                   | Powder in gelatine | 1      |
| 66          | Garlic                              | BE                  | Garlic extract, mistletoe extract             | Powder in gelatine | 1      |
| 67          | Omega 3                             | BE                  | Fish oil, rosemary extract                    | Oil in gelatine | 2      |
| 68          | Maca forte                          | BE                  | Maca extract, rice powder                     | Solid pills   | 1      |
| 69          | Cranberry                           | BE                  | Extract                                       | Solid pills   | 1      |
| 70          | Ginseng forte + maca                | BE                  | Extract of ginseng root and maca              | Solid pills   | 1      |
| 71          | Cat’s claw                          | BE                  | Cat’s claw, Echinacea, quarana powder        | Solid pills   | 1      |
| 72          | Shark’s oil                         | PL                  | Oil from shark’s liver + garlic               | Oil in gelatine | 2      |
| 73          | Propolis mixture                    | IE                  | Honey powder, propolis extract                | Liquid        | 3      |
| 74          | Spirulina                           | IE                  | 100% pure powder                              | Powder        | 1      |

(continued)
edible oils (Table 1). All samples were stored in their original container at room temperature prior to analysis.

Reagents and standards

All chemicals were of at least analytical grade, purchased from either Sigma-Aldrich (St. Louis, MO, USA) or VWR (Radnor, PA, USA). Sodium polyacrylate cross-linked and sand 50–70 mesh particle size were obtained from Sigma-Aldrich (St. Louis, MO, USA). Celite® 545 particle size 0.02–0.1 mm was from Merck (Darmstadt, Germany). Syringe filters Acrodisc® with 1 μm PTFE membrane were purchased from Pall Life Sciences (Ann Arbor, MI, USA) and SPE cartridges Extract Clean™ Silica 500 mg/

Table 1. Continued.

| Sample code | Name                        | Country of purchase | Comments                                      | Type of pills | Group* |
|-------------|-----------------------------|---------------------|-----------------------------------------------|---------------|--------|
| 75          | Bioactive Spirulina IE      | 100% pure powder    | Powder                                        | 1             |
| 76          | Premium Spirulina IE        | 100% pure powder    | Solid pills                                   | 1             |
| 77          | Propolis herbal IE         | Honey, propolis extract, peppermint oil | Liquid | 3         |
| 78          | Propolis liquid IE         | Propolis extract    | Liquid                                        | 3             |
| 79          | Bioactive Spirulina IE      | –                   | powder                                        | 1             |
| 80          | Spirulina IE               | Blue-green algae    | Solid pills                                   | 1             |
| 81          | Children’s propolis herbal IE | Honey, propolis extract | Liquid | 3         |
| 82          | Propolis extract IE        | Purified propolis extract | Liquid | 3         |
| 83          | Evening primrose oil IE    | –                   | Oil in gelatine                               | 2             |
| 84          | Acidophilus IE             | Lactobacillus       | Powder in gelatine                            | 1             |
| 85          | Spirulina IE               | 100% pure powder    | Powder                                        | 1             |
| 86          | Propolis liquid IE         | Propolis extract    | Liquid                                        | 3             |
| 87          | Propolis liquid IE         | Propolis extract    | Liquid                                        | 3             |
| 88          | Premium Spirulina IE       | 100% pure powder    | Powder                                        | 1             |
| 89          | Blood capsules IE          | Vitamins and minerals for better blood formation | Powder in gelatine | 1         |
| 90          | Dietary supplements – B1 IE| Peppermint, fennel, ginger, chamomile, ginseng, aniseed, oregano | Powder in gelatine | 1         |
| 91          | Dietary Supplements – C1 IE| Psyllium husk, bushkorn bark, sweet flag root, dandelion, chamomile, etc. | Powder in gelatine | 1         |
| 92          | Dietary supplements – L1 IE| Psyllium husk, dandelion, ginger root, ginseng, centaury herb | Powder in gelatine | 1         |
| 93          | Dietary supplements – Y1 IE| Burdock powder, myrrh gum, garlic, slippery elm powder, oregano powder, olive leaf | Powder in gelatine | 1         |
| 94          | Spirulina IE               | 100% Hawaiian spirulina | Solid pills | 1         |
| 95          | Primrose oil IN            | Evening primrose oil | Oil in gelatine                               | 2             |
| 96          | Germ & cod liver oil IN    | Wheat germ oil, cod liver oil + vitamins/others | Semi-solid in gelatine | 2         |
| 97          | Multicomponent extract IN  | Ginseng, Ginkgo biloba, citrus bioflavonoids, green tea, garlic powder + vitamins/minerals | Semi-solid in gelatine | 1         |
| 98          | Green tea & garlic IN      | Green tea, garlic powder + vitamins/minerals | semi-solid in gelatine | 1         |
| 99          | Multicomponent extract IN  | Polypodium leucotomos, green tea, French marine pine bark, konjac powder + vitamins/minerals | Semi-solid in gelatine | 1         |
| 100         | Grape & green tea IN       | Grape seed, green tea + vitamins/minerals | Semi-solid in gelatine | 1         |
| 101         | Botanicals extract IN      | Gulancha, Anantamul, shetverenda, Sajina, Rakta Punarnava | Solid pills | 1         |
| 102         | Wheat germ plus IN         | Wheat germ oil, citrus bioflavonoids, green tea + vitamins/minerals | Semi-solid in gelatine | 2         |
| 103         | Grape seed plus IN         | Grape seed extract + vitamins/minerals | Solid pills | 1         |
| 104         | Ginseng IN                 | Ginseng extract powder + vitamins/minerals | Semi-solid in gelatine | 1         |
| 105         | Ginseng IN                 | Ginseng extract + vitamins and Zn | Powder in gelatine | 1         |

Note: *1, botanical/herbal food supplement; 2, fish/edible oil supplement; 3, propolis extract/bee products.
4 ml from Grace Davison Discovery Sciences (Deerfield, IL, USA).

