Report on the 8th inter-laboratory comparison organised by the European Union Reference Laboratory

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Radoslav Lizak, Szilard Szilagyi, Philippe Verlinde, and Thomas Wenzl *

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1   Summary

This report presents the results of the eighth inter-laboratory comparison (ILC) organised by the European Union Reference Laboratory for Polycyclic Aromatic Hydrocarbons (EU-RL PAHs) on the determination of the four marker PAHs, benz[a]anthracene (BAA), benzo[a]pyrene (BAP), benzo[b]fluoranthene (BBF) and chrysene (CHR) in a dry extract of St. John's wort. It was conducted in accordance with ISO Standard 17043.

In agreement with National Reference Laboratories (NRLs), the test material used in this exercise was a commercial product, naturally incurred with PAHs.

Both officially nominated National Reference Laboratories and official food control laboratories (OCLs) of the EU Member States were admitted as participants. Twenty-five NRLs and 23 OCLs subscribed for participation. One of the NRLs did not report results.

The participants were free to choose the method for the analysis of the materials. The four marker PAHs were chosen as target analytes as limits for the sum of these four contaminants were recently introduced in European legislation. The determination of the mentioned four PAHs was mandatory for the participants who had also to report the sum of the four analytes. The performance of the participating laboratories in the determination of the target PAHs in the test material was expressed by both z-scores and zeta-scores.

Participants also received a solution of PAHs in solvent of their choice (either toluene or acetonitrile) with undisclosed content for the verification of their instrument calibration.

A summary of the performance of the participants in the determination of the four marker PAHs in the food supplement test material is given in the following table.

Participant group	Reporting laboratories	Calculated z-scores	z-scores ≤ |2|	z-scores ≤ |2|	Calculated zeta-scores	zeta-scores ≤ |2|	zeta-scores ≤ |2|
#	#	#	#	%	#	#	%
NRLs	24	116	83	72	111	68	61
OCLs	23	109	70	64	51	29	57

In some cases, a bias was discovered; in particular, chrysene caused problems for laboratories applying gas chromatography with mass spectrometric detection.

2 Introduction

The Institute for Reference Materials and Measurements (IRMM) of the European Commission's Joint Research Centre hosts the European Union Reference Laboratory for Polycyclic Aromatic Hydrocarbons in Food (EU-RL-PAH). One of its core tasks is to organise inter-laboratory comparisons (ILCs) for the National Reference Laboratories (NRLs) [1, 2].

Polycyclic aromatic hydrocarbons (PAHs) constitute a large class of organic substances. The chemical structure of PAHs consists of two or more fused aromatic rings. PAHs may be formed during the incomplete combustion of organic compounds and can be found in the environment. In food, PAHs may be formed during industrial food processing and domestic food preparation, such as smoking, drying, roasting, baking, frying, or grilling.

In 2002, the European Commission's Scientific Committee on Food identified 15 individual PAHs as being of major concern for human health. These 15 EU priority PAHs should be monitored in food to enable long-term exposure assessments and to verify the validity of the use of the concentrations of benzo[*a*]pyrene (BAP) as a marker for a "total-PAH content" [3]. The toxicological importance of these compounds was confirmed in 2010 by the International Agency for Research on Cancer (IARC), which classified BAP as carcinogen to human beings (IARC group 1), and the other three target PAHs as possibly carcinogenic to human beings (group 2b) [4].
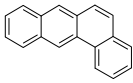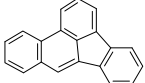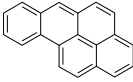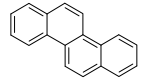
As a consequence, the European Commission (EC) issued Commission Regulation (EC) No 1881/2006 setting maximum levels of benzo[*a*]pyrene in food, and Commission Regulation (EC) No 333/2007 laying down sampling methods and performance criteria for methods of analysis for the official control of benzo[*a*]pyrene levels in foodstuffs [5, 6].

To evaluate the suitability of BaP as a marker for occurrence and toxicity of PAHs in food, the European Commission asked the European Food Safety Authority (EFSA) for a review of the previous risk assessment on PAHs carried out by the Scientific Committee on Food (SCF).

The scientific opinion on polycyclic aromatic hydrocarbons in food was published by EFSA's Panel on Contaminants in the Food Chain in June 2008 [7]. The Contaminants Panel concluded that benzo[*a*]pyrene was not a suitable indicator for the occurrence of PAHs in food and that, based on the currently available data relating to occurrence and toxicity, four (PAH4) or eight substances (PAH8) were the most suitable indicators of PAHs in food, with PAH8 not providing much added value compared to PAH4. Following these conclusions, an approach for risk management was agreed in the Standing Committee on the Food Chain and Animal Health. It was agreed that maximum levels should be set for four PAHs (PAH4) - BAA, BAP, BBF, and CHR. In addition, maximum levels for BAP would be maintained to ensure comparability of data. Consequently, maximum levels for the sum of the four PAHs were included in Commission Regulation (EU) No 835/2011, which amends Commission Regulation (EC) No 1881/2006 [8]. Coherently, also the Commission Regulation (EC)

No 333/2007 [5] laying down performance criteria of analysis methods for the official control of food, was extended to all four EU marker PAHs [9].The target PAHs are listed in **Table 1** together with the acronyms used in this report, and with their chemical structures.

Table 1: Names and structures of the four EU marker PAHs.

1	Benz[*a*]anthracene (BAA)		3	Benzo[*b*]fluoranthene (BBF)	
2	Benzo[*a*]pyrene (BAP)		4	Chrysene (CHR)	

3 Scope

As specified in Regulation (EC) No 882/2004 on official controls performed to ensure the verification of compliance with food and feed law, animal health and animal welfare rules [2], one of the core duties of EU-RLs is to organise inter-laboratory comparison tests (ILCs).

This inter-laboratory comparison study aimed to evaluate the comparability of analysis results reported by National Reference Laboratories (NRLs) and EU official food control laboratories (OCLs) for the four EU marker PAHs in a commercial food supplement sample, and to assess the influence of standard preparation and instrument calibration on the performance of individual participants. The appropriateness of the reported measurement uncertainty was also tested as this parameter is important in the compliance assessment of food with EU maximum levels.

The ILC was designed and evaluated according to ISO Standard 17043 and the International Harmonized Protocol for the Proficiency Testing of Analytical Chemistry Laboratories, further denoted as Harmonized Protocol [10, 11].

The IRMM is a PT provider accredited according to ISO Standard 17043 [10].

4   Participating Laboratories

Officially nominated National Reference Laboratories (NRLs) and official food control laboratories of the EU Member States were admitted as participants. The participants are listed in **Table 2** and **Table** 3.

Table 2: List of participating National Reference Laboratories

Institute	*Country*
AGES GmbH	AUSTRIA
WIV-ISP (Scientific Institute of Public Health)	BELGIUM
SGL - State General Laboratory, Environmental and other Food Contamination Laboratory	CYPRUS
State Veterinary Institute Prague	CZECH REPUBLIC
Danish Plant Directorate, Laboratory for Feed and Fertilizers	DENMARK
Technical University of Denmark (DTU)	DENMARK
Tartu Laboratory of Health Protection Inspectorate	ESTONIA
Finnish Food Safety Authority Evira	FINLAND
LABERCA, Laboratoire d'Etude des Résidus et des Contaminants dans les Aliments	FRANCE
Bundesamt für Verbraucherschutz und Lebensmittelsicherheit (BVL)	GERMANY
General Chemical State Laboratory	GREECE
Central Agricultural Office, Food & Feed Safety Directorate, Feed Investigation NRL	HUNGARY
Central Agricultural Office Food & Feed Safety Directorate, Food Residues Toxicological Dept	HUNGARY
Public Analyst's Laboratory Dublin	IRELAND
Istituto superiore di sanità	ITALY
Institut of Food Safety, Animal Health and Environment	LATVIA
National Food and Veterinary Risk Assessment Institute	LITHUANIA
National Institute of Public Health - National Institute of Hygiene	POLAND
Instituto Nacional de Recursos Biológicos	PORTUGAL
State Veterinary and Food Institute Dolny Kubin	SLOVAKIA
Zavod za zdravstveno varstvo Maribor	SLOVENIA
Centro Nacional de Alimentación	SPAIN
Livsmedelsverket (SLV)	SWEDEN
RIKILT	The NETHERLANDS
Food Environment Research Agency	UNITED KINGDOM

One of the 25 NRLs did not report results for this PT.

Table 3: List of participating Official Food Control Laboratories

Institute	Country
Federal Laboratory for the Safety of the Food Chain	BELGIUM
LARECO S.A.	BELGIUM
Laboratorium ECCA NV	BELGIUM
MTT Agrifood Research Finland	FINLAND
LEAV - Laboratoire de l'Environnement et de l'Alimentation	FRANCE
LDA56	FRANCE
IDAC	FRANCE
Laboraroire Departemental de la Sarthe	FRANCE
LDA 22	FRANCE
LDA 26	FRANCE
SCL Ile de France-Massy Laboratory	FRANCE
CVUA Freiburg	GERMANY
CVUA-Münsterland-Emscher-Lippe	GERMANY
Landesbetrieb Hessisches Landeslabor	GERMANY
LAVES Lebensmittelinstitut Braunschweig	GERMANY
Chemisches Untersuchungsamt Hagen	GERMANY
Landesamt für Verbraucherschutz Sachsen-Anhalt	GERMANY
Thüringer Landesamt für Lebensmittelsicherheit und Verbraucherschutz	GERMANY
LTZ Augustenberg	GERMANY
Landesuntersuchungsanstalt Sachsen	GERMANY
Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit	GERMANY
State Veterinary and Food Institute, Košice	SLOVAKIA
ANFACO-CECOPESCA	SPAIN

5   Time frame

The ILC was agreed with the NRLs at the EU-RL PAH workshop held in Brussels on 6 April 2011. It was announced on the IRMM web page (see ANNEX 1) and registration was opened on 15 May 2011 (see ANNEX 2). Test samples were dispatched (see ANNEX 3) on the 18 July 2011 and the deadline for reporting of results was set to finally 22 September 2011.

6   Confidentiality

The identities of participants are kept confidential unless the participant provides a letter of consent to the PT organiser giving permission to disclose his/her details and results to a third party.

7   Test materials

7.1   Preparation of test materials

The test material of this PT round was a food supplement, in particular a dry extract of St John's wort, in the following denoted as <u>FS</u>.

The test material was prepared from commercial products at the EU-RL PAH laboratories. About 60 units containing each 120 pills of a dry extract of St John's wort were acquired at local stores. They were then ground and homogenised with a Loedige Ploughshare® mixer. Portions of at least 15 g of the homogenised powder were packed into amber glass screw cap vials.

Participants also received a solution of the 15+1 EU priority PAHs in either acetonitrile or toluene (according to their choice) with disclosed concentrations, which served for checking instrument calibration. The technical specifications (see ANNEX 4) of the chosen solution were dispatched together with the test samples.

Besides the standard solution with disclosed content, a solution with undisclosed content was prepared. The participants were requested to report the analyte content of this solution to the organiser of the PT. This information was used to identify potential reason for bias.

7.2   Assigned values and standard deviation for proficiency assessment

The assigned values were determined from in-house measurements at the EU-RL PAH applying bracketing calibration, and from measurements conducted by a subcontracted external laboratory. The measurements at the EU-RL were conducted on two different days. Four replicate samples were analysed by bracketing calibration using a commercially available standard solution (Dr. Ehrenstorfer Standard 183) in each of the two analysis sessions. The contract laboratory had to perform three replicate analyses of two different samples, each composed of three units of PT test samples. The analyses had to be performed on two different days.

In cases of almost identical analysis results (difference between own results and results of contract laboratory < 5%) the results from the in-house measurements were applied as assigned values (applicable to BAA and BBF). If the difference between the two results exceeded 5 %, the average of the two results was applied as assigned value (applicable to BAP and CHR), and the combined uncertainty of in-house and external measurements was associated with the assigned value.

The sum of PAH 4 was calculated from the individually assigned values, and the corresponding uncertainty from the uncertainties of the assigned values according to equation 1. The results of in-house measurements and the results reported by the contract laboratory as well as the assigned values of the target PAHs are listed in Table 4.

Equation 1 $$u_{sum} = \sqrt{u_{BAA}^2 + u_{BAP}^2 + u_{BBF}^2 + u_{CHR}^2}$$

where u_{sum} refers to the standard uncertainty of the sum of the four PAHs and u_{BAA}, u_{BAP}, u_{BBF}, and u_{CHR} refer to the standard uncertainty of the individual analytes

The standard deviation for proficiency assessment (σ_P) was set for the four individual analytes equal to the maximum tolerated standard measurement uncertainty U_f (see Equation 2) as defined by Commission Regulation (EC) No 333/2007 [5] and Commission Regulation (EU) No 836/2011 [9].

Equation 2 $$U_f = \sqrt{(LOD/2)^2 + (\alpha C)^2}$$

where U_f relates to the maximum tolerated standard measurement uncertainty, LOD to the required limit of detection, α to a numeric factor depending on the concentration C.

The application of Equation 2 with the assigned values listed in Table 4, the maximum tolerated value of LOD of 0.30 μg/kg, and α equal to 0.2 results in U_f values as reported in Table 4.

The Uf values of the individual analytes are propagated in analogy to equation 1 for the determination of the Uf value for the sum of the four PAHs, which was used as standard deviation for proficiency assessment for the sum parameter.

Table 4: Analyte contents of the food supplement test material

			Average of two sessions, bracketing calibration	External evaluation	Assigned value[#] and σ_P
BAA	**Cont**	**µg/kg**	2.98	2.97	**2.98**
	U	**µg/kg**	0.24	0.41	**0.24**
	σ_P	**µg/kg**			**0.62**
BAP	**Cont**	**µg/kg**	1.57	1.74	**1.65**
	U	**µg/kg**	0.20	0.16	**0.26**
	σ_P	**µg/kg**			**0.36**
BBF	**Cont**	**µg/kg**	2.92	2.89	**2.92**
	U	**µg/kg**	0.28	0.34	**0.28**
	σ_P	**µg/kg**			**0.60**
CHR	**Cont**	**µg/kg**	4.24	3.89	**4.07**
	U	**µg/kg**	0.57	0.57	**0.81**
	σ_P	**µg/kg**			**0.83**
Sum PAH4	**Cont**	**µg/kg**			**11.62**
	U	**µg/kg**			**0.93**
	σ_P	**µg/kg**			**1.25**

\#   from chemical analysis. Sum of the four analytes   contents for the SUM

σ_p standard deviation for proficiency assessment. For the individual analytes is equal to the uncertainty function - *Uf* according to Ref [9]

U   expanded uncertainty of the assigned value (k=2).

The gravimetrical preparation concentrations were used as assigned values for the standard solutions with undisclosed content. Table 5 contains the assigned values and associated expanded uncertainties of the standard solutions with undisclosed content.

Table 5: Analyte contents of the standard solutions with undisclosed content.

	Solution in toluene		Solution in acetonitrile	
	Content	U (k=2)	Content	U (k=2)
	µg/kg	µg/kg	µg/kg	µg/kg
benz[*a*]anthracene	43.17	0.39	47.64	0.43
benzo[*c*]fluorene	73.42	0.61	80.52	0.67
chrysene	29.04	0.31	31.79	0.34
cyclopenta[*cd*]pyrene	25.58	0.50	28.18	0.55
benzo[*a*]pyrene	28.97	0.38	31.86	0.42
benzo[*b*]fluoranthene	29.26	0.26	31.99	0.29
benzo[*ghi*]perylene	22.14	0.23	24.38	0.26
benzo[*j*]fluoranthene	43.53	0.38	47.63	0.41
benzo[*k*]fluoranthene	21.43	0.17	23.80	0.19
dibenzo[*a,e*]pyrene	57.35	0.84	63.26	0.93
dibenzo[*a,h*]anthracene	22.15	0.32	24.45	0.35
dibenzo[*a,h*]pyrene	21.69	0.29	23.94	0.32
dibenzo[*a,i*]pyrene	36.91	1.00	40.35	1.09
dibenzo[*a,l*]pyrene	46.27	0.57	51.04	0.63
indeno[1,2,3-*cd*]pyrene	37.93	0.36	41.76	0.39
5-methylchrysene	85.89	0.87	94.43	0.96

Prep. conc.: concentration from gravimetrical preparation; U: expanded uncertainty of the assigned value; k: coverage factor

7.3 Homogeneity and stability

Homogeneity of the food supplements test sample was evaluated according to ISO Standard 13528. Ten units of the St John's wort extract test material were selected randomly and analysed by isotope dilution gas chromatography mass spectrometry (GC-MS). The test material was rated sufficiently homogeneous (see ANNEX 5).

The contributions of the uncertainties of homogeneity (relative uncertainties below 1 %) to the total uncertainties of the assigned values were found negligible and were therefore not considered in the calculations.

The stability of the test materials was evaluated applying an isochronous experimental set up. This comprised the storage of test samples both at room temperature and cooled in the fridge. The content values of the cooled sample were used as reference for the stability evaluation. The materials were stored from dispatch of test samples to the participants until expiry of the deadline for reporting of results. Afterwards the samples were analysed under repeatability conditions, and results were compared. The content values of the cool-stored sample and the sample stored at room temperature were not statistically significantly different. Hence stability of the samples over the whole study period can be assumed. The analysis data are presented in ANNEX 5.

The uncertainty of stability of the test material was considered negligible compared to the uncertainty of the characterisation measurements. Therefore, this parameter was not taken into account in the determination of the uncertainties of the assigned values.

7.4 Sample dispatch

Samples were dispatched at room temperature. Each participant received at least

- one ampoule of the solution of the 15+1 EU priority PAHs in the chosen solvent with disclosed content (2 ml),
- one ampoule of the 15+1 EU priority PAHs in the chosen solvent with undisclosed content (2 ml),
- one screw cap vial with the food supplement test material.

Each parcel contained a sample receipt form, the outline of the study, an appropriate material safety data sheets, and a unique code that was required for reporting of results. The documents sent to the participants are presented in ANNEX 6.

8 Design of the proficiency test

The design of the PT foresaw triplicate analyses of the test sample and reporting of the individual results of replicate analyses for the single analytes, in the following denoted as FS_REP, and additionally a "value for proficiency assessment", in the following denoted as "final value - FS_FIN", for both the single analytes and the sum of the four marker PAHs. Both FS_REP results and FS_FIN results had to be reported corrected for recovery (and recovery had to be stated in the questionnaire, which participants were asked to fill in, together with other parameters of the method applied); the latter had also to be accompanied by the respective expanded measurement uncertainty. The FS_FIN results were used for performance assessment.

Besides analysis results participants were also asked to report details of the applied analysis method and to provide answers to a questionnaire (see ANNEX 7 for the template and the compiled returned questionnaires).

9 Evaluation of the results

The results reported by participants are listed in ANNEX 8.

9.1 General

The most important evaluation parameter was the performance of the laboratories in the determination of the target PAHs in the food supplement test material, which was expressed by z-scores and zeta-scores.

The compliance of method performance characteristics specified by the participants with provisions given in legislation was evaluated too.

9.2 Evaluation criteria

The participants were requested to report for all analytes the results of replicate measurements and a "value for proficiency assessment", which is the result they wish to be applied for the calculation of performance indicators. z-Scores and zeta-scores were attributed only to these results. The individual results of replicate analyses were not rated.

z-Scores

z-scores were calculated based on the FS_FIN values. Equation 1 presents the formula for calculation of z-scores.

Equation 1
$$z = \frac{\left(x_{lab} - X_{assigned}\right)}{\sigma_P}$$

where z refers to the z-score, x_{lab} to the reported "value for proficiency assessment", $X_{assigned}$ to the assigned value, and σ_P to the standard deviation for proficiency testing.

zeta-Scores

In addition to z-scores zeta-scores were calculated. In contrast to z-scores, zeta-scores describe the agreement of the reported result with the assigned value within the respective uncertainties. Unsatisfactorily large zeta-scores might be caused by underestimated measurement uncertainties, large bias, or a combination of both. Zeta-scores were calculated according to Equation 3.

Equation 3
$$zeta = \frac{x_{lab} - X_{assigned}}{\sqrt{u_{lab}^2 + u_{assigned}^2}}$$

where *zeta* refers to the zeta-score, x_{lab} to the reported "value for proficiency assessment", $X_{assigned}$ to the assigned value, u_{lab} to the measurement uncertainty reported by the laboratory, and $u_{assigned}$ to the uncertainty of the assigned value.

The performance of the laboratories was classified according to ISO/IEC 17043:2010 [10]. Following scheme is applied for the interpretation of zeta scores and z-scores:

|score| ≤ 2.0 = satisfactory performance

2.0<|score| < 3.0 = questionable performance

|score| ≥ 3.0 = unsatisfactory performance

9.3 Performance of participants

The 48 participants in the study reported in total 225 results for proficiency evaluation, which equals to about 94 % of the 240 possible results. About 68 % of the reported results were rated as satisfactory with regard to z-scores.

Figure 1 and **Figure 2** give an overview of the z-scores assigned to the respective results. The larger the triangles, the larger were the differences to the assigned values. Red triangles indicate z-scores above an absolute value of three, whereas yellow triangles represent z-scores in the questionable performance range. The corresponding score values are plotted next to the triangles. About 40 % of the 72 non-satisfactory results were reported by seven laboratories only, e.g. the performance of participant 3608 was not satisfactory for the majority of target analytes.

It is also obvious that most problems were encountered in the determination of chrysene and the sum of the four analytes. Potential reasons for the difficulties related to chrysene are discussed further down.

The ideal situation would be that deviations from the assigned values of the results reported for the individual analytes are small and randomly distributed around zero. This will give a satisfactory performance statement for the sum parameter. Deviations of opposite sign could also cancel out and lead to a satisfactory performance for the sum parameter (e.g. participants 5303, 5304, and 5305).

However, in most cases a single underperformance for one analyte led also to non-satisfactory rating for the sum parameter.

The numerical values of the calculated z-scores are compiled in **Table 6** and **Table 7**. z-Scores with an absolute value of above 2 are given in red, bold font.

The results of the data evaluation for the individual analytes are given in ANNEX 8.

For each analyte the figure shows the individual analysis results of the three replicate determinations. The assigned value is shown as dotted line. The blue boxes represent the expanded uncertainties as reported by participants for the "value for proficiency assessment". The arithmetic mean of the results of the individual participants is indicated in the blue boxes by a blue line. The satisfactory performance range is located between the two red lines.

The individual results of the replicate measurements and the "value for proficiency assessment" with its accompanying expanded measurement uncertainty (k=2) are listed in the tables in ANNEX 8 as well.

Figure 1: Graphical presentation of z-scores corresponding to the "values for proficiency assessment" reported by the **NRLs** for the contents of BAA, BAP, BBF, and CHR in the St John's wort extract test material.

Blue triangles indicate satisfactory performance; yellow triangles indicate questionable performance; red triangles indicate non-satisfactory performance; z-score values are presented above the triangles for the latter two performance categories.
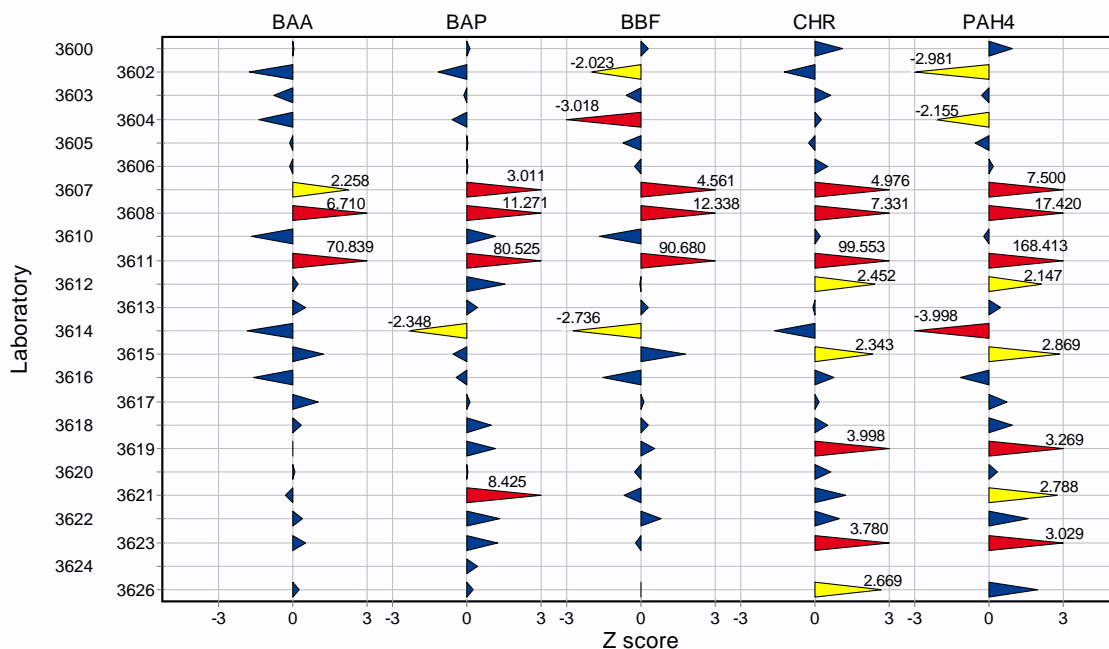
Figure 2: Graphical presentation of z-scores corresponding to the "values for proficiency assessment" reported by the **OCLs** for the contents of BAA, BAP, BBF, and CHR in the St John's wort extract test material.

Blue triangles indicate satisfactory performance; yellow triangles indicate questionable performance; red triangles indicate non-satisfactory performance; z-score values are presented above the triangles for the latter two performance categories.

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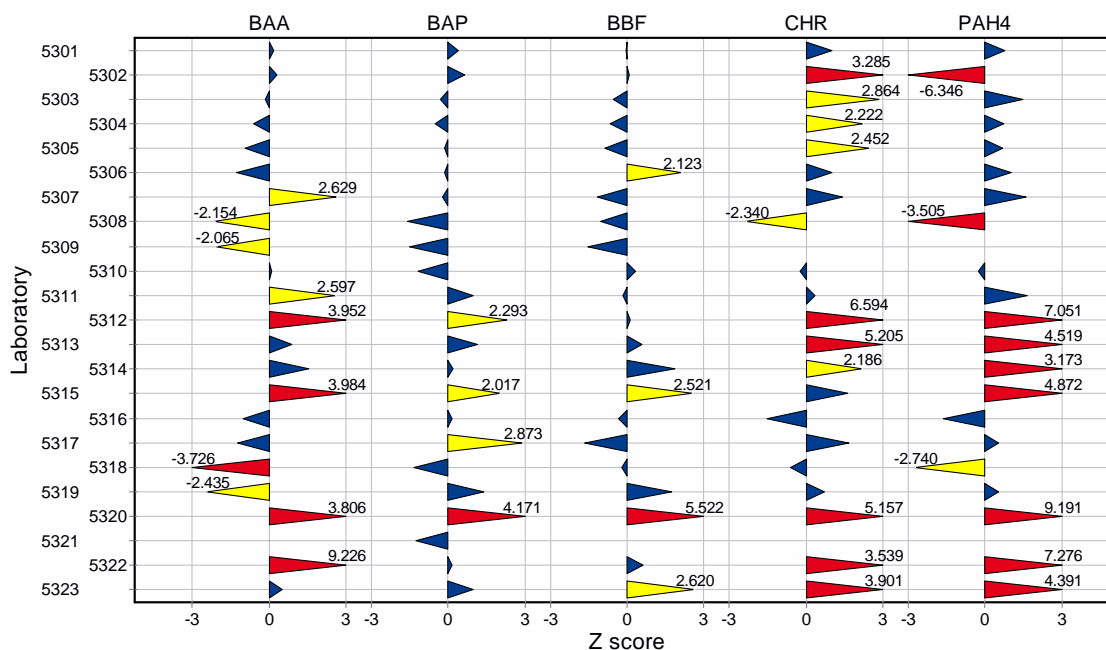
Table 6: Compilation of z-scores calculated from the "results for proficiency assessment" reported by the NRLs for food supplement test material: z-scores outside the satisfactory range (|z| > 2) are indicated by red font. Values are reproduced as reported by the laboratories. Empty cells denote analytes for which results were not reported.

Measurand		benz[a]anthracene		benzo[a]pyrene		benzo[b]fluoranthene		chrysene		Sum PAH 4	
Assigned value	µg/kg	2.98		1.65		2.92		4.07		11.62	
Standard deviation for proficiency assessment	µg/kg	0.62		0.36		0.60		0.83		1.25	
Laboratory code		Result	z-score	Result	z-score	Result	z-score	Result	z-score	Result	z-score
		µg/kg		µg/kg		µg/kg		µg/kg		µg/kg	
3600		3	0.0	1.7	0.1	3.1	0.3	5	1.1	12.8	0.9
3602		1.9	-1.8	1.24	-1.1	1.7	**-2.0**	3.04	-1.2	7.9	**-3.0**
3603		2.51	-0.8	1.61	-0.1	2.56	-0.6	4.59	0.6	11.27	-0.3
3604		2.13	-1.4	1.43	-0.6	1.1	**-3.0**	4.29	0.3	8.93	**-2.2**
3605		2.91	-0.1	1.67	0.1	2.47	-0.7	3.86	-0.3	10.91	-0.6
3606		2.89	-0.1	1.67	0.1	2.76	-0.3	4.51	0.5	11.84	0.2
3607		4.38	**2.3**	2.74	**3.0**	5.67	**4.6**	8.19	**5.0**	20.98	**7.5**
3608		7.14	**6.7**	5.73	**11.3**	10.36	**12.3**	10.14	**7.3**	33.36	**17.4**
3610		1.96	-1.7	2.06	1.1	1.91	-1.7	4.24	0.2	11.38	-0.2
3611		46.9	**70.8**	30.8	**80.5**	57.6	**90.7**	86.5	**99.6**	221.8	**168.4**
3612		3.1	0.2	2.2	1.5	2.9	0.0	6.1	**2.5**	14.3	**2.1**
3613		3.3	0.5	1.8	0.4	3.1	0.3	4	-0.1	12.2	0.5
3614		1.85	-1.8	0.8	**-2.3**	1.27	**-2.7**	2.71	-1.6	6.63	**-4.0**
3615		3.76	1.3	1.45	-0.6	4	1.8	6.01	**2.3**	15.2	**2.9**
3616		2	-1.6	1.5	-0.4	2	-1.5	4.7	0.8	10.2	-1.1
3617		3.62	1.0	1.69	0.1	2.99	0.1	4.22	0.2	12.52	0.7
3618		3.2	0.4	2	1.0	3.1	0.3	4.5	0.5	12.8	0.9
3619		2.99	0.0	2.07	1.2	3.26	0.6	7.38	**4.0**	15.7	**3.3**
3620		3.02	0.1	1.66	0.0	2.76	-0.3	4.6	0.6	12.04	0.3
3621		2.8	-0.3	4.7	**8.4**	2.5	-0.7	5.1	1.2	15.1	**2.8**
3622		3.22	0.4	2.13	1.3	3.4	0.8	4.88	1.0	13.61	1.6
3623		3.3	0.5	2.1	1.2	2.8	-0.2	7.2	**3.8**	15.4	**3.0**
3624				1.8	0.4						
3626		3.14	0.3	1.74	0.2	2.92	0.0	6.28	**2.7**	14.09	2.0

Table 7: Compilation of z-scores calculated from the "results for proficiency assessment" reported by the OCLs for food supplement test material: z-scores outside the satisfactory range (|z| > 2) are indicated by red font. Values are reproduced as reported by the laboratories. Empty cells denote analytes for which results were not reported.

Measurand		benz[a]anthracene		benzo[a]pyrene		benzo[b]fluoranthene		chrysene		Sum PAH 4	
Assigned value	µg/kg	2.98		1.65		2.92		4.07		11.62	
Standard deviation for proficiency assessment	µg/kg	0.62		0.36		0.60		0.83		1.25	
Laboratory code		Result	z-score	Result	z-score	Result	z-score	Result	z-score	Result	z-score
		µg/kg		µg/kg		µg/kg		µg/kg		µg/kg	
5301		3.1	0.2	1.8	0.4	2.9	0.0	4.9	1.0	12.6	0.8
5302		3.16	0.3	1.88	0.6	2.98	0.1	6.79	**3.3**	3.7	**-6.3**
5303		2.876	-0.2	1.535	-0.3	2.586	-0.6	6.441	**2.9**	13.438	1.5
5304		2.61	-0.6	1.46	-0.5	2.53	-0.6	5.91	**2.2**	12.51	0.7
5305		2.4	-0.9	1.6	-0.1	2.4	-0.9	6.1	**2.5**	12.5	0.7
5306		2.2	-1.3	1.6	-0.1	4.2	**2.1**	4.9	1.0	12.9	1.0
5307		4.61	**2.7**	1.57	-0.2	2.21	-1.2	5.24	1.4	13.63	1.6
5308		1.6446	**-2.2**	1.07009	-1.6	2.29848	-1.0	2.13243	**-2.3**	7.2456	**-3.5**
5309		1.7	**-2.1**	1.1	-1.5	2	-1.5				
5310		3.05	0.1	1.23	-1.2	3.13	0.3	3.86	-0.3	11.27	-0.3
5311		4.59	**2.6**	2	1.0	2.81	-0.2	4.37	0.4	13.67	1.6
5312		5.43	**4.0**	2.48	**2.3**	2.99	0.1	9.53	**6.6**	20.42	**7.1**
5313		3.53	0.9	2.07	1.2	3.28	0.6	8.38	**5.2**	17.26	**4.5**
5314		3.94	1.6	1.72	0.2	4.04	1.9	5.88	**2.2**	15.58	**3.2**
5315		5.45	**4.0**	2.38	**2.0**	4.44	**2.5**	5.42	1.6	17.7	**4.9**
5316		2.35	-1.0	1.7	0.1	2.73	-0.3	2.82	-1.5	9.6	-1.6
5317		2.22	-1.2	2.69	**2.9**	1.92	-1.7	5.45	1.7	12.29	0.5
5318		0.67	**-3.7**	1.17	-1.3	2.79	-0.2	3.57	-0.6	8.2	**-2.7**
5319		1.47	**-2.4**	2.16	1.4	3.96	1.7	4.65	0.7	12.25	0.5
5320		5.34	**3.8**	3.16	**4.2**	6.25	**5.5**	8.34	**5.2**	23.09	**9.2**
5321				1.2	-1.2						
5322		8.7	**9.2**	1.7	0.1	3.3	0.6	7	**3.5**	20.7	**7.3**
5323		3.3	0.5	2	1.0	4.5	**2.6**	7.3	**3.9**	17.1	**4.4**

Table 8 and **Table 9** present the respective zeta-scores. As for the z-scores, data outside the satisfactory performance range are given in red, bold font. Zeta scores were only calculated for laboratories that provided measurement uncertainty data as well as a numerical value for the coverage factor. Zeta scores were not calculated when the reported uncertainty value exceeded the content value, which is probably linked to mixing of reporting units (µg/kg and %). However, the participants are reminded that the measurement uncertainty shall be expressed in the same unit as the content value.

The assessment of the performance of the participants based on the reported measurement uncertainty gave a less favourable picture compared to z-scores. Only 60% of the zeta-scores are within the satisfactory performance range. The distribution of satisfactory performance ratings between NRLs and OCLs is not balanced. It was higher for the NRLs.

It has to be noted that the magnitude of the zeta-scores were for some participants much higher than the z-scores attributed to the same results. Consequently the laboratories perform according to fitness-for-purpose criterion specified in legislation, which forms the basis for the z-scores, but seem to have difficulties in deducing realistic measurement uncertainty values. E.g. the relative standard measurement uncertainties reported by the NRLs ranged from 2.5 % to 20 %., with a median value of about 10 %. Only two OCLs reported measurement uncertainties that exceeded the maximum tolerable uncertainty (U_f). The respective data is highlighted in yellow in **Table 9**.

However, the EU-RL PAHs will continue to pay special attention to measurement uncertainty in future ILCs, as it has major implications on the assessment of compliance of food with European legislation.

Table 8: Compilation of zeta-scores calculated from the "results for proficiency assessment" reported by the NRLs for test material St John's wort, the reported measurement uncertainty, and the uncertainty of the analyte content of the test material: zeta-scores outside the satisfactory range (|zeta| > 2) are indicated by red bold font. Empty cells denote analytes for which either results or measurement uncertainties were not reported.

Measurand	benz[a]anthracene				benzo[a]pyrene				benzo[b]fluoranthene				chrysene				Sum PAH 4			
Assigned value (µg/kg)	2.98				1.65				2.92				4.07				11.6			
U assigned (k=1) (µg/kg)	0.12				0.13				0.14				0.41				0.47			
Laboratory code	Result (µg/kg)	U (µg/kg)	k	zeta-score	Result (µg/kg)	U (µg/kg)	k	zeta-score	Result (µg/kg)	U (µg/kg)	k	zeta-score	Result (µg/kg)	U (µg/kg)	k	zeta-score	Result (µg/kg)	U (µg/kg)	k	zeta-score
3600	3	0.5	2	0.1	1.7	0.3	2	0.3	3.1	0.9	2	0.4	5	0.7	2	1.7	12.8	2.4	2	0.9
3602	1.9	0.21	2		1.24	0.15			1.7	0.17			3.04	0.36			7.9	1.26		
3603	2.51	0.22	2	**-2.9**	1.61	0.15	2	-0.3	2.56	0.25	2	-1.9	4.59	0.48	2	1.1	11.27	0.96	2	-0.5
3604	2.13	0.71	2	**-2.3**	1.43	0.4	2	-0.9	1.1	0.28	2	**-9.2**	4.29	1.19	2	0.3	8.93	2.58	2	**-2.0**
3605	2.91	0.76	2	-0.2	1.67	0.57	2	0.1	2.47	0.74	2	-1.1	3.86	0.85	2	-0.4	10.91	3.05	2	-0.4
3606	2.89	0.64	2	-0.3	1.67	0.31	2	0.1	2.76	0.46	2	-0.6	4.51	1.24	2	0.6	11.84	2.38	2	0.2
3607	4.38	0.96	2	**2.8**	2.74	0.55	2	**3.6**	5.67	1.13	2	**4.7**	8.19	1.8	2	**4.2**	20.98	4.2	2	**4.4**
3608	7.14	1.49	2	**5.5**	5.73	2.26	2	**3.6**	10.36	3.26	2	**4.5**	10.14	2.84	2	**4.1**	33.36	3.25	2	**12.9**
3610	1.96	0.39	2	**-4.5**	2.06	0.42	2	1.7	1.91	0.38	2	**-4.3**	4.24	0.85	2	0.3	11.38	1.7	2	-0.2
3611	46.9	9.6	2	**9.1**	30.8	3.6	2	**16.2**	57.6	7.5	2	**14.6**	86.5	6.1	2	**26.8**	221.8	25.9	2	**16.2**
3612	3.1	0.6	2	0.4	2.2	0.5	2	2.0	2.9	0.6	2	-0.1	6.1	1.2	2	**2.8**	14.3	2.9	2	1.8
3613	3.3	0.7	2	0.9	1.8	0.5	2	0.5	3.1	0.6	2	0.5	4	0.8	2	-0.1	12.2	2	2	0.5
3614	1.85	0.37	2	**-5.1**	0.8	0.16	2	**-5.6**	1.27	0.22	2	**-9.3**	2.71	0.54	2	**-2.8**	6.63	1.29	2	**-6.3**
3615	3.76	0.75	2	2.0	1.45	0.29	2	-1.0	4	0.8	2	**2.5**	6.01	1.2	2	**2.7**	15.2	3.04	2	**2.3**
3616	2	0.1	2	**-7.5**	1.5	0.3	2	-0.8	2	0.1	2	**-6.2**	4.7	0.5	2	1.3	10.2	0.3	2	**-2.9**
3617	3.62	0.46	2	**2.5**	1.69	0.23	2	0.2	2.99	0.35	2	0.3	4.22	0.65	2	0.3	12.52	1.69	2	0.9
3618	3.2	0.5	2	0.8	2	0.2	2	**2.1**	3.1	0.5	2	0.6	4.5	0.6	2	0.9	12.8	1.8	2	1.2
3619	2.99	0.48	2	0.0	2.07	0.75	2	1.1	3.26	0.64	2	1.0	7.38	1.18	2	**4.6**	15.7	3.05	2	**2.6**
3620	3.02	0.45	2	0.2	1.66	0.17	2	0.1	2.76	0.69	2	-0.4	4.6	0.92	2	0.9	12.04	1.2	2	0.6
3621	2.8	0.4	2	-0.8	4.7	0.5	2	**10.8**	2.5	0.4	2	-1.7	5.1	0.6	2	2.0	15.1	1.9	2	**3.3**
3622	3.22	0.52	2	0.8	2.13	0.38	2	**2.1**	3.4	0.52	2	1.6	4.88	0.8	2	1.4	13.61	1.15	2	**2.7**
3623	3.3	0.7	2	0.9	2.1	0.3	2	**2.3**	2.8	0.2	2	-0.7	7.2	0.5	2	**6.6**	15.4	0.9	2	**5.8**
3624					1.8	0.31	2	0.7												
3626	3.14	1.26	2	0.2	1.74	0.7	2	0.2	2.92	1.17	2	0.0	6.28	2.51	2	1.7	14.09	5.6	2	0.9

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Table 9: Compilation of zeta-scores calculated from the "results for proficiency assessment" reported by the OCLs for test material St John's wort, the reported measurement uncertainty, and the uncertainty of the analyte content of the test material: zeta-scores outside the satisfactory range (|zeta| > 2) are indicated by red bold font. yellow highlighted cells: u>Uf, Empty cells denote analytes for which results were not reported.

Measurand	benz[a]anthracene				benzo[a]pyrene				benzo[b]fluoranthene				chrysene				Sum PAH 4			
Assigned value (µg/kg)	2.98				1.65				2.92				4.07				11.6			
u assigned (k=1) (µg/kg)	0.12				0.13				0.14				0.41				0.47			
Laboratory code	Result (µg/kg)	U (µg/kg)	k	zeta-score	Result (µg/kg)	U (µg/kg)	k	zeta-score	Result (µg/kg)	U (µg/kg)	k	zeta-score	Result (µg/kg)	U (µg/kg)	k	zeta-score	Result (µg/kg)	U (µg/kg)	k	zeta-score
5301	3.1	0.6	2	0.4	1.8	0.4	2	0.6	2.9	0.6	2	-0.1	4.9	1	2	1.3	12.6			
5302	3.16	0.63	2	0.5	1.88	0.38	2	1.0	2.98	0.6	2	0.2	6.79	1.36	2	**3.4**	3.7	0.74	2	**-13.3**
5303	2.876	27	62		1.535	19	80		2.586	22	78		6.441	24	70		13.44	92	2	
5304	2.61	5.7	2	-0.1	1.46	6.9	2	-0.1	2.53	8.5	2		5.91	8.5	2		12.51	8.5	2	
5305	2.4	20			1.6	15			2.4	15			6.1	20			12.5	20		
5306	2.2	1.1	2	-1.4	1.6	0.8	2	-0.1	4.2	2.1	2	1.2	4.9	2.5	2	0.6	12.9	6.5	2	0.4
5307	4.61				1.57				2.21				5.24				13.63			
5308	1.645				1.07				2.298				2.132				7.246			
5309	1.7				1.1				2											
5310	3.05	0.15	2	**3.6**	1.23	0.06	2		3.13	0.16	2		3.86	0.19	2		11.27	0.56	2	
5311	4.59	15	2		2	15	2		2.81	15	2		4.37	15	2		13.67	15	2	
5312	5.43	1.34	2	**4.4**	2.48	0.5	2	**2.9**	2.99	0.75	2	0.2	9.53	1.91	2	**5.3**	20.42	4.08	2	**4.2**
5313	3.53				2.07				3.28				8.38				17.26			
5314	3.94	5	1		1.72	11	1		4.04	34	1		5.88	18	1		15.58			
5315	5.45	1.1	2	**4.4**	2.38	0.5	2	**2.6**	4.44	0.9	2	**3.2**	5.42	1.1	2	2.0	17.7	3.5	2	**3.4**
5316	2.35	0.22	2	**-3.9**	1.7	0.16	2	0.3	2.73	0.18	2	-1.1	2.82	0.6	2	**-2.5**	9.6	0.73	2	**-3.4**
5317	2.22	0.07	2	**-6.1**	2.69	0.12	2	**7.3**	1.92	0.06	2	**-7.0**	5.45	0.46	2	**3.0**	12.29	0.66	2	1.2
5318	0.67	0.1	2	**-17.8**	1.17	0.2	2	**-2.9**	2.79	0.4	2	-0.5	3.57	0.5	2	-1.1	8.2	0.5	2	**-6.5**
5319	1.47	0.29	2	**-8.0**	2.16	0.43	2	2.0	3.96	0.79	2	**2.5**	4.65	1.16	2	0.8	12.25	2.67	2	0.4
5320	5.34	4.8			3.16	7.8			6.25	1.4			8.34	9.9			23.09	4.1		
5321					1.2															
5322	8.7	35	2		1.7	35	2		3.3	35	2		7	35	2		20.7	35	2	
5323	3.3	1.6	2	0.4	2	1	2	0.7	4.5	2.7	2	1.2	7.3	3.7	2	1.7	17.1	10.3	2	1.1

9.4 Evaluation of the influence of analysis method

The information on the applied analysis method (ANNEX 11) was used to group the performance indicators attributed to the reported results according to the applied analysis technique. Two groups of data were formed. They represent results obtained by high performance liquid chromatography-fluorescence detection (HPLC-FD, about 40 %) and by gas chromatography-mass spectrometry (GC-MS, about 60 %) respectively. Kernel density plots were constructed based on the respective z-scores. Figure 3A to 3E show the different Kernel density plots.

Figure 3: Kernel density plots of z-scores attributed to the results for proficiency assessment, which were reported for the food supplements test sample. Results were classified according to the applied analysis technique.
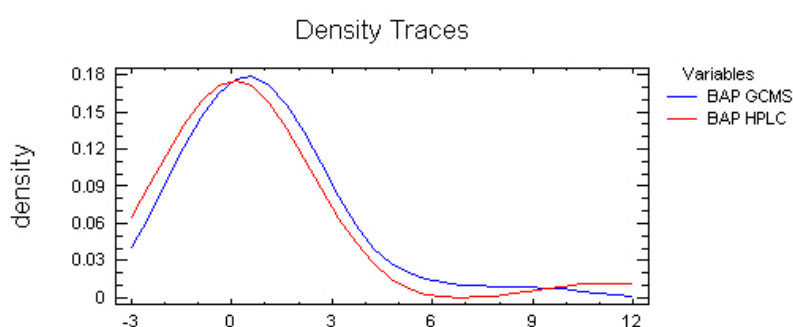
A) benz[*a*]anthracene
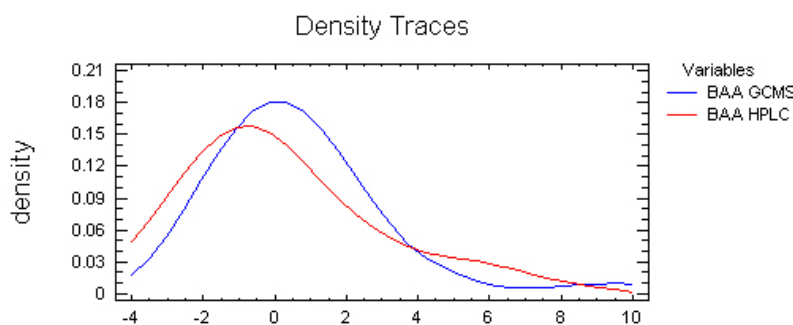
B) benzo[a]pyrene

Figure 3: continued

C) benzo[*b*]fluoranthene
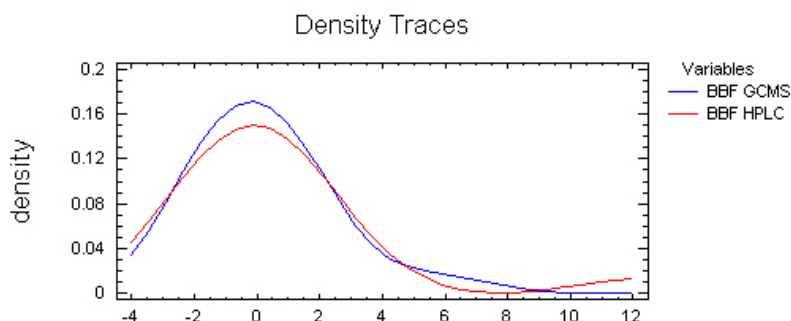
D) chrysene
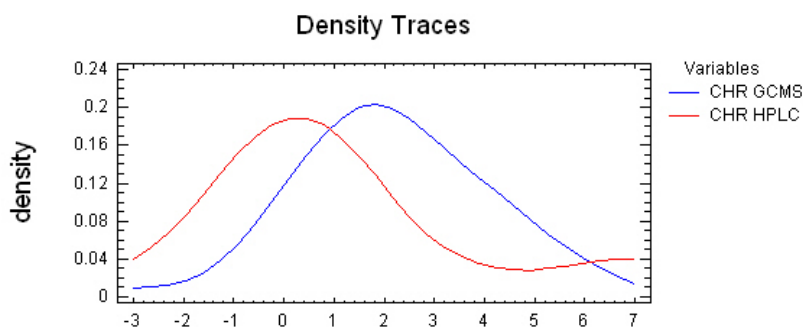
E) sum of four marker PAHs
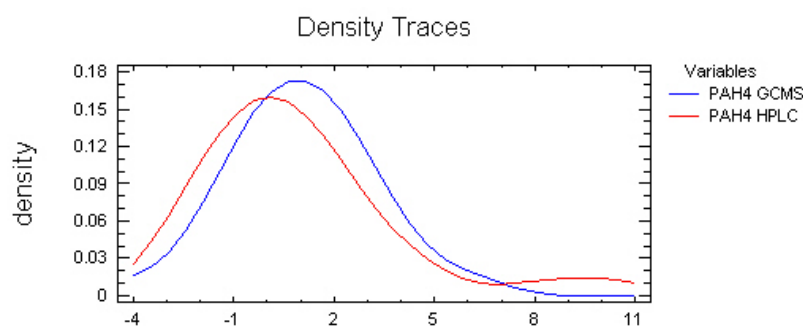
The kernel density plots for BAP and BBF are almost identical and centred at or close to a z-score value of zero, as can be seen in Figure 3B and 3C. Therefore, bias introduced by the type of analysis method is unlikely for these two analytes. The situation is different for BAA and CHR. In case of BAA, the kernel density distribution of z-scores based on HPLC-FD is shifted to negative values

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(Figure 3A), which indicates a tendency to underestimate the BAA content in this particular sample/matrix when applying HPLC-FD. This might be reasoned by separation/integration problems between BAA and CHR peaks. A few participants (from the HPLC-FD subgroup) that got negative z-scores for BAA overestimated the CHR content. This caused the slight shift of the density trace for HPLC-FD towards negative values. Removing this data from the evaluation would result in a density trace with the mode at zero.

The opposite is the case for CHR (Figure 3D). The density distribution of the z-scores attributed to HPLC-FD data is centred almost at zero. However, the distribution for data based on GC-MS is shifted to positive values, indicating strong positive bias. This bias might be caused by a lack of selectivity of the analysis method. The test material contains triphenylene, which is isobaric to chrysene. Consequently, it cannot be distinguished from chrysene by means of mass spectrometry. These two compounds have to be separated chromatographically, which is not easy. They coelute on unpolar and on most mid-polar capillary columns. Columns that allow their separation were commercialised only recently, and might not be available in all laboratories. Therefore, laboratories, which determine the four EU marker PAHs by means of GC-MS shall pay attention to the chromatographic separation of triphenylene and chrysene. A lack of chromatographic resolution might lead to strong positive bias of GC-MS methods and to false conclusions on the marketability of the tested sample.

The chromatographic separation of chrysene and triphenylene is not a problem with HPLC, which is reflected in the position of the density plot for chrysene. However, attention has to be paid in HPLC-FD analysis to a proper clean up of the sample extract, in order to eliminate interferences that show similar retention as BAA and CHR.

9.5   Evaluation of the influence of calibration

The participants received together with the food supplement test sample two standard solutions in, depending of their choice, either acetonitrile or toluene. The analyte content of one solution was disclosed to the participants, whereas the content of the other solution was kept undisclosed. The content values of the latter are presented in Table 5. Analysis results for the solution with undisclosed content had to be reported to the PT organiser. The respective data are presented in Annex 9.

Percent deviations of the reported results from the assigned values were calculated for both the food supplement test sample and the standard solution with undisclosed analyte content. Youden plots were prepared in order to visualize the influence of instrument calibration on the results reported for the food supplement sample. Two plots are discussed in the following. The whole set of Youden plots are given in Annex 9.

Figure 4: Youden plot of the percent deviations of values reported for benzo[*a*]pyrene from the assigned values of the food supplement test material and the standard solution in toluene with undisclosed content.
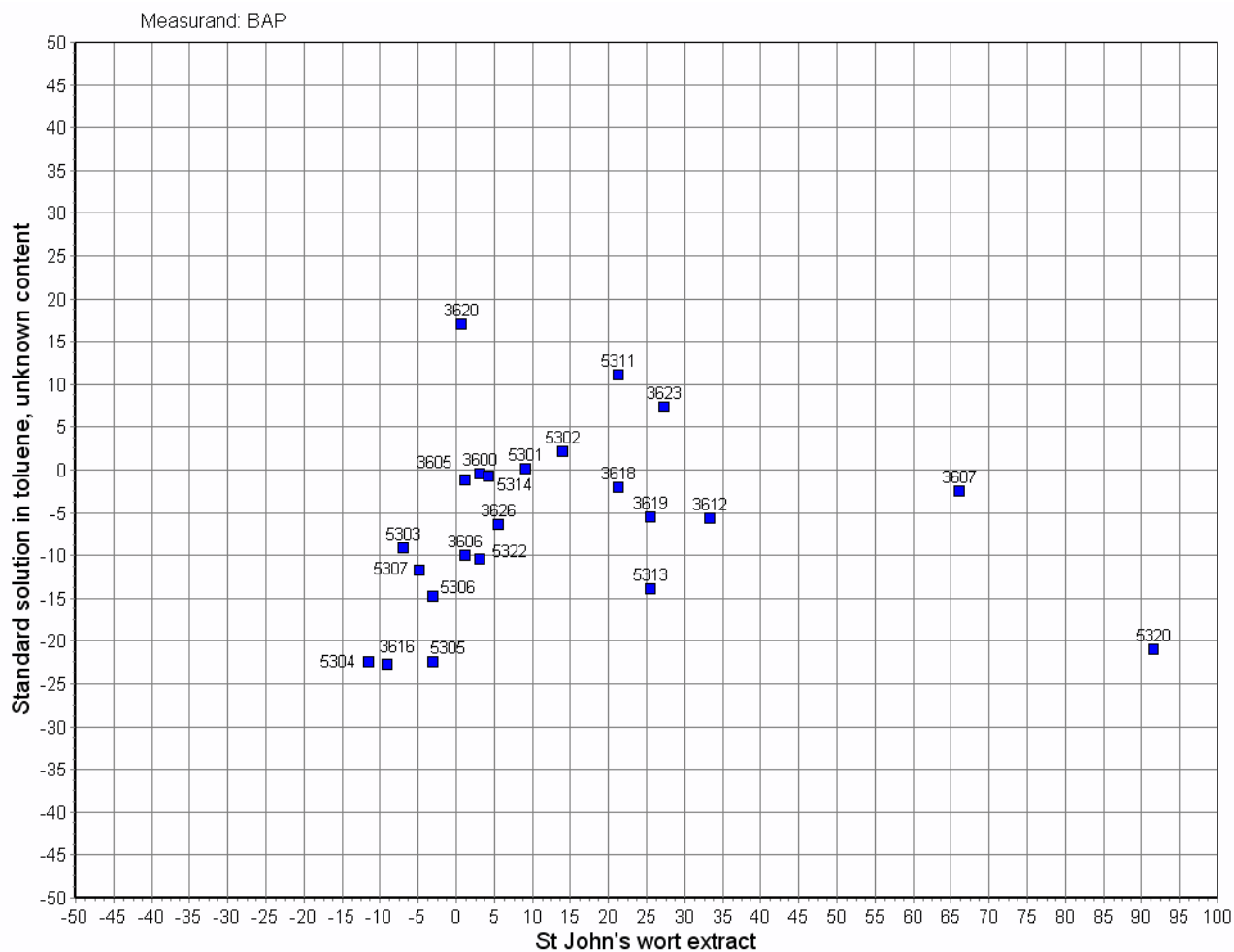
Figure 4 reveals that a number of participants reported results with similar percent deviations from the assigned values of both the results for the food supplement material and the standard solution in toluene with undisclosed content. This correlation indicates that over- respectively underestimation of the analyte content of the food supplement material might be caused by biased instrument calibration. This finding underpins the importance of thorough calibration standard preparation. However, the data was not in all cases conclusive, as some laboratories performed well for the food supplement samples, but reported biased results for the standard solution with undisclosed content (e.g. Figure 4 participants 3620 and 5305). This fact cannot be explained yet.

Figure 5 presents the respective Youden plot for chrysene in the food supplement test material and the standard solution in toluene with undisclosed content. From this graph it can be concluded that the overestimation of the chrysene content of the food supplement material was not caused by instrument calibration. The results for the solvent solution of many laboratories, which underperformed for the

food supplement sample, agreed well with the assigned value. This supports the conclusion that the overestimation is caused by the determination of an interferent, most probably triphenylene.

Figure 5: Youden plot of the percent deviations of values reported for chrysene from the assigned values of the food supplement test material and the standard solution in toluene with undisclosed content.

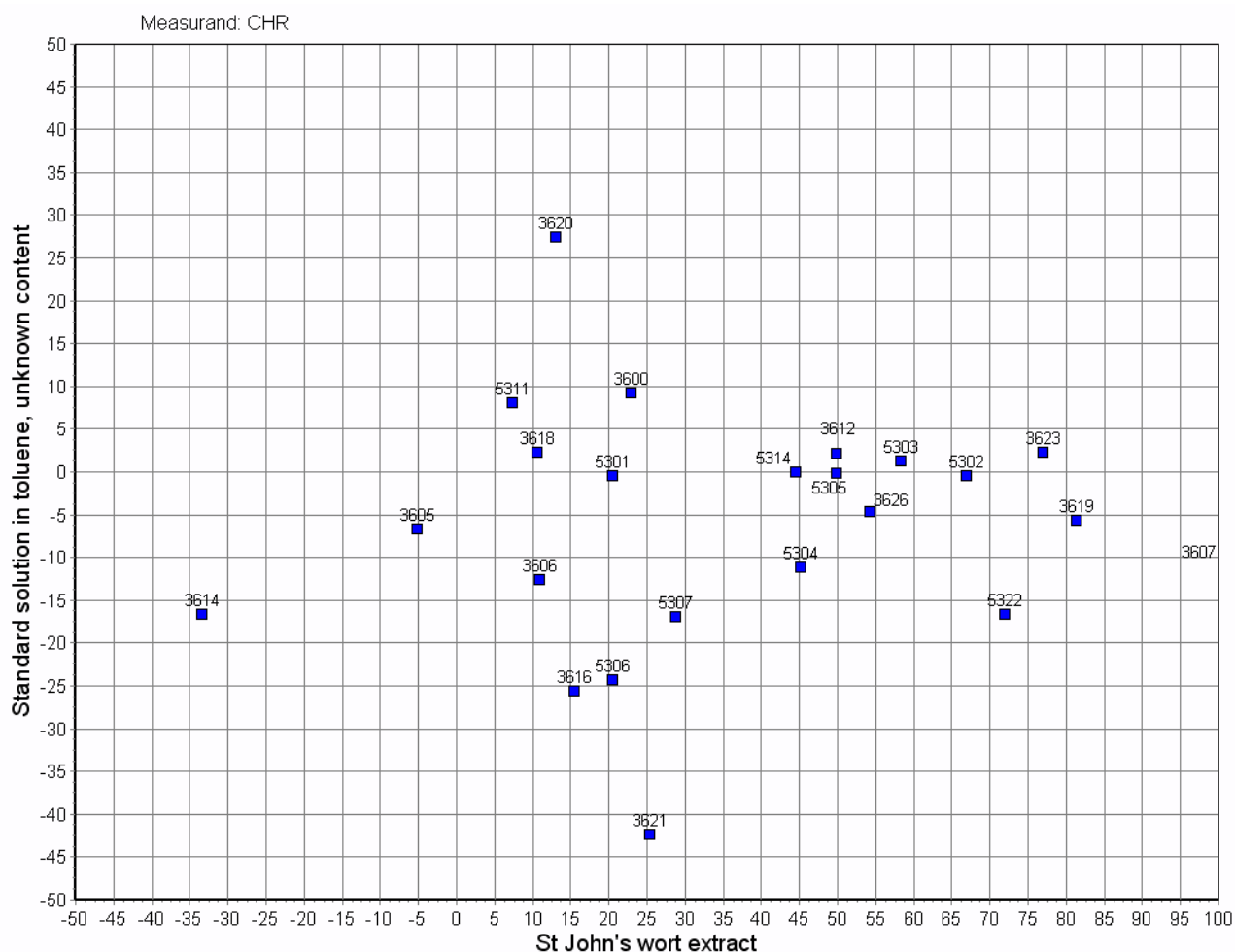## 9.6 Evaluation of compliance with legislation

The method performance data reported for the four marker PAHs were evaluated for compliance with the provisions given in Commission Regulation (EC) No 836/2011. Table 18 in ANNEX 10 gives an overview on the results of the evaluation. Data were not reported in case of empty cells. Non-compliant data are highlighted in Table 18. It should be noted that Regulation (EU) No 836/2011 specifies LOD and LOQ with two decimals. This was not respected by all participants, which hampered the compliance assessment.

In summary it can be stated that the majority of participants reported method performance characteristics for the determination of the four marker PAHs in the food supplement test sample that are compliant with legislation. However, some laboratories need to improve their analysis methods.

The laboratories in question are urged to adapt to the provisions given in the new legislation.

10   Follow-up actions for underperforming laboratories

All laboratories that got "questionable" or "non-satisfactory" performance ratings are urged to perform root cause analysis, and to implement corrective actions.

As agreed during the EU-RL PAH workshop 2011, the EU-RL PAH will set up follow-up measures in due time for all NRLs that received for at least one of the four PAHs (BAA, BAP, BBF, and CHR) z-scores > |3|, as required by Regulation (EC) 882/2004, and by the Protocol for management of underperformance in comparative testing and/or lack of collaboration of National Reference Laboratories (NRLs) with European Union reference laboratories (EU-RLs) activities.

These laboratories shall perform as an immediate action root-cause-analysis, and shall report to the EU-RL PAH in writing the identified cause for their underperformance and corrective actions they are going to take. Additionally, they shall participate to an independent (non-EU-RL) proficiency test on the determination of PAHs in food and shall communicate the outcome of this exercise to the EU-RL PAH.

11 Conclusions

Fourty seven participants reported results back to the EU-RL. The performance of the majority of participants was good. In total 153 out of 225 attributed z-scores were below an absolute value of two, which equals to almost 68 %. However, this percentage is much below the percentages of satisfactory performance gained in previous studies. This might be the consequence of the higher level of difficulty with respect to matrix composition. In addition bias can be concluded from the pattern of performance indicators of some laboratories.

In general, the determination of chrysene caused most difficulties to the participants.

zeta-Scores were calculated besides z-scores. They indicate the agreement of the reported result with the assigned value with respect to the stated measurement uncertainty. The outcome of this rating was worse than for the z-scores, which indicates that the measurement uncertainty estimates were not realistic. Therefore, participants underperforming with regard to zeta scores are urged to adapt their measurement uncertainty statements.

The great majority of participants in this inter-laboratory comparison applied analytical methods which complied with EU legislation with regard to performance characteristics.

12 Acknowledgements

13   References

1   EU, *COMMISSION REGULATION (EC) No 776/2006 of 23 May 2006 amending Annex VII to Regulation (EC) No 882/2004 of the European Parliament and of the Council as regards Community reference laboratories.* Official Journal of the European Union, 2006. **L 136**: p. 3-8. Available from:

http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:136:0003:0008:EN:PDF

2   EU, *Regulation (EC) No 882/2004 of the European Parliament and of the Council of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules.* Official Journal of the European Communities, 2004. **L191**: p. 1-52. Available from:

http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2004:191:0001:0052:EN:PDF

3   *EU, Opinion of the Scientific Committee on Food on the risks to human health of Polycyclic Aromatic Hydrocarbons in food.* (2002). Available from:

http://europa.eu.int/comm/food/fs/sc/scf/out153_en.pdf

4   International Agency for Research on Cancer, *IARC Monographs on the Evaluation of*

Carcinogenic Risks to Humans, Some Non-heterocyclic Polycyclic Aromatic Hydrocarbons and Some Related Exposures, Volume 92 (2010). Available from:

http://monographs.iarc.fr/ENG/Monographs/vol92/mono92.pdf

5   *EU, Commission Regulation (EC) No 333/2007 of 28 March 2007 laying down the methods of sampling and analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and benzo(a)pyrene in foodstuffs.* Official Journal of the European Union, 2007. **L 88**: p. 29-38. Available from:

http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2007:088:0029:0038:EN:PDF

6   *EU, Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs.* Official Journal of the European Union, 2006. **L 364**: p. 5-24. Available from:
http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:364:0005:0024:EN:PDF

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

http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902034842.htm

8   *EU, Commission Regulation (EU) No 835/2011 amending Regulation (EC) No 1881/2006 as regards maximum levels for polycyclic aromatic hydrocarbons in foodstuffs Official Journal of the European Union, 2011.* **L 215**: p. 4-8

   http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:215:0004:0008:EN:PDF

9   *EU, Commission Regulation (EU) No 836/2011 amending Regulation (EC) No 333/2007 laying down the methods of sampling and analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and benzo(a)pyrene in foodstuffs Official Journal of the European Union, 2011. L 215: p. 9-16*
   http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:215:0009:0016:EN:PDF

10 *ISO/IEC 17043:2010 (2010).* Conformity assessment -- General requirements for proficiency testing providers. Geneva, Switzerland

11 Thompson, M., Ellison, S.L.R., and Wood, R., The International Harmonized Protocol for the Proficiency Testing of Analytical Chemistry Laboratories. Pure Appl. Chem., (2006) 78(1): p. 145–196.

   Available from: http://media.iupac.org/publications/pac/2006/pdf/7801x0145.pdf

14 ANNEXES

ANNEX 1 – Announcement of the PT on the IRMM webpage

ANNEX 2 – Announcement via e-mail and invitation

ANNEX 3 – Announcement of sample dispatch

ANNEX 4 – Technical specifications of the calibration solutions

ANNEX 5 – Homogeneity and stability of the test material

ANNEX 6 – Acknowledgement of sample receipt

ANNEX 7 – Questionnaire on details of analysis method

ANNEX 8 – Results reported for the food supplement sample

ANNEX 9 – Results reported for the standard solutions with undisclosed analyte content

ANNEX 10 – Method performance characteristics reported by participants

ANNEX 11 – Details of analysis methods reported by participants

EU-RL PT 1101: PAHs in food supplements

Proficiency Test on the determination of 4 EU priority PAHs in a food supplement

The European Union Reference Laboratory for Polycyclic Aromatic Hydrocarbons organises a proficiency test on the determination of 4 EU priority PAHs in a food supplement.

The objective of this study is to evaluate the capabilities of official food control laboratories (OCLs) in the determination of 4 EU priority PAHs in food supplements.

Only national reference laboratories (NRLs) for PAHs and EU official food control laboratories (OCLs) can participate in the study.

Participation is admitted to maximum 50 official food control laboratories, which will be accepted in the order of registration.

Participation is free of charge for NRLs for PAHs. The participation fee for other official food control laboratories, which do not have national reference laboratory status, is **EUR 250** (two hundred fifty) per registration!

Test material and analytes

The test material is a commercial dry extract of St. John's wort containing the target analytes (see Table 1). One amber glass screw cap vial containing about 15 g of the dry extract of St. John's wort will be sent to the participants in the second half of June 2011. In addition participants will get an ampoule with a solution of the target PAHs with undisclosed analyte content, in, depending on their preference, either ecetonitrile or toluene. A third ampoule with a solution of the target analytes in the preferred solvent, with disclosed analyte content, will be supplied to the participants to allow the participants verifying instrument calibration against an independent standard.

The measurands are the 4 EU priority PAHs as listed in Table 1.

Results have to be reported at least for the contents of the individual analytes as well as for the sum of the four PAHs. All results have to be reported corrected for recovery, and have to be accompanied by the respective measurement uncertainty.

Table 1: the 4 target PAHs

benz[a]anthracene (BaA)	benzo[a]pyrene (BaP)
benzo[b]fluoranthene (BbF)	chrysene (CHR)

General outline

Participants are requested to perform three independent analyses of the St. John's wort extract and at least one analysis of the standard solution with undisclosed analyte content using a method of their choice. The analyses shall be performed on the same day.

Performance assessment:

The performance of the participants in the determination of 4 EU priority PAHs in a food supplement will be rated by z-scores and zeta-scores.

The standard deviations for proficiency assessment will be derived:
- for all four analytes from the fitness-for-purpose function given in Commission Regulation (EC) No 333/2007 assuming a value of 0.3 μg/kg for the limit of detection

Performance indicators will not be calculated for the standard solutions.

Confidentiallity

The identity of participants will be treated confidentially. They may be disclosed only upon provision in writing of the consent of the respective participants.

Registration

Registration shall be done via this link

Schedule

Registration	Sample dispatch	Reporting of results	Report
15 June 2011	second half of June 2011	deadline 15/09/2011	December 2011

Dear Madame/Sir,

Registration for participation in the inter-laboratory comparison study organised by the EU-RL PAH on the determination of the 4 EU priority PAHs in a food supplement and solvent solution has been opened. Participation is mandatory and free of charge for **national reference laboratories** (NRLs) for PAHs.

In support to the NRLs, to facilitate fulfilling their tasks according to Regulation No 882/2004, **EU official food control laboratories** falling under the responsibility of the NRLs may participate in the study. The **participation fee** for official food control laboratories is 250 Euro per participation. For reasons of logistics maximum 50 EU official food control laboratories can be accepted.

The target analytes are the 4 EU priority PAHs benz[*a*]anthracene, benzo[*a*]pyrene, benzo[*b*]fluoranthene, and chrysene. Results have to be reported corrected for recovery and accompanied by the respective measurement uncertainty for the individual PAHs as well as for the sum of the four PAHs.

Each participant will be provided with one amber glass screw cap bottle containing ~ 15 g of a dry extract of St. John's wort (~15 mL), an unknown solution of the target analytes in, depending on their preference, either acetonitrile or toluene, with to them undisclosed content. A third ampoule with a standard solution in either acetonitrile or toluene with disclosed content may be used for verification of instrument calibration. Information on the preferred solvent will be requested via a separate email.

Detailed information can be found (from 23 May 2011 on) on the EU-RL website:
http://irmm.jrc.ec.europa.eu/html/CRLs/crl_pah/interlaboratory_comparisons/index.htm

Timing:
Deadline for registration: 15 June 2011
Dispatch of samples: End of June 2011
Deadline for reporting of results: 15 September 2011

Registration procedure:
Participants shall register via this link:
https://irmm.jrc.ec.europa.eu/ilc/ilcRegistration.do?selComparison=720

Access of NRLs to performance data of official food control laboratories:
Two options:
1) *NRL enrols official food control laboratories* and covers participation fees: NRL submits to EU-RL list of participants including name and address of laboratory, and details of the contact person (name, address (no post box!), email and telephone number). The coverage of the participation fees has to be confirmed and details for invoicing (e.g. order number) have to be provided. It shall be made clear, that the full participation fee is payable upon dispatch of the test samples. In return the performance data of the respective official food control laboratories will be disclosed to the NRL.
2) The *official food control laboratory enrols itself* in the inter-laboratory comparison and covers the participation fee: The official food control laboratory shall register via this link:
https://irmm.jrc.ec.europa.eu/ilc/ilcRegistration.do?selComparison=720
The NRL will get access to performance data of the official food control laboratory only upon providing a letter of consent.

Distribution of information:
The NRLs are kindly requested to distribute this information to the official food control laboratories under their responsibility, and to assist the EU-RL in identifying laboratories that are eligible to participate in the study.

In case you may wish clarification of open questions, please do not hesitate to contact the EU-RL team via:
JRC-IRMM-CRL-PAH@ec.europa.eu

With best regards
Thomas Wenzl

ANNEX 3 - Announcement sample dispatch

Dear Madame/Sir,

The test samples for the **proficiency test on the determination of four PAHs in a food supplement** (dry extract of St John's wort) were dispatched today.

Explanation on the design of the study as well as <u>instructions for sample storage, and for reporting of results are in the parcel</u>. You will also find your <u>participant key</u> in the parcel, which is required for reporting of results.

Please check the parcel after arrival for completeness, and report the receipt of the test samples as well as their status to us by applying the sample <u>receipt form, which is in the parcel</u>.

You are kindly requested to inform us by email, if you do not receive the parcel by end of this week.
My colleague Donata Lerda will then take care of it.

With best regards
Thomas Wenzl

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Geel, 07.07.2011

Standard solution specification sheet	**Product ID: ACN-FS-K**
Date of production: 06/07/2011	Total volume: 3 mL
Expiry date: *December 2011*	

Standard solution composition:

	Product name	CAS	Conc.*	Conc.*	U**
			(ng/g)	(ng/mL)	± %
1	5-methylchrysene	3697-24-3	63.8	49.8	2
2	Benz[a]anthracene	56-55-3	63.7	49.7	1
3	Benzo[a]pyrene	50-32-8	63.8	49.8	2
4	Benzo[b]fluoranthene	205-99-2	65.0	50.7	1
5	Benzo[c]fluorene	205-12-9	62.9	49.1	1
6	Benzo[ghi]perylene	191-24-2	65.1	50.8	2
7	Benzo[j]fluoranthene	205-82-3	64.4	50.2	1
8	Benzo[k]fluoranthene	207-08-9	64.5	50.3	1
9	Chrysene	218-01-9	63.9	49.8	2
10	Cyclopenta[c,d]pyrene	27208-37-3	67.3	52.5	2
11	Dibenzo[a,e]pyrene	192-65-4	63.9	50.3	2
12	Dibenz[a,h]anthracene	53-70-3	63.9	49.9	2
13	Dibenzo[a,h]pyrene	189-64-0	64.2	50.1	2
14	Dibenzo[a,i]pyrene	189-55-9	65.2	50.9	3
15	Dibenzo[a,l]pyrene	191-30-0	64.4	50.2	2
16	Indeno[c,d]pyrene	193-39-5	63.1	49.2	1

* The concentrations were calculated taking into account the purity statements of the single products. The concentration value given in ng/mL is based on the gravimetrical preparation data and the nominal volume of the applied volumetric flask.

** U is the expanded uncertainty calculated using the coverage factor 2 (corresponding to a confidence interval of 95%) multiplied by the combined standard uncertainty. The standard uncertainty is equal to the square root of the sum of the squares of the uncertainties associated with each single operation involved in the preparation of this standard solution.

Solvent: Acetonitrile

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European Union Reference Laboratory
Polycyclic Aromatic Hydrocarbons

Geel, 07.07.2011

Standard solution specification sheet	Product ID: TOL-FS-K
Date of production: 06/07/2011	Total volume: 3 mL
Expiry date: *December 2011*	

Standard solution composition:

	Product name	CAS	Conc.* (ng/g)	Conc.* (ng/mL)	U** ± %
1	5-methylchrysene	3697-24-3	57.7	50.0	2
2	Benz[a]anthracene	56-55-3	57.7	50.1	1
3	Benzo[a]pyrene	50-32-8	57.8	50.1	2
4	Benzo[b]fluoranthene	205-99-2	58.9	51.0	1
5	Benzo[c]fluorene	205-12-9	57.0	49.4	1
6	Benzo[ghi]perylene	191-24-2	59.0	51.1	2
7	Benzo[j]fluoranthene	205-82-3	58.3	50.5	1
8	Benzo[k]fluoranthene	207-08-9	58.4	50.6	1
9	Chrysene	218-01-9	58.1	50.4	2
10	Cyclopenta[c,d]pyrene	27208-37-3	60.6	52.5	2
11	Dibenzo[a,e]pyrene	192-65-4	57.7	50.0	2
12	Dibenz[a,h]anthracene	53-70-3	59.0	51.1	2
13	Dibenzo[a,h]pyrene	189-64-0	57.9	50.2	2
14	Dibenzo[a,i]pyrene	189-55-9	59.2	51.3	3
15	Dibenzo[a,l]pyrene	191-30-0	58.4	50.6	2
16	Indeno[c,d]pyrene	193-39-5	57.2	49.5	1

* The concentrations were calculated taking into account the purity statements of the single products. The concentration value given in ng/mL is based on the gravimetrical preparation data and the nominal volume of the applied volumetric flask.

** U is the expanded uncertainty calculated using the coverage factor 2 (corresponding to a confidence interval of 95%) multiplied by the combined standard uncertainty. The standard uncertainty is equal to the square root of the sum of the squares of the uncertainties associated with each single operation involved in the preparation of this standard solution.

Solvent: Toluene

Homogeneity of the test material

Benz[*a*]anthracene - BAA

BaA

	n =	10			
	mean =	2.95793	21%	= σ-trg(%)	
0.001576	s_x =	0.0397	0.618503	= σ-trg	
√MSW =	s_w =	0.087586			
	s_s =	0.047535	0.185551	= 0,3*σ	

ISO-13528   passed

F = 0.410906 3.020383 = Fcrit
passed

IUPAC
(MSB-MSW)/2 -0.00226 0.072475 = F1*(0,3*σ)²+F2*MSW
passed

Bottle	Result a	Result b	diff	sum	avg
IF-001	2.99	2.92	0.07	5.91	2.95
IF-008	2.99	2.82	0.17	5.82	2.91
IF-016	2.99	2.82	0.17	5.82	2.91
IF-021	2.99	2.95	0.04	5.94	2.97
IF-028	3.06	2.96	0.10	6.02	3.01
IF 039	3.10	2.93	0.17	6.03	3.02
IF-044	3.00	2.94	0.06	5.94	2.97
IF-053	2.99	2.91	0.08	5.90	2.95
IF-060	3.01	2.82	0.19	5.83	2.91
IF-069	3.01	2.95	0.06	5.95	2.98

Σ(diff)² = 0.153426
var(sum)/2 =   0.003152 =MSB
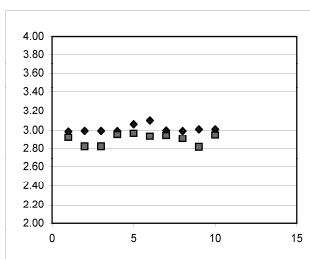
Benzo[*a*]pyrene - BAP

BaP

	n =	10			
	mean =	1.65633	25%	= σ-trg(%)	
0.002091	s_x =	0.045731	0.414248	= σ-trg	
√MSW =	s_w =	0.04955			
	s_s =	0.029389	0.124274	= 0,3*σ	

ISO-13528   passed

F = 1.703553 3.020383 = Fcrit
passed

IUPAC
(MSB-MSW)/2 0.000864 0.031515 = F1*(0,3*σ)²+F2*MSW
passed

Bottle	Result a	Result b	diff	sum	avg
IF-001	1.64	1.64	0.00	3.28	1.64
IF-008	1.61	1.59	0.02	3.20	1.60
IF-016	1.63	1.74	-0.11	3.37	1.69
IF-021	1.65	1.71	-0.05	3.36	1.68
IF-028	1.71	1.67	0.04	3.38	1.69
IF-039	1.83	1.67	0.16	3.50	1.75
IF-044	1.62	1.60	0.02	3.22	1.61
IF-053	1.58	1.65	-0.07	3.23	1.62
IF-060	1.64	1.66	-0.03	3.30	1.65
IF-069	1.64	1.64	-0.01	3.28	1.64

Σ(diff)² = 0.049104
var(sum)/2 =   0.004183 =MSB
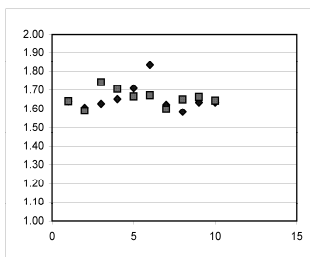
Benzo[*b*]fluoranthene - BBF

BbF

n =	10		
mean =	2.863228	20%	**= σ-trg(%)**
0.007553 **s$_x$ =**	0.08691	0.580663	**= σ-trg**
√MSW = **s$_w$ =**	0.11189		
s$_s$ =	0.035968	0.174199	**= 0,3*σ**

ISO-13528   passed

F = 1.206671   3.020383 = Fcrit
         passed

IUPAC
(MSB-MSW)/2 0.001294 0.069694 = F1*(0,3*σ)^2+F2*MSW
         passed

Bottle	Result a	Result b	diff	sum	avg
IF-001	2.87	2.83	0.04	5.70	2.85
IF-008	2.76	2.63	0.12	5.39	2.70
IF-016	2.90	3.08	-0.18	5.99	2.99
IF-021	2.87	2.84	0.03	5.71	2.86
IF-028	2.88	2.83	0.05	5.71	2.85
IF 039	3.11	2.81	0.30	5.91	2.96
IF-044	2.93	2.88	0.04	5.81	2.90
IF-053	2.89	2.64	0.25	5.52	2.76
IF-060	2.95	2.76	0.19	5.71	2.85
IF-069	2.95	2.86	0.09	5.81	2.91
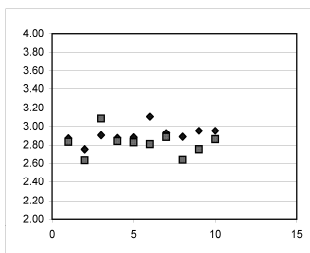
Σ(diff)^2 = 0.250388
var(sum)/2 = 0.015107 =MSB

Chrysene - CHR

CHR

n =	10		
mean =	4.396042	22%	**= σ-trg(%)**
0.016574 **s$_x$ =**	0.128741	0.981636	**= σ-trg**
√MSW = **s$_w$ =**	0.198059		
s$_s$ =	0.05513	0.294491	**= 0,3*σ**

ISO-13528   passed

F = 0.845043   3.020383 = Fcrit
         passed

IUPAC
(MSB-MSW)/2 -0.00304 0.202662 = F1*(0,3*σ)^2+F2*MSW
         passed

Bottle	Result a	Result b	diff	sum	avg
IF-001	4.45	4.49	-0.04	8.94	4.47
IF-008	4.27	4.34	-0.07	8.61	4.30
IF-016	4.46	4.43	0.04	8.89	4.45
IF-021	4.40	3.85	0.55	8.26	4.13
IF-028	4.28	4.49	-0.22	8.77	4.38
IF 039	4.85	4.42	0.43	9.27	4.63
IF-044	4.28	4.51	-0.24	8.79	4.40
IF-053	4.59	4.17	0.42	8.76	4.38
IF-060	4.39	4.51	-0.13	8.90	4.45
IF-069	4.39	4.35	0.04	8.73	4.37
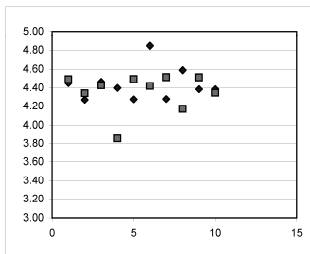
Σ(diff)^2 = 0.784545
var(sum)/2 = 0.033149 =MSB

Stability of the test material:

Table 10: Content of the four target analytes in test materials stored under different temperatures

		BaA	BaP	BbF	CHR*
		µg/kg	µg/kg	µg/kg	µg/kg
Storage at room temperature	Content	3.1	1.6	3.1	6.9
	U (k=2)	0.5	0.2	0.5	1.7
Storage at reference temperature	Content	3.1	1.5	2.9	6.8
	U (k=2)	0.4	0.3	0.4	1.0

* The applied capