Residue2Heat

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DISCLAIMER

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Executive summary

In view of the production of large quantities of fast pyrolysis bio-oil (FPBO) and the use of these oils in various applications, the FBPO should have a REACH (Registration, Evaluation and Authorization of Chemicals) registration according to European legislation. Within the REACH registration a SIP (Substance Identity Profile) is incorporated giving amongst other things the maximum allowable concentration of certain components in the FPBO. Large quantities (> 1000 kg/y) of pyrolysis liquids can only be produced and traded as FPBO if they meet the criteria given in this registration. Furthermore in the REACH registration, an EU producer should indicate if a substance is toxic (and to what extend) and how this substance should be handled safely by e.g. the user. So in view of the application of various pyrolysis liquids as boiler fuels (FPBO's) in this project, this would of course be of great importance. One of the component groups mentioned in the SIP are the PAH's (poly aromatic hydrocarbons). PAH's are notorious of having carcinogenic and/or mutagenic effects on the human body, and allowable concentration are therefore very low. Due to the general complexity of the FPBO, it is difficult to apply standard methods e.g. used in PAH's analysis of water and mineral oils samples. The alternative analytical methods that were applied to determine the PAH's in FPBO were however not yet properly evaluated. This deliverable describes the development and validation of a method suitable for qualification and quantification of the PAH's in FPBO. FPBO samples were analysed by 3 different laboratories and compared mutually and with data obtained in the earlier BIOTOX project for method evaluation. This method validation was to a large extend already performed in earlier projects (Empyro & Pyrobest). In this deliverable the analysis and results of these separate tests are combined, compared and evaluated. Actual analysis of *Residue2Heat* samples will be carried out soon, and reported separately.

Contents

List of Abbreviations

DMSO	Dimethyl sulfoxide
ECHA	European Chemicals Agency
EPA	Environmental Protection Agency
FID	Flame Ionization Detector
FPBO	Fast Pyrolysis Bio-oil
GC	Gas chromatography
HPLC	High-performance liquid chromatography
JD	Joint Dossier
MS	Mass Spectrometry
PAH	Poly Aromatic Hydrocarbon
PPM	Parts Per Million
PPORD	Process Orientated Research and Development
REACH	Registration, Evaluation, Authorization of Chemicals
RRF	Relative Response Factors
SIM	Single Ion Monitoring
SIP	Substance Identity Profile
SR&D	Scientific Research and Development
UV	Ultra Violet
UVCB	Unknown or Variable composition, Complex reaction products or Biological materials

1 Introduction

1.1 Scope

REACH is the abbreviation for Registration, Evaluation, Authorization of Chemicals, and it addresses the production and use of chemical substances and their potential impacts on both human health and the environment. REACH was introduced in 2007, and registration is mandatory for each new chemical that enters the market and requires the submission of a dossier with results of various analytical tests. In 2013 a Fast Pyrolysis Bio-oil (FPBO) REACH consortium of FPBO producing/trading companies was established, with the goal of obtaining REACH registration for FPBO at the European Chemicals Agency (ECHA). According to REACH, FPBO is a substance of Unknown or Variable composition, Complex reaction products or Biological materials (UVCB substance). The objective of REACH is to combine different European regulations and directives and in this way inter alia; create a higher level of protection to human health and environment, making producers and importers more responsible, enhancing innovation in (EU) chemical industry, and to promote the use of alternative assessment methods of chemicals/substances. Authorities can banish or restrict certain substances with high risks, and in this way also stimulate the introduction of less problematic alternative substances. A disadvantage is that REACH registration could potentially reduce the possibilities to introduce new substances, especially if these are produced in relatively small volumes by a pioneering company (expensive procedure) however there are some possibilities to get exceptions for development (SR&D, PPORD) [ECHA 17], [HSE 17]. In 2018 all substances should be REACH registered. In the REACH registration an EU producer should indicate if a substance is toxic (and to what extend) and how this substance should be handled safely by e.g. the user. REACH registration of substances also forces companies making the same product to join forces on this matter. Al substances produced or imported (in EU) in amounts larger than 1000 kg/y will be obligated for the REACH registration [RO 17], [EC 17].

1.2 REACH registration FPBO

FPBO is difficult to quantify in detail, due to its complex compositions (matrix of several hundred components) and its variability. Due to this fact a definition for FPBO was agreed (30/11/2012, Fortum, Finland) focusing on the used raw material and the process conditions applied. The present definition of FPBO is as follows: “Liquid condensate recovered by thermal treatment of lignocellulosic biomass, at short hot vapour residence time (typically less than about 10 seconds) typically at between 450-600°C close to atmospheric pressure or below in the absence of oxygen” [Kärki 12], [Saari 14]. In 2013 the consortium submitted a Joint Dossier (JD) in which the first characteristics and properties (chemical safety assessments etc.) of FPBO were published. Data was obtained by performing laboratory analysis and also by applying the data generated in the BIOTOX project [Blin 05]. From the JD a properties and composition Table (see Table 1) for FPBO was extracted.

Table 1: Properties and composition of FPBO [Saari 14] (From Substance Identity Profile (SIP) for fast Pyrolysis Bio-oil)

pH	> 2 – 3.5
Water content	< 40 ww %
Ash content	< 0.5 ww %
Solids content	< 5.0 ww %
Viscosity (40°C)	< 200 mm^2/s
Density (kg/dm^3)	1.1 – 1.3 kg/dm^3
Polar components	
Formaldehyde	< 0.5 ww %
Methanol	< 3 ww %
Non-polar components	
PAH13 **a**	< 35 ppm
Bentso[a]Pyrene	< 0.01 ww %
Dibenz[a,h]anthracene	< 0.01 ww %
Sum of Carc. 1B classified substances **b**	< 0.1 ww %
Sum of Carc. 2 classified substances **c**	< 1.0 ww %

a Sum PAH13: Anthracene, Benz[a]anthracene, Benzo[a]pyrene, Benzo[a] fluoranthene, Benzo[k]fluoranthene, Benzoperylene, Chrysene, Dibenz[a,h] anthracene, Fluorene, Fluoranthene, Indenopyrene, Phenantrene, Pyrene
b Carc. 1B classified substances (Annex VI of CLP regulation 1272/2008): e.g. of sum PAH13: Benz[a]anthracene, Benzo[a]pyrene, Benzo[k] fluoranthene, Chrysene, Dibenz[a,h]anthracene
c Carc. 2 classified substances (Annex VI of CLP regulation 1272/2008): e.g. Formaldehyde, Acetaldehyde, Furfural

In Table 1, the properties and composition of (wood derived) FPBO is given. The chemical composition is divided in two groups, being the polar components and the non-polar components. The polar component group includes formaldehyde and methanol and the validation of methods to determine these, is described in deliverable D3.9 [Ohra-aho 17]. The other group, the non-polar components, includes the PAH's. PAH's are poly aromatic hydrocarbons, which can be formed by the incomplete combustion or high temperature pyrolysis of materials such as biomass, plastics and fuels. PAH's are also naturally occurring, e.g. in coals or formed during forest fires and volcano eruptions. PAH's are complex fused aromatic ring structures with the simplest being naphthalene and they are notorious of having carcinogenic and/or mutagenic effects on the human body. Typically, PAH's are highly non-polar and therefore have a relative low solubility in water. From literature it is known that in general fast pyrolysis oils have a low total PAH's concentration of below 35 ppm (PAH-13), slow pyrolysis oils tend to have much higher concentrations (100 ppm). Not only the residence time, but also the temperature applied in pyrolysis is a real important factor on PAH's formation. Especially at temperatures above 700 °C, large quantities of PAH's are being formed due to secondary thermochemical reactions [WHO 00], [Garcia-Perez 08]. Apparently the cellulose structures in biomass tend to produce more PAH's via the decomposition of char than lignin's do [Fabri 10]. Frequently encountered PAH's are the PAH's in the so called PAH-16 group, which are known to be of serious health safety concern. The PAH-16 were first identified by the US-Environmental Protection Agency in the seventies and these 16 PAH's are therefore often referred to as the 16-EPA-PAH's [Jing 16].

The most important PAH's of this group in terms of toxicity are; benzo[α]pyrene (group 1B), naphthalene, crysene, ben[α]anthracene, benzo[k]fluoranthene and benzo[b]fluoranthene (group 2B), these are carcinogenic or highly suspected of being carcinogenic. Standard PAH-analysis applied e.g. for bitumen, water samples, liquid smoke or foodstuffs, are mainly based on HPLC- or GC-methods. In HPLC often fluorescence is used for detection, most PAH's namely emit light of a certain wavelength when excited. Also UV detection is used in the analysis of PAH's by HPLC, PAH's show very characteristic UV-spectra. Often the two detection methods are also combined in the analysis of PAH's by HPLC. The GC methods applied to do PAH's analysis are often based on GC-MS combined with GC-FID or by GC-MS-SIM. In the combination of MS and FID, the MS-detector is used to qualify the components, and subsequently the FID applied for further qualification. In the GC-MS-SIM analysis, a MS is used for both qualification and quantification, by applying the single ion monitoring mode. This means that when a component is ionized, characteristic fragments are produced in specific amounts. Qualification is done by comparing these ion fragments (qualifier ions) to the library, and quantification is performed by using the peak area of the most abundant ion fragment (quantifier) to calculate the concentration (by applying standards) [Hussein 16], [Fabri 10], [Jing 16], [Rupert 06], [Wenzl 06]. In this deliverable the focus will be mainly on the GC-methods, this due to the nature and complexity of the samples, and outcomes of earlier performed analysis tests with HPLC.

1.3 Objectives

The objective is to determine the polar and non-polar compounds according to REACH registration, since the analysis techniques proposed in REACH for polar and non-polar compounds are not yet properly evaluated. Furthermore, hardly any data on FPBO's from non-wood biomass has been included. In this deliverable results are presented on the validation of the PAH's analysis in FPBO samples with varying methods at different laboratories.

2 Experimental

2.1.1 Laboratory 1

Method 1 (M1, Table 1) was performed according a standard HPLC method (PAH-16) used by Laboratory 1 (Lab1), typically used for PAH's analysis of wastewater samples. The FPBO sample was prepared for analysis by dissolving it in acetone and subsequently dilute the mixture with a substantial amount of water and extract it with petroleum ether. The petroleum ether was then removed and the remaining components (PAH's) were dissolved in acetonitrile and analysed. In method 2 (M2), the FPBO is first dissolved in a 1 mol/l NaOH-solution and subsequently extracted with petroleum ether. The petroleum ether was then removed and residues dissolved in acetonitrile and analysed.

2.1.2 Laboratory 2; Adaptation of "BIOTOX" method:

The PAH-components (16 pieces) were analyzed by Laboratory 2 (Lab2) from an n-hexane extract of the sample, possibly the FPBO sample was first dissolved in ethanol before extraction (so not with cyclohexane in alkaline circumstances, as in BIOTOX). The sample was transferred to an extraction funnel and 4 internal deuterated PAH standards were added. The extract was then cleaned by using DMSO liquid-liquid partitioning. The PAH-compounds were analyzed from the cleaned n-hexane extract using Gas-Chromatography-Mass Spectrometry (GC-MS) running in SIM-mode (Selected-Ion Monitoring). The quantitation was made using the Internal Standard Method.

2.1.3 RuG (Rijkuniversiteit Groningen) method

The FPBO samples are first dissolved in ethanol, then four deuterated internal standards are added and subsequently this liquid is extracted with n-hexane and washed with DMSO. The PAH-compounds were analyzed from the cleaned n-hexane extract using Gas-Chromatography-Mass Spectrometry (GC-MS) running in SIM-mode. For all PAH's the relative response factors (RRF) were determined by making a 5 point calibration line in which the amount of internal standard is kept constant. For each PAH, the peak area is determined and divided by the peak area of the internal standard. Similar to the areas also the concentrations are divided by the concentration of the internal standard. Then the values are plotted (concentration against area) in a graph with the obtained slope being the RRF.

Additional information on the last two methods mentioned can be found in appendixes.

3 Results

3.1 Non-polar components/PAH's analysis

As mentioned earlier, PAH's analysis were performed at three different laboratories. Initially PAH's analysis were performed at Lab1; a Dutch laboratory with some earlier experience on the analysis of PAH's in pyrolysis oil derived products. Subsequently samples were analysed by Lab2, using a method based on the method developed in the BIOTOX-project. Finally, analysis were performed at the RuG, which used an own interpretation of the Lab2 method (Lab2 method was only briefly described).

3.1.1 PAH's analysis by Lab1

At Lab1, two methods were tested to determine the PAH's in FPBO (see Table 2, M1 and M2). In M2 a sample pre-treatment adaption was performed because of the complex sample matrix observed during the HPLC-analysis. There was a strong indication that phenolic components were co-extracted and possibly overlapping PAH's peaks in the HPLC-chromatogram. In earlier test analysis this problem was also observed (initial Lab1 analysis), when FPBO derivatives were analysed for PAH's. Possibly the use of acetone to make the FPBO soluble in water contributes to the transfer of lignin derived phenols to the petroleum ether in the subsequent extraction. It is expected that when FPBO is dissolved in a NaOH-sol and followed by the extraction with petroleum ether the lignin derived phenols (being weak acids) will largely remain in the watery phase (= M2 in Table 2). When comparing data for the PAH's-13 and PAH's-16 obtained with M1 and M2, it can clearly be observed in Table 2 that the amount of PAH's is roughly reduced by > 60% when applying M2, this is especially caused by the large reduction of the amount of phenantrene (from 38.3 ppm → 6.5 ppm). Nevertheless the total PAH's (13 and 16) are higher compared to the data obtained by the other methods or to the BIOTOX data (except for M3a).

3.1.2 PAH's analysis by Lab2

In Table 2 the results are given for the PAH's analysis (M3) performed by Lab2. Lab2 also performed the FPBO analysis (polar and non-polar compounds) for the establishment of the SIP profile (Table 1) of FPBO, and therefore the methods used by Lab2 are (up to now) the methods to use. The PAH's method used by Lab2 is based on the method developed in the BIOTOX-project, both methods are based on GC (MS-SIM) instead of HPLC. The main difference between the two methods is the sample preparation. In the BIOTOX method the sample was extracted with cyclohexane after dissolving the FPBO in a NaOH-solution. In the Lab2 method the FPBO was extracted with n-hexane. Also a different sample clean-up and different internal standards were used. Noticeable is that for fluorene and naphthalene relative high values were obtained (24.6 ppm and 39.5 ppm) compared to the values found using M1-M2. As a matter of fact the high fluorene value contributes to almost 60% of the total PAH 13 and the naphthalene to 40% of the total PAH-16. In the BIOTOX project, 21 different FPBO's were analysed i.a. for PAH-13, none of the FPBO samples showed that high concentrations for fluorene (highest observed: 5 ppm) [Blin 05]. Only for a slow pyrolysis oil sample a fluorine value of 39.0 was obtained. In view of the SIP profile (Table 1) the high value for fluorene is much more of a concern here than that of naphthalene. Also here it might be possible that a component (phenols) other than the PAH contributes to this relative large value. In Table 2 the BIOTOX data for four different pyrolysis oils is given for comparison (M5a, M5b, M5c, M5d).

Table 2: Results of PAH's analysis.

Component	PAH no:	Lab1 (HPLC) M1 (ppm)[1]	Lab1 (HPLC) M2 (ppm)[1]	Lab2 (GC-MS-SIM) M3a (ppm)	Lab2 (GC-MS-SIM) M3b (ppm)	Lab2 (GC-MS-SIM) M3c (ppm)	RuG[3] (GC-MS-SIM) M4a (ppm)	RuG[3] (GC-MS-SIM) M4b (ppm)	BioTox (GC-MS-SIM) M5a (ppm)[4]	BioTox (GC-MS-SIM) M5b (ppm)[5]	BioTox (GC-MS-SIM) M5c (ppm)[6]	BioTox (GC-MS-SIM) M5d (ppm)[7]
Anthracene	1	1.58	3.84	2.13	0.1	0.2	0.65	0.9	0.15	0.79	0.31	0.29
Benzo[a]anthracene	2	3.58	1.35	1.68	0.1	0.1	<0.1	1.4	0.02	0.23	0.15	0.06
Benzo[a]pyrene	3	0.23	0.11	Nd[2]	0.1	0.1	0.23	1.0	0.04	0.16	0.17	0.47
Benzo[b]fluoranthene	4	1.17	0.02	Nd[2]	0.1	0.1	<0.1	0.6	0.03	0.05	0.06	0.03
Benzo[k]fluoranthene	5	0.08	0.02	Nd[2]	0.1	0.1	<0.1	0.9	0.03	0.05	0.06	0.01
Benzo[g,h,i]perylene	6	0.08	0.02	0.221	0.1	0.1	<0.1	0.7	0.02	0.06	0.08	0.03
Crycene	7	9.17	4.94	0.871	0.1	0.1	0.49	0.1	0.03	0.15	0.12	0.10
Dibenzo[a,h]anthracene	8	0.21	0.02	Nd[2]	0.1	0.1	<0.1	8.0	Nd[2]	Nd[2]	Nd[2]	Nd[2]
Fluorene	9	1.92	1.40	24.6	0.9	1.6	2.90	4.1	0.89	2.18	0.90	2.81
Fluoranthene	10	3.83	1.85	1.75	0.2	0.4	0.40	0.5	0.11	0.36	0.23	0.35
Indeno[1,2,3-cd]pyrene	11	0.08	0.02	Nd[2]	0.1	0.1	<0.1	0.4	0.01	0.08	0.08	0.03
Phenantrene	12	38.33	6.49	7.75	0.6	1.0	1.30	1.9	0.46	2.53	1.07	0.97
Pyrene	13	3.42	1.95	2.76	0.2	0.4	0.51	0.7	0.18	0.75	0.46	0.35
Tot PAH (13)		63.69	22.01	41.8	2.8	4.4	7.08	21.2	1.97	7.40	3.70	5.50
Naphtalene	14	10.00	8.48	39.5	1.6	2.3	4.10	4.0	-	-	-	-
Acetnaphtylene	15	0.42	0.09	7.14	0.2	0.3	0.63	0.8	-	-	-	-
Acetnaphtene	16	1.17	0.60	10.1	0.1	0.3	0.92	1.4	-	-	-	-
Tot PAH (16)		75.28	31.18	98.5	4.7	7.3	12.7	27.4	-	-	-	-

M1: Lab1 standard; M2: Lab1 adapted; M3a: Lab2 sample FPBO1 (BTG FPBO); M3b Lab2 sample FPBO2 (Empyro FPBO); M3c Lab2 sample FPBO3 (Empyro FPBO). [1]: PPM calculated from µg/l with ρ (assumption): 1.2 kg/l; [2]: Nd: Not detected; [3]: M4a-b: FPBO's from Empyro., [4]: M5a: BIOTOX sample 4 (Circulating Fluidised Bed, pine sawdust, 500 °C); [5]: M5b: BIOTOX sample 5 (Fluidised Bed, pine/spruce, 500 °C); [6]: M5c: BIOTOX sample 9 (Fluidised Bed, beech, 480 °C); [7]: M5d: BIOTOX sample 18 (Circulating Fluidised Bed, beech, 500 °C, , see BIOTOX p. 16). Note: Samples Lab1, Lab2 and BIOTOX were not identical.

3.1.3 PAH's analysis by RuG

In Table 2, standard FPBO's produced by Empyro in Hengelo were analysed by the RuG (M4). The RuG method is an interpretation of the Lab2 method, which was only briefly described. For the second sample (M4b) analysed by the RUG, the value for dibenz[a,h]anthracene is high compared to all other analysis performed. The measurements performed are analysed by GC-MS in the SIM (single ion monitoring) mode. This means that when a component is ionized, characteristic fragments are produced in specific amounts. In the SIM mode, these most abundant fragments are used to qualify and quantify the component. Qualification is done by comparing these ion fragments (qualifier ions) to the library, and quantification is done by using the peak area of the most abundant ion fragment (quantifier) to calculate the concentration (by applying standard).

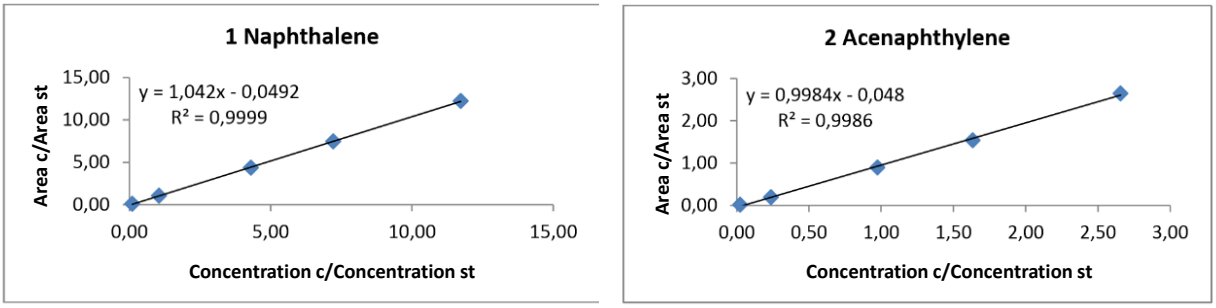

Figure 1: Examples of calibration curves

In Figure 1, the (5 point) calibration curves for napthalene and acenaphtylene are illustrated as an example. All the PAH calibration curves showed good linear regression and correlation coefficients of > 0.99. The individual calibration curves are used to determine the RRF (relative response factor) of each PAH. The RRF is the slope of the graph in which the values obtained by dividing the PAH peak area by the peak area of the internal standard are plotted against the values obtained by dividing the PAH concentration by the concentration of the internal standard.

When analysis measured data it is important to check the ratio(s) of qualifier and quantifiers (calculated from the standards), if this value is deviant to the theoretic value for that certain component, overlapping of ion fragments by other present components might be interfering the results.

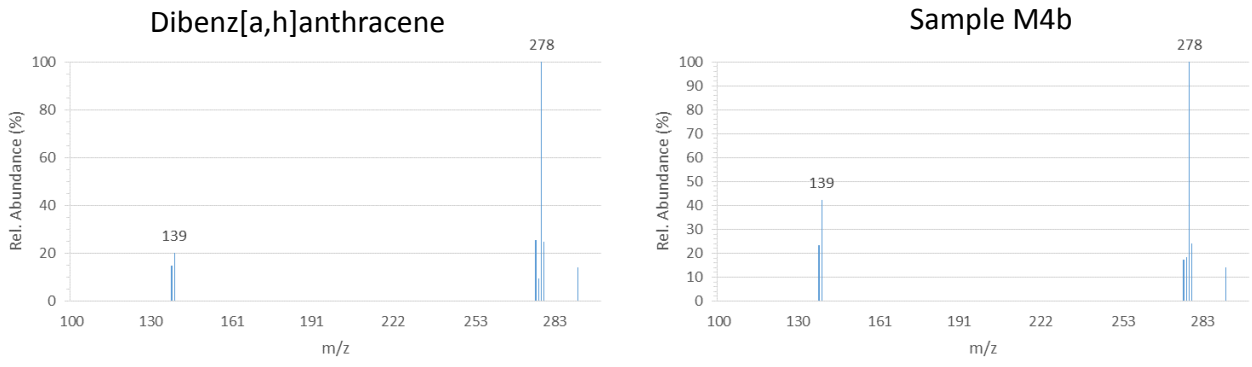

Figure 2: MS Spectrum of standard and spectrum of sample M4b

In Figure 2, the MS spectrum of dibenz[a,h]anthracene and sample 4b are illustrated. Here peak (mass) 139, 278 and 279 are used for qualification, peak 278 is also used as the quantifier ion. Comparing the standard

with the sample it can be seen that ration between e.g. Peak 139 and peak 278 (set at 100 %) is different. This means that the PAH peak in the chromatogram probably contains another component which has the same retention time and gives the same ion-fragments. The recoveries of the PAH components were determined by spiking sample with known amounts of individual components. The recoveries of the PAH's are given in Table 3. The recovery of indeno[1,2,3-cd]pyrene showed to be quite high (196 %), the reason for this is probably the low amount of component added. Therefore in a second measurement, more of this component was added, which resulted in a decrease to 136%. In general, the recovery values obtained are deviating (from 100%) to some extent, nevertheless these values/deviations are comparable to data found in literature for comparable PAH's analysis with similar techniques [Simon 05].

Table 3: Recovery of PAH's in RuG analysis (for M4a)

Component	PAH no:	RuG (GC-MS-SIM) Recovery %
Anthracene	1	88
Benzo[a]anthracene	2	106
Benzo[a]pyrene	3	109
Benzo[b]fluoranthene	4	105
Benzo[k]fluoranthene	5	110
Benzo[g,h,i]perylene	6	104
Crycene	7	97
Dibenzo[a,h]anthracene	8	113
Fluorene	9	120
Fluoranthene	10	88
Indeno[1,2,3-cd]pyrene	11	196, 136[1]
Phenantrene	12	80
Pyrene	13	86
Naphtalene	14	74
Acetnaphtylene	15	84
Acetnaphtene	16	148

[1] After adaption of internal standard concentration (adding more spike component)

4 Conclusions

In 2013 the FPBO REACH consortium submitted a Joint Dossier in which the first characteristics, properties and maximum limits of certain components and component groups (e.g. PAH's etc.) of FPBO were published. In establishing this SIP, information was extracted from the BIOTOX project and analysis were performed by Lab2. However, most of the analytical methods applied to the FPBO were not yet properly evaluated. In this deliverable results are presented on the validation of the PAH's analysis of FPBO samples applying 3 varying methods at 3 different laboratories.

FPBO is of course an extremely complex mixture, and it is therefore very difficult to separate individual components. Especially in the PAH's analysis often components in the oil are co-extracted and can cause interference/overlap in the HPLC/GC chromatogram, possibly resulting in higher PAH value's than the actual value. For instance the two HPLC analysis performed by Lab1 (M1 & M2) already showed large differences in concentrations, although the same oil was used. Most probably this is caused by the overlap of the PAH's in the chromatogram by lignin derived phenolic components. Therefore the selection of the pre-treatment of this complex sample matrix is of crucial importance in the PAH-analysis, maybe even more important than the discussion whether GLC or HPLC is the appropriate technique to use, because PAH's can be well determined by both methods. The nature and complexity of the sample and with that the pre-treatment will finally decide the best method to use. In the determination of PAH's in wood derived FPBO's, the GC-MS (sim) method (and it's pre-treatment) seems to be the most suitable method to apply.

The pre-treatment step applied uses several extraction and (re-)dissolving steps largely eliminating interfering components. The method also nicely combines qualification and quantification in one run, and is quite good in discriminating between overlapping components occurring in the chromatogram. Nevertheless, also here overlap can occur resulting in deviant results. According to the PAH's analysis performed by Lab2 (GC-MS sim) one oil sample provided by BTG showed a total PAH-13 of 42 ppm. The high obtained value for PAH-13 is mainly due to a high value found for fluorene, this high value was not found using the other methods. In the BIOTOX project high fluorene values were especially observed in FPBO's produced by slow pyrolysis and not in fast pyrolysis oils. The discussable high fluorene value found for BTG's sample is therefore questionable, and might well be due to component/peak overlap in analysis. Nonetheless bentso[a]pyrene, dibenzo[a,h]anthracene could not be detected and the sum of Carc. 1B (see Table 1) is very low (2.56 ppm). Due to the fact that the exact procedure of the Lab2 method was not exactly known, the method developed, and used by the RuG was an interpretation of this method. In cooperation with the RuG this method was tested, evaluated and further modified/adapted and validated. In the analysis by the RuG (GC-MS-sim), the FPBO's analysed (M5a and M5b) did meet the PAH'-13 criteria of < 35 ppm, although the value for Dibenz[a,h]anthracene was high. Like for the high fluorene value in the Lab2 analysis, also here overlapping of other components in the MS spectrum is probably causing this deviating value as explained earlier. Nevertheless, all other values for the PAH's are within the given limits. In a coming contribution, also FPBO's produced from other types of biomass feeds will be analysed at the RuG with this comprehensive method, the produced data will subsequently be evaluated and reported.

References

[Blin 05] Joël Blin, Biotox Final Technical Report, 2005, p23-26, available at: http://www.pyne.co.uk/Resources/user/docs/BIOTOX%20Final%20Publishable%20report.pdf

[EC 17] http://ec.europa.eu/enterprise/sectors/chemicals/reach/index_en.htm

[ECHA 17] https://echa.europa.eu/regulations/reach/

[Fabri 10] Daniele Fabri *et a*l., GC-MS determination of polycyclic aromatic hydrocarbons evolved from pyrolysis of biomass, Anal. Bioanal. Chem., 2010, p309-317

[Garcia-Perez 08] M. Garcia-Perez, The formation of Polyaromatic Hydrocarbons and Dioxins During Pyrolysis, Washington State University, June 2008, available at: https://research.libraries.wsu.edu/xmlui/bitstream/handle/2376/5966/TheFormationOfPolyaromaticHydrocarbonsAndDioxinsDuringPyrolysis.pdf?sequence=1&isAllowed=y

[HSE 17] http://www.hse.gov.uk/reach/whatisreach.htm

[Hussein 16] Hussein, I. Abdel-Shafy *et al*., A review on polycyclic aromatic hydrocarbons: Source, environmental impact, effect on human health and remediation., Egypt, J. Pet., 2016, 25, p107-123

[Jing 16] Chen Jing, Determination of PAHs in Edible Oils by DACC-HPLC with Fluorescence Detection, Application note, 2016, available at: https://tools.thermofisher.com/content/sfs/brochures/AN-196-LC-PAHs-Edible-Oils-AN71492-EN.pdf

[Kärki 12] Sara Kärki, REACH registration of fast pyrolysis bio-oil, PyNe Issue 32, December 2012, p27-29

[Ohra-aho 17] Taina Ohra-aho, Anja Oasmaa, Method development and validation of formaldehyde, methanol, furfural determination by GC, Residue2Heat Deliverable D3.9, available at: http://www.residue2heat.eu/

[Rupert 06] Simon Rupert *et al*., Single-laboratory validation of a gas chromatography-mass spectrometry method for quantitation of 15 European priority polycyclic aromatic hydrocarbons in spiked smoke flavourings., J. Chromatogr. A, 1103, 2006, p307-313

[RO 17] http://www.rijksoverheid.nl/onderwerpen/gevaarlijke-stoffen/vraag-en-antwoord/wat-is-reach.html

[Saari 14] Pia Saari, REACH registration of fast pyrolysis bio-oil, PyNe Issue 34, January 2014, p4-5

[Simon 05] Rupert Simon *et al*., Composition and analysis of liquid smoke flavouring primary products, J. Sep. Sci., 2005, 28, p871-882

[Wenzl 06] Thomas Wenzl *et al*., Analytical methods for polycyclic aromatic hydrocarbons (PAHs) in food and the environment needed for new food legislation in the European Union, Trends Anal. Chem., Vol. 25, No. 7, 2006, p716-725

[WHO 00] http://www.euro.who.int/data/assets/pdf_file/0015/123063/AQG2ndEd_5_9PAH.pdf

Appendix: Additional information of applied methods

A. Laboratory 2 method; Adaptation of "BIOTOX"method:

The PAH-components (16 pc.) were analysed from a n-hexane (possibly FPBO sample was dissolved in ethanol before hexane extraction) extract of the sample (so not with cyclohexane in alkaline circumstances, as in BIOTOX). The sample was transferred to a extraction funnel and the 4 internal deuterated PAH standards were added (d8-naphtalene, d10-anthracene, d12-chrycene and d14-dibenzo[a,h]anthracene). The extract was then cleaned by using DMSO liquid-liquid partitioning. The PAH-compounds were analysed from the cleaned n-hexane extract using Gas-Chromatography-Mass Spectrometry (GC-MS) running in SIM-mode (Selected-Ion Monitoring). The quantitation was made using the Internal Standard Method.

A.1 Method: Extraction based on ISO 16703 standard (original BIOTOX procedure):

The BIOTOX method:

- Pour 5 g pyrolysis oil in a beaker
- add 1 ml internal standard solution with benzo(A)anthracene D12, c = 4 µg/ml
- add 1 ml internal standard solution with anthracene D10, c = 4 µg/ml
- mix with 10 ml NaOH and pour into separation funnel
- extract with 60 ml cyclohexane
- remove NaOH phase
- add 10 ml NaOH extract; remove NaOH phase
- dry cyclohexane extract over Na_2SO_4
- condition 2 g Florisil-cartrige (MgO_3Si) with 15 ml cyclohexane
- add cyclohexane-extract on cartridge
- use 50 ml cyclohexane for elution
- add keeper (100 µl DMF) to eluate evaporate solvent to dryness
- dissolve in 1ml cyclohexane and fill vial for GC-MS

B. "RUG"method:

To determine the retention times of the 16 PAH's, a known mixture of PAH's is injected in the GC with the MS detector in scan-modus (TIC). Subsequently a SIM method was developed in which the 16 PAH's are subdivided into four SIM-groups by looking at the retention times and the mass fragments observed. To each of the SIM-group an internal standard is added. For all 16 PAH's the relative response factors (RRF) are determined by making a 5 point calibration line in which the amount of internal standard is kept constant. Of each PAH, the peak area is determined and divided by the peak area of the internal standard. Similar to the areas also the concentrations are divided by the concentration of the internal standard. Then the values are plotted (concentration against area) in a graph with the obtained slope being the RRF (see Table 3 and Figure 2).

For samples: 1 part FPBO is diluted with 1 part of ethanol, to this mixtures the four internal standards (Naphtalene-d8, Anthracene-d10, Benzo[a]anthracene and Dibenz[1,2,3-cd]pyrene) are added. Subsequently this mixture is extracted with 8 parts of hexane, and the extract is washed in a 1:1 ratio with DMSO. The washed solution is then evaporated under a nitrogen atmosphere and the residue re-dissolved in toluene. The PAH's are qualified by their retention times and by abundance ration of the qualifier ions. To validate the PAH's analysis, a known amount of PAH's is added to the FPBO. This is done by adding 1 ml of calibration solution to the sample oil. This calibration solution already contains the internal standard and is therefore not extra added. The FPBO with calibration solution is then extracted and analyzed as described earlier and the recovery of the added PAH's is determined.

Table 4: Standards, PAH's, quantifier and qualifier ions used.

SIM Ion Group	#	Code	PAHs	Retention time (min)	Quantifier Ion, m/z	Qualifier Ions, m/z	m/z window Dwell time = 0.05 seconds
1	Istd 1	Nap-d8	Naphthalene-d8	6.54	136	136	127, 128, 129, 136, 151, 152, 153, 154, 165, 166, 167
	1	Nap	Naphthalene	6.56	128	128, 129, 127	
	2	Acy	Acenaphthylene	9.46	152	152, 151, 153	
	3	Ace	Acenaphthene	9.88	154	154, 153, 152	
	4	Flu	Fluorene	11.27	166	166, 165, 167	
2	5	Ph	Phenanthrene	14.38	178	178, 179, 176	101, 176, 178, 179, 188, 200, 202, 203
	Istd 2	An-d10	Anthracene-d10	14.48	188	188	
	6	An	Anthracene	14.57	178	178, 176, 179	
	7	Flt	Fluoranthene	19.13	202	202, 101, 203	
	8	Py	Pyrene	20.05	202	202, 200, 203	
3	9	BaA	Benzo[a]anthracene	25.50	228	228, 229, 226	125, 226, 228, 229, 240, 252, 253
	Istd 3	Chry-d12	Chrysene-d12	25.49	240	240	
	10	Chry	Chrysene	25.65	228	228, 226, 229	
	11	BbF	Benzo[b]fluoranthene	30.10	252	252, 253, 125	
	12	BkF	Benzo[k]fluoranthene	30.23	252	252, 253, 125	
	13	BaP	Benzo[a]pyrene	31.35	252	252, 253, 125	
4	14	InPy	Indeno[1,2,3-cd]pyrene	35.38	276	276, 138, 277	138, 139, 276, 277, 278, 279, 292
	Istd 4	DiahA-d14	Dibenz[a,h]anthracene-d14	35.54	292	292	
	15	DiahA	Dibenz[a,h]anthracene	35.57	278	278, 139, 279	
	16	BghiP	Benzo[g,h,i]perylene	36.21	276	276, 138, 277	