The analytes benz[a]anthracene (BaA) CAS#56-55-3, chrysene (CHR) CAS#218-01-9, benzo[b]fluoranthene (BbF) CAS#205-99-2 and benzo[a]pyrene (BaP) CAS#50-32-8 were commercially available BCR-certified reference materials (IRMM, Geel, Belgium). The isotopically labelled compounds benz[a]anthracene (13C6, 99%), chrysene (13C6, 99%), benzo[b]fluoranthene (13C6, 99%) and benzo[a]pyrene (13C6, 99%) were obtained from LGC Promochem GmbH (Wesel, Germany). 9-Fluorobenzo[k]fluoranthene (FBkF), which was used as an injection standard, was purchased from Chiron AS (Trondheim, Norway). Certified standard reference material® 2260a (aromatic hydrocarbons in toluene) was purchased from NIST (Maryland, USA). Stock solution of the individual PAHs were gravimetrically prepared in toluene.

**Equipment**

A pressurised liquid extractor ASE 300 (Dionex, Sunnyvale, CA, USA) was used for the extraction of the samples. An automated gel permeation chromatography (GPC) system of LC Tech (Delft, Netherlands) equipped with a GPC column (25 mm id, 320 mm length) was filled with 50 g Bio-Beads S-X3 from Bio-Rad Laboratories (Hercules, CA, USA). A FW-20 UV detector (LC Tech) operated at 254 nm and an automated concentrator CPC 2000-II Vacuumbrand (Wertheim, Germany) was used for the first step of the sample purification. Samples were evaporated after ASE on a Turbo Vap® workstation (Zymark, Hopkinton, MA, USA), respectively, after SPE on a Techne Sample Concentrator Dri-Block® DB-3D (Bibby Scientific, Staffordshire, UK).

A gas chromatograph (GC) HP 6890N (Agilent Technologies, Santa Clara, CA, USA) with programmable temperature vapourisation (PTV) injection port (septumless head) equipped with a J&W Select PAH GC column (15 m × 150 μm × 0.10 μm; Agilent Technologies) was used for the analysis of the target PAHs. The GC was coupled to an Agilent 5975B single quadrupole mass spectrometer (Agilent Technologies) operated in electron ionisation (EI) mode at 70 eV. Injection was carried out using an automated GC PAL injection system (CTC Analytics, Zwingen, Switzerland). Acquired data were evaluated by MSD ChemStation E.02.00.493 (Agilent Technologies).

**Sample preparation**

For botanical and propolis food supplements, 5 g of sample was weighed and mixed with 5 g of polyacrylic acid and 15 g of sand. The test portion was spiked with 200 μl of a mix of 13C-labelled analogues of the four target PAHs (125 ng/ml), homogenised and transferred to the 33 ml PLE extraction cell. In case of products in very fine powder form, two filters were used instead of one and a layer of Celite® was placed on the bottom of the cell. The extraction was carried out with n-hexane at 100°C in 2 cycles of 10 min static time with 60% purge volume and 120 s purge time. After the extraction the extract was evaporated in a Turbo Vap® workstation at 40°C using a steam of nitrogen to a small volume of approximately 0.5 ml. The concentrated extract was brought up to 5 ml with a mixture of cyclohexane:ethyl acetate (1:1 v/v), filtered through a PTFE filter and cleaned up by GPC. For the GPC separation 5 ml of the sample was injected and eluted by cyclohexane:ethyl acetate (1:1 v/v) at a flow rate of 4 ml/min. The PAH-containing fraction was collected between 36 and 63 min of the 85 min total run time and concentrated to 4 ml by an automated concentrator integrated in the GPC system. After the addition of 200 μl of toluene as a keeper, the extract was gently evaporated by a stream of nitrogen at 40°C in the sample concentrator to 200 μl. The evaporated extract was diluted with 800 μl of cyclohexane to a final volume of 1 ml. Consecutive clean-up was performed by SPE on silica. The SPE cartridge was conditioned with 2 ml of cyclohexane, then the sample was loaded and PAHs were eluted with 10 ml of cyclohexane. The collected SPE fraction was evaporated to 200 μl with a stream of nitrogen at 40°C and transferred to a GC autosampler vial while rinsing the collection tube with an additional volume of 200 μl of toluene. Finally, 100 μl of injection standard solution (FBkF, 80 ng/ml) was added into the autosampler vial and the sample was analysed by GC-MS.

For fish oil and other edible oil food supplements, 1 g of the sample was weighed directly to the GC vial, 200 μl isotopically labelled 4PAHs solution added and the final volume adjusted to 5 ml by dissolving with cyclohexane:ethyl acetate mixture (1:1, v/v). The prepared test portion was directly applied for GC cleanup. Further sample treatment was carried out as described above.

For each batch of samples, a procedural blank was prepared to assess interferences deriving from the applied reagents and apparatus. The system was considered suitable for the analysis of food supplement samples if the analyte content in the procedural blank did not exceed the limit of detection of the method. A well-defined quality control sample, St. John’s Wort, was included in each sample batch, for checking the method performances along time. All samples were processed in duplicate.

**GC/MS analysis**

For the GC/MS analysis, 10 μl of sample extract was injected into the PTV injector in split-vent mode at an initial temperature of 55°C for 0.5 min at a vent flow of 100 ml/min; the temperature was then ramped up at 600° C/min to 400°C with a 15 min static time. Helium was used as carrier gas at a flow rate of 1 ml/min, and the transfer line temperature was set to 325°C. The oven
temperature programme was as follows: initial temperature 60°C hold for 1 min, 60°C/min till 180°C (held for 0 min), 28°C/min till 280°C (held for 3 min), 14°C/min till 325°C (held for 10 min). PAHs were detected in selected ion monitoring mode (SIM) recording the \( m/z \) values: 8–20 min \( m/z \) 114, 226, 228 for BaA and CHR, \( m/z \) 116, 234 for \( ^{13}C_6 \)-BaA and \( ^{13}C_6 \)-CHR, 2035 min \( m/z \) 126, 250, 252 for BbF and BaP, \( m/z \) 129, 258 for \( ^{13}C_4 \)-BbF, \( m/z \) 128, 256 for \( ^{13}C_4 \)-BaP and \( m/z \) 135, 270 for FBkF.

Results and discussion

Method performance

The method used for the determination of the four EU marker PAHs is routinely used in the EURL PAHs and is accredited according to EN ISO/IEC 17025:2005 (ISO 2005; BELAC 2013). The performance of the method was validated for several types of food matrices including food supplements for the working range of 0.5–12.5 µg/kg. In case the analyte content was above the method working range, the sample intake was lowered. The limit of detection (LOD) and limit of quantification (LOQ) of the method was set to 0.25 and 0.5 µg/kg for the individual PAHs, respectively. The combined relative standard uncertainties for individual PAHs at content levels of 0.9 and 11.1 µg/kg taking into account the contributions of intermediate precision, calibration, standard solutions and trueness. The estimated combined relative standard uncertainties were for all analytes compliant with the “fitness-for-purpose” approach defined in Commission Regulation (EU) No. 836/2011 (EU 2011b). The expanded uncertainty was obtained by multiplying the combined standard uncertainty by a factor of two, which provides a confidence level of 95% (Table 2).

Quality control tools

The determination of low quantities of PAHs in complex matrices needs a comprehensive set of quality assurance tools to prove the reliability of analytical results. Certified reference materials (CRMs) are most suitable for method performance verification as they provide the best estimate of the true value and its uncertainty. The availability of CRMs for PAHs in food matrices is limited; they currently comprise food matrices such as mussels, fish, tuna and coconut oil.

Hence, a well-characterised proficiency test material, comprising St. John’s Wort, was used for monitoring of the performance of the analysis method. The assigned values were 2.98 ± 0.62 µg/kg for BaB, 4.07 ± 0.83 µg/kg for CHR, 2.92 ± 0.60 µg/kg for BbF and 1.65 ± 0.36 µg/kg for BaP. The material was left over from a proficiency test organised by the EURL PAH in 2011. This quality control sample was analysed with each batch of samples and the obtained results were inserted into QC charts. A means to assess the agreement of the measured results with the assigned values are zeta-scores, calculated as the difference between the measured value and the assigned value divided by the quadratically propagated standard uncertainty of the measured value and of the assigned value. The warning limit was set to a value of ±2 and the action limit to ±3 (Figure 1). Measured values over the whole duration of the study did not exceed the warning limit; hence, the method was regarded as being under control.

An important factor in chromatography is selectivity. Criteria for mass spectrometric detection as laid down in Commission Decision 2002/657/EC were applied for the

| Analyte | Combined relative standard uncertainty (%) | Expanded relative uncertainty (%) |
|---------|-------------------------------------------|---------------------------------|
| BaA     | 13.1                                      | 26.1                            |
| CHR     | 9.7                                       | 19.5                            |
| BbF     | 18.3                                      | 36.5                            |
| BaP     | 16.2                                      | 32.3                            |

Figure 1. Example of quality control chart for benzo[a]pyrene measured in quality control sample (St. John’s Wort).
assessment of selectivity even though those requirements are, strictly speaking, applicable to residues in products of animal origin only (EC 2002). Accordingly, at least three ions had to be monitored for the identification and confirmation of the target analytes. Maximum permitted tolerances for relative ion intensities expressed as a percentage of the intensity of the most intense ion (base peak) were defined for the measured mass/charge ratios and applied as identification criteria (Table 3). A chromatographic peak eluting at the retention time of a target PAH was not considered identified, unless all qualifier ions were present and the ratios between quantifier (base peak) and qualifier ions were within the acceptable range.

As there is a lack of CRMs for PAHs in food supplements, the trueness of the method was verified via spiking experiments for three classes of food supplements (botanical supplement, fish oil, propolis extract). The group of botanical food supplement was represented by sample No. 30 (Maqui Berry), fish oil by sample No. 34 and propolis extract by sample No. 77 (Propolis herbal elixir). The three samples were spiked with a mix of the four target PAHs in toluene so as to double the native content, properly mixed, and left standing overnight to evaporate the residual solvent. Both native and spiked samples were analysed in five replicates. An absolute difference of the nominal (spiked) content value and the measured value of less than two times the standard measurement uncertainty was defined as acceptance criterion. The agreement between the measured levels and the expected results was acceptable for all analytes. The calculated recoveries were in the range of 92.8%–103.2% for botanical food supplement, 91.4%–101.0% for fish oil and 92.9%–105.2% for propolis extract.

Calibration standards prepared from neat substances were checked against NIST SRM® 2260a in order to verify the correctness of instrument calibration (Figure 2). Linearity of the calibration curves was verified by applying Mandel’s test and visual inspection of the plot of residuals. Mandel’s tests were passed for all four target analytes at the 99% confidence level and no trend was observed in any plot of residuals.

In addition to recoveries determined from spiked samples, recoveries of PAHs in commercial food supplement samples were assessed for each individual sample by means of the recovery of labelled PAHs, whose analytical behaviour was considered similar to that of native PAHs. Recoveries of labelled PAHs were calculated from the relative response factors of the labelled PAHs and the injection standard (FBkF). Recovery values between 50% and 120% were considered acceptable. Results were not reported if this recovery interval was not met.

**PAHs in food supplements**

When analysing complex samples such as food supplements, many other matrix components are unavoidably co-extracted together with the target analytes. Oils, waxes, essential oils and natural pigments such as carotenoids and chlorophylls are the most typical matrix components occurring in extracts obtained from samples of plant origin. Taking into account the complexity and variety of food supplements (e.g. powder, oils, liquids), a robust method based on pressurised liquid extraction (PLE), size exclusion chromatography and solid-phase extraction (SPE) was applied. Special care had to be paid to the products in very fine powder form as during the PLE extraction some of those samples tend to cross the PLE cell and block the extractor tubes and capillaries. The most critical aspect when using a GC for separation of target PAHs is co-elution with other PAHs present in the sample (Poster et al. 2006; Gómez-Ruiz & Wenzl 2009). By using the PAH Select® capillary column, resolution problems were partly resolved; however, full baseline separation of

![Figure 2](image-url)

*Figure 2. Example of calibration for benzo[a]pyrene prepared from calibration standards and SRM® 2260a.*
some of the target PAHs from interferents was not achieved; namely, the peaks of triphenylene and chrysene, and benzo[b]fluoranthene–benzo[j]fluoranthene and benzo[k]fluoranthene still overlapped partially (Figure 3).

The levels of the four EU marker PAHs measured in the food supplements purchased on the European market are summarised in Table 4. In general, a huge variation between the products was observed with contents ranging from <0.25 to 709.4 µg/kg for BaP and from <0.25 to 709.4 µg/kg for ΣPAH4. Content levels below the method LOD or LOQ were found in a significant number of products. Overall, 48% of samples did not exceed the level of 0.5 µg/kg for BaP and 28% for PAH4. All values below the LOD or LOQ were replaced by zero in order to describe the whole sample set by descriptive statistics (lower bound), including arithmetic mean, median, 90th percentile and the 95th percentile. The arithmetic mean value calculated for ΣPAH4 was 35.8 µg/kg, median 3.4 µg/kg, 90th percentile 95.0 µg/kg and 95th percentile 133.3 µg/kg. For BaP only were following values calculated: arithmetic mean 5.2 µg/kg, median 0.54 µg/kg, 90th percentile 12.0 µg/kg and 95th percentile 26.2 µg/kg. Out of the 4 EU marker PAHs chrysene was most abundant, ranging from <0.25 to 280.1 µg/kg with a mean of 13.6 µg/kg, median 1.4 µg/kg and 90th percentile 36.9 µg/kg. For calculation of upper bound arithmetic mean and median, values below the LOD or LOQ were replaced by the respective LOD (0.25 µg/kg) and LOQ (0.5 µg/kg) levels. In this case the arithmetic mean for ΣPAH4 and BaP was 36.3 µg/kg and 5.4 µg/kg and median 3.5 µg/kg and 0.54 µg/kg, respectively. The highest PAH concentrations were determined in two samples of propolis extract (sample nos. 78 and 61) and spirulina powder (sample no. 88), in which ΣPAH4 reached the levels of 709.4, 656.9 and 275.2 µg/kg, respectively.

Products collected in EU member states were categorised into three groups: botanical and herbal food supplements, fish and edible oils, and propolis and bee products. Based on the results fish and edible oil food supplements could be assumed as low contaminated. Determined values for BaP in this group of products ranged from <0.25 to 3.0 µg/kg (mean <0.5 µg/kg, 90th percentile 0.56 µg/kg) and ΣPAH4 varied between <0.25 and 20.9 µg/kg (mean 2.4 µg/kg, 90th percentile 4.4 µg/kg). However, some oil-based food supplements would exceed EU maximum levels at present applicable to edible oils and fats intended for direct human consumption or used as an ingredient in food (2.0 µg/kg BaP and 10.0 µg/kg ΣPAH4). Most fish oil samples did not exceed the LOQ level. The low contamination of fish oil might be explained by the ability of fish to metabolise PAHs (Ramalhosa et al. 2012). Others edible oil-based products did not contain any of the target PAHs, except few products of garlic oil mixed with soybean or sunflower oil (ΣPAH4 levels up to 4.1 µg/kg), sea buckthorn oil (ΣPAH4 level 20.9 µg/kg) and two multicomponent products containing oil from apricot kernel. Sources for contamination of edible oils with PAHs might be artificial drying of oil seeds and heating during oil production and processing. However, contamination should be avoided by precautionary measures such as indirect drying and good temperature control (Moret & Conte 2000).
Table 4. Measured concentrations (μg/kg) of four EU marker PAHs in food supplements products and their sum (ΣPAH4) including the measurement uncertainties (μg/kg).

| Code | Name                        | BaA ± U* | CHR ± U* | BbF ± U* | BaP ± U* | Σ4PAHs ± U* |
|------|-----------------------------|----------|----------|----------|----------|-------------|
| 1    | Aloe vera                   | n.d.     | n.d.     | n.d.     | n.d.     |             |
| 2    | Green tea extract           | 1.2      | 0.3      | 1.8      | 0.4      | 1.0 0.4     |
| 3    | Siberian ginseng            | 1.0      | 0.3      | 1.4      | 0.3      | 0.8 0.3     |
| 4    | Ginger                      | n.d.     | 1.0      | 0.2      | <0.5     | n.d. 1.0    |
| 5    | Green coffee                | <0.5     | 0.9      | 0.2      | n.d.     | n.d. 0.9    |
| 6    | Cat’s claw                  | n.d.     | <0.5     | n.d.     | n.d.     |             |
| 7    | Super green tea             | <0.5     | 0.7      | 0.1      | <0.5     | <0.5 0.7    |
| 8    | Valerian                    | 36.5     | 9.5      | 43.1     | 8.1      | 34.3 12.5   |
| 9    | St. John’s wort             | 31.6     | 8.2      | 49.5     | 9.6      | 30.1 11.0   |
| 10   | Kelp                        | n.d.     | n.d.     | n.d.     | n.d.     |             |
| 11   | Cha-de-burge                | 1.6      | 0.4      | 3.1      | 0.6      | 1.3 0.5     |
| 12   | Pu-erh tea                  | 15.6     | 4.1      | 18.4     | 3.6      | 9.0 3.3     |
| 13   | Cod liver oil               | n.d.     | n.d.     | n.d.     | n.d.     |             |
| 14   | Green tiger                 | n.d.     | n.d.     | n.d.     | n.d.     |             |
| 15   | Fenugreek powder            | n.d.     | n.d.     | n.d.     | n.d.     |             |
| 16   | Sillybium marianum          | 0.7      | 0.2      | 1.0      | 0.2      | <0.5 <0.5   |
| 17   | Ginkgo biloba               | 0.5      | 0.1      | 1.3      | 0.3      | <0.5 <0.5   |
| 18   | Ginseng                     | 0.9      | 0.2      | 1.2      | 0.2      | 0.5 0.2     |
| 19   | Pumpkin                     | n.d.     | <0.5     | n.d.     | n.d.     |             |
| 20   | Green tea                   | n.d.     | <0.5     | n.d.     | n.d.     |             |
| 21   | Propolis                    | 8.31     | 2.2      | 12.4     | 2.4      | 8.1 2.9     |
| 22   | Honey bee booster           | 1.8      | 0.5      | 3.9      | 0.8      | 1.6 0.6     |
| 23   | Valerian                    | 10.7     | 2.8      | 14.1     | 2.7      | 8.4 3.1     |
| 24   | Yam-root                    | 1.3      | 0.3      | 2.0      | 0.4      | 1.4 0.5     |
| 25   | Day & Night                 | 2.6      | 0.7      | 3.10     | 0.6      | 2.0 0.7     |
| 26   | Salmon oil                  | n.d.     | n.d.     | n.d.     | n.d.     |             |
| 27   | Omega-3 – see Fish oil      | 0.6      | 0.1      | 0.9      | 0.2      | <0.5 n.d.   |
| 28   | Dandelion juice             | n.d.     | <0.5     | n.d.     | n.d.     |             |
| 29   | Maca powder                 | n.d.     | n.d.     | n.d.     | n.d.     |             |
| 30   | Maqui berry organic         | 2.7      | 0.7      | 3.6      | 0.7      | 1.0 0.4     |
| 31   | Ginseng                     | 1.0      | 0.3      | 1.5      | 0.3      | <0.5 n.d.   |
| 32   | Pangamin                    | n.d.     | n.d.     | n.d.     | n.d.     |             |
| 33   | Omega-3 & omega-6           | n.d.     | n.d.     | n.d.     | n.d.     |             |
| 34   | Omega 3                     | 0.9      | 0.2      | 1.4      | 0.3      | 0.7 0.3     |
| 35   | Oyster mushroom             | 0.8      | 0.2      | 1.0      | 0.2      | 0.6 0.2     |
| 36   | Apricots                    | 1.1      | 0.3      | 1.9      | 0.4      | 1.0 0.4     |
| 37   | Ginkgo biloba               | 2.7      | 0.7      | 4.6      | 0.7      | 2.4 0.9     |
| 38   | Cranberries                 | 1.0      | 0.3      | 1.1      | 0.2      | 0.6 0.2     |
| 39   | St. John’s wort             | <0.5     | 0.7      | 0.1      | <0.5     | n.d. 0.7    |
| 40   | Bovine colostrum            | n.d.     | n.d.     | n.d.     | n.d.     |             |
| 41   | Skeleton                    | 1.3      | 0.3      | 1.9      | 0.4      | 1.1 0.4     |
| 42   | Green tea                   | <0.5     | 1.4      | 0.3      | <0.5     | n.d. 1.4    |
| 43   | Chlorella organic           | 0.9      | 0.2      | 2.5      | 0.5      | 1.7 0.6     |
| 44   | Spirulina organic           | 6.8      | 1.8      | 17.1     | 3.3      | 14.7 5.4    |
| 45   | Evening primrose & borage   | n.d.     | <0.5     | n.d.     | n.d.     |             |
| 46   | Sea buckthorn oil           | 5.1      | 1.3      | 9.1      | 1.8      | 3.7 1.3     |
| 47   | Evening primrose oil        | n.d.     | n.d.     | n.d.     | n.d.     |             |
| 48   | Linseed oil                 | n.d.     | <0.5     | n.d.     | n.d.     |             |
| 49   | Pangamin                    | <0.5     | 0.5      | 0.1      | <0.5     | n.d. 0.5    |
| 50   | Garlic super                | 0.7      | 0.2      | 0.8      | 0.2      | <0.5 n.d.   |
| 51   | Vegenex                     | <0.5     | 1.2      | 0.2      | 0.6      | 0.2 n.d.    |
| 52   | Sillybium marianum          | n.d.     | n.d.     | n.d.     | n.d.     |             |
| 53   | Young barley BIO            | 6.9      | 1.8      | 11.4     | 2.2      | 9.9 3.6     |
| 54   | Garlic                      | 1.3      | 0.3      | 1.6      | 0.3      | 0.7 0.3     |
| 55   | Yucca                       | n.d.     | 0.6      | 0.1      | <0.5     | n.d. 0.6    |
| 56   | Acai berry                  | 0.8      | 0.2      | 1.4      | 0.3      | 0.7 0.3     |
| 57   | Garcinia                    | 1.6      | 0.4      | 2.8      | 0.5      | 1.6 0.6     |
| 58   | Ganoderma lucidum           | 15.2     | 4.0      | 45.3     | 8.8      | 32.7 11.9   |
| 59   | Birch                       | 8.9      | 2.3      | 16.1     | 3.1      | 11.1 4.1    |

(continued)
Propolis and bee products were highly contaminated, in which the concentration of BaP ranged from <0.25 to 87.3 μg/kg (mean 25.9 μg/kg, median 10.0 μg/kg, 90th percentile 84.6 μg/kg) and ΣPAH4 from <0.25 to 709.4 μg/kg (mean 188.2 μg/kg, median 63.2 μg/kg, 90th percentile 662.2 μg/kg). These amounts were approximately in the same range of those found in propolis extracts from Italy published by Moret et al. (2010) (BaP <0.3–41.8 μg/kg). Propolis is a sticky, resinous substance collected by bees from different kinds of trees and plants, variable in colour and composed of resins, waxes, volatile oils, pollen, vitamins, minerals and plant chemicals, especially concentrated flavonoids. The main source of propolis contamination represents environmental pollution due to combustion processes, industrial and vehicle emissions. Besides that, sometimes bees collect man-made materials of similar consistency, such as road tar or varnish (Bees 2014). The level of PAH

| Code | Name                        | BaA ± U* | CHR ± U* | BbF ± U* | BaP ± U* | Σ4PAHs ± U* |
|------|-----------------------------|---------|---------|---------|---------|------------|
| 60   | Cat’s claw                  | n.d.    | <0.5    | n.d.    | n.d.    | –          |
| 61   | Propolis intense            | 214.0   | 55.6    | 239.8   | 46.8    | 87.3       |
| 62   | Fish oil                    | n.d.    | n.d.    | n.d.    | n.d.    | –          |
| 63   | Echinacea forte             | 5.6     | 1.4     | 13.5    | 2.6     | 4.8        |
| 64   | St. John’s wort             | 3.4     | 0.9     | 9.0     | 1.8     | 3.9        |
| 65   | Ginkgo complex              | 4.1     | 1.1     | 13.2    | 2.6     | 7.0        |
| 66   | Garlic                      | 0.6     | 0.2     | 1.0     | 0.2     | 1.0        |
| 67   | Omega 3                     | n.d.    | n.d.    | n.d.    | <0.5    | –          |
| 68   | Maca forte                  | n.d.    | n.d.    | n.d.    | n.d.    | –          |
| 69   | Cranberry                   | 16.8    | 4.4     | 22.9    | 4.5     | 10.3       |
| 70   | Ginseng forte + maca        | 2.7     | 0.7     | 3.7     | 0.7     | 2.6        |
| 71   | Cat’s Claw                  | 2.5     | 0.6     | 4.0     | 0.8     | 2.1        |
| 72   | Shark’s oil                 | 0.7     | 0.2     | 1.0     | 0.2     | 0.5        |
| 73   | Propolis mixture            | 2.6     | 0.7     | 3.6     | 0.7     | 1.8        |
| 74   | Spirulina                   | 2.3     | 0.6     | 7.7     | 1.5     | 6.9        |
| 75   | Bioactive Spirulina         | n.d.    | 0.8     | 0.2     | <0.5    | –          |
| 76   | Premium Spirulina           | 1.4     | 0.4     | 4.1     | 0.8     | 4.4        |
| 77   | Propolis herbal             | 2.3     | 0.6     | 2.6     | 0.5     | 1.3        |
| 78   | Propolis liquid             | 157.7   | 41.0    | 280.1   | 54.6    | 187.4      |
| 79   | Bioactive Spirulina         | n.d.    | <0.5    | n.d.    | n.d.    | –          |
| 80   | Spirulina                   | 29.9    | 7.8     | 39.6    | 7.7     | 34.9       |
| 81   | Children’s propolis herbal  | <0.5    | 0.7     | 0.1     | n.d.    | –          |
| 82   | Propolis extract            | 79.6    | 20.7    | 91.6    | 17.9    | 46.1       |
| 83   | Evening primrose oil        | n.d.    | n.d.    | n.d.    | n.d.    | –          |
| 84   | Acidophilus                 | n.d.    | n.d.    | n.d.    | n.d.    | –          |
| 85   | Spirulina                   | 19.8    | 5.2     | 36.6    | 7.1     | 23.3       |
| 86   | Propolis liquid             | 22.9    | 5.9     | 37.0    | 7.2     | 17.9       |
| 87   | Propolis liquid             | 25.6    | 6.6     | 37.6    | 7.3     | 19.1       |
| 88   | Premium Spirulina           | 58.6    | 15.2    | 96.6    | 18.8    | 52.5       |
| 89   | Blood capsules              | 1.7     | 0.4     | 2.8     | 0.6     | 3.0        |
| 90   | Dietary supplements – B1    | 9.6     | 2.5     | 22.4    | 4.4     | 9.1        |
| 91   | Dietary supplements – C1    | 3.4     | 0.9     | 5.7     | 1.1     | 3.2        |
| 92   | Dietary supplements – L1    | 3.0     | 0.8     | 6.6     | 1.3     | 2.4        |
| 93   | Dietary Supplements – Y1    | 4.1     | 1.1     | 8.6     | 1.7     | 4.4        |
| 94   | Spirulina                   | n.d.    | n.d.    | n.d.    | n.d.    | –          |
| 95   | Primrose oil                | n.d.    | n.d.    | n.d.    | n.d.    | –          |
| 96   | Germ & cod liver oil        | 1.0     | 0.3     | 2.0     | 0.4     | 4.6        |
| 97   | Multicomponent extract      | 1.5     | 0.4     | 3.7     | 0.7     | 1.1        |
| 98   | Green tea & garlic          | n.d.    | 0.6     | 0.1     | n.d.    | –          |
| 99   | Multicomponent extract      | <0.5    | –       | 0.7     | 0.1     | <0.5       |
| 100  | Grape & green tea           | <0.5    | –       | 0.7     | 0.1     | <0.5       |
| 101  | Botanicals extract          | 0.7     | 0.2     | 1.7     | 0.3     | 0.6        |
| 102  | Wheat germ plus             | <0.5    | –       | 0.9     | 0.2     | <0.5       |
| 103  | Grape seed plus             | n.d.    | n.d.    | n.d.    | n.d.    | –          |
| 104  | Ginseng plus                | <0.5    | –       | 0.8     | 0.2     | <0.5       |
| 105  | Ginseng plus                | n.d.    | –       | 0.6     | 0.1     | <0.5       |

*Note: * Uncertainties are given as expanded (k = 2) uncertainties according to the ISO Guide to the Expression of Uncertainty (GUM), corresponding to an approximate 95% confidence interval; n.d., below LOD.
contamination could also be influenced by beekeeper practices, especially smoking the hive before honey collection (Moret et al. 2010).

ΣPAH4 occurred in the group of botanical and herbal food supplements without any systematics ranging from <0.25 to 275.2 µg/kg (mean 21.0 µg/kg, median 3.2 µg/kg, 90th percentile 52.2 µg/kg). ΣPAH4 levels varied also within the same type of products. There was no evidence of contamination of certain kinds of products which could support any concrete conclusions. The principal pathway for contamination of fruits and vegetables with PAHs is gas and particle deposition, while in some cases a considerable uptake of PAHs from soil was monitored (Rojo Camargo & Toledo 2003). Fruits and vegetables growing in highly industrialised areas or near roadways, or irrigated with wastewater generally showed higher levels than those from rural areas (Khan et al. 2008). Another source of PAH contamination is from the processing techniques, mainly inappropriate drying processes. Furthermore, relatively low PAH levels in botanical material may become magnified when subjected to concentration steps during production, which also concentrate PAHs (Danyi et al. 2009). Our findings confirmed the observation of non-systematic ΣPAH4 values measured in botanical supplements, as the contamination is connected with environmental pollution and processing techniques.

In addition to analysed food supplements products available on the European market, the PAH contamination of food supplements was examined on randomly selected products from an Indian market. Eleven mainly multicomponent food supplements with botanicals or oil ingredients were collected and analysed for ΣPAH4 content. Measured levels of ΣPAH4 varied within <0.25 and 15.4 µg/kg, with the lower bound arithmetic mean of 2.8 µg/kg. The majority of Indian samples (67%) did not contain BaP, only one oil product (wheat germ/cod liver oil) contained 7.8 µg/kg. The lower bound arithmetic mean calculated for BaP was 0.9 µg/kg. PAH contamination of Indian food supplements did not show any differences compared to European food supplements (Table 1).

The relevance of exposure to PAHs by food supplements is frequently questioned due to their low consumption in comparison to other food categories such as edible oils or seafood. However, to clarify this point, the potential intake of PAHs was evaluated for the 10 most contaminated products analysed in this study (Table 5). The manufacturers’ maximum recommended dose was used for intake calculation and results were compared to general dietary exposure estimates. EFSA (2008) estimated the overall average dietary exposure to ΣPAH4 across European countries to 1168 ng/day and average exposure to BaP to 235 ng/day. EFSA identified cereals and cereal products and seafood and seafood products as the two highest contributors to dietary exposure with ΣPAH4 values of 257 ng/day and 289 ng/day, respectively (EFSA 2008). The highest hypothetical daily intakes of BaP and ΣPAH4 were determined among the studied food supplements for product no. 88 “Premium Spirulina” with 608 ng/day BaP and 2477 ng/day ΣPAH4, respectively. The high exposure through this product is apparently caused by the high daily dosage (9.0 g/day) recommended by the manufacturer. The second highest ΣPAH4 intake estimation was calculated for product no. 61 “Propolis intense” with a value of 1486 ng/day. With an exception of propolis no. 87, the estimated daily intake of ΣPAH4 was for the other eight products in the range between about 200 and about 260 µg/kg. Based on the assumptions that spirulina product no. 88 is consumed at the maximum recommended dose, it may be concluded that the average daily exposure to ΣPAH4 would be more than twice as much as the exposure due to consuming an average diet. The daily intake through each of the next eight highest contaminated food supplement products would contribute to the average daily exposure to PAHs at the same level as EFSA concluded for cereal or seafood products (EFSA 2008).

| Code | Name                  | BaP (µg/kg) | ΣPAH4 (µg/kg) | Maximum dose (g/day) | Intake BaP (ng/day) | Intake ΣPAH4 (ng/day) |
|------|-----------------------|-------------|---------------|----------------------|---------------------|-----------------------|
| 78   | Propolis liquid       | 84.3        | 709.4         | 0.3                  | 29                  | 245                   |
| 61   | Propolis intense      | 87.3        | 656.9         | 2.3                  | 197                 | 1486                  |
| 88   | Premium Spirulina     | 67.5        | 275.2         | 9.0                  | 608                 | 2477                  |
| 82   | Propolis extract      | 48.1        | 265.5         | 1.0                  | 47                  | 258                   |
| 8    | Valerian              | 31.1        | 143.1         | 1.6                  | 50                  | 230                   |
| 80   | Spirulina             | 23.5        | 128.0         | 1.6                  | 38                  | 205                   |
| 9    | St. John’s wort       | 15.4        | 126.5         | 1.9                  | 29                  | 236                   |
| 58   | Ganoderma lucidum     | 6.2         | 99.4          | 2.0                  | 13                  | 203                   |
| 87   | Propolis liquid       | 15.3        | 97.6          | 0.3                  | 5                   | 34                    |
| 85   | Spirulina             | 16.8        | 96.5          | 2.0                  | 34                  | 193                   |

Table 5. Examples of daily intake levels of BaP and ΣPAH4 by the 10 most contaminated food supplements when assuming an intake of the maximum daily dose recommended by the manufacturers.
Literature data on PAHs in food supplements were in good agreement with those observed in this survey. Data collected from different European countries reported by EFSA (2008) covered overall 325 food supplement products. The arithmetic mean reported for BaP was 2.5 μg/kg and the maximum value 270 μg/kg, with 46% of the samples showing detectable levels of this compound. The highest contents were found for CHR with an arithmetic mean of 9.1 μg/kg and a maximum of 590 μg/kg. The maximum PAH content was recorded for a product named “Arkopharma thé noir”, the second highest for a spirulina product and the third highest for a propolis extract (EFSA 2008).

Chrysene was also the most abundant among ΣPAH4 in dietary supplements studied by FSA (2005). With very few exceptions, PAHs were undetected or rarely present in fish oils. The highest concentration of BaP was found in a spirulina sample (44 μg/kg).

In the Dutch study on food supplements running from 2003 to 2007, BaP was present in 44% of food supplements with a mean level of 3.4 μg/kg. In a follow-on survey (2008 to 2009), 63% of the food supplements contained BaP with a mean value of 5.3 μg/kg (Martena et al. 2011).

PAH levels in propolis-based supplements are overall relatively high. Raw propolis samples collected in Italy in 2008 contained quite variable PAH amounts, with BaP ranging from 0.7 to 1371 μg/kg. However, CHR was always the most abundant PAH with amounts between 4.7 and 3176 μg/kg. Lower levels of BaP were found in propolis extracts ranging from below LOQ to 41.8 μg/kg (Moret at al. 2010). High levels of CHR in propolis and royal jelly samples were observed also in the Dutch market study in 2008 and 2009, where the mean concentration of CHR was 58.4 μg/kg compared to BaP of 7.6 μg/kg. Certain food supplements when used at the maximum recommended dose, especially those with botanicals or propolis, can contribute significantly to dietary PAH exposure. However, when the lower bound mean of ΣPAH4 was assumed, the estimated PAH intake was found to be less substantial. In this case food supplements will be at the lower end of the range of contributions among the food groups that contribute only modestly to ΣPAH4 exposure (Martena et al. 2011).

Conclusions
In this study the applicability of a stable-isotope dilution GC/MS method was evaluated for the determination of the four EU marker PAHs in various kinds of food supplements. A stringent quality control programme was applied in the analysis of commercial food supplements comprising oil-, botanical- and bee product-based food supplements to ensure the generation of reliable occurrence data.

The analysed food supplements showed significant variability in PAH levels. The levels for ΣPAH4 ranged between below <0.25 and 709.4 μg/kg. The priority PAH with the highest arithmetic mean content was chrysene with a value of 13.6 μg/kg and a maximum level of 280.1 μg/kg. Propolis extracts and bee products were more highly contaminated compared to other products with a ΣPAH4 mean value of 188.2 μg/kg. Low contents of ΣPAH4 (mean 2.4 μg/kg) were determined in fish and edible oil supplements. No specific tendency in PAH contamination was found for botanical products. Many of the analysed products did not contain the target PAHs at levels exceeding the LOQ.

Nevertheless, in some samples, considerably high ΣPAH4 amounts were found, which could remarkably increase the daily exposure of consumers to PAHs. This is in agreement with literature data, demonstrating the need for continuous monitoring of ΣPAH4 in food supplements. Sources of contamination should be identified for highly contaminated products and remediation measures taken.

Disclosure statement
No potential conflict of interest was reported by the authors.

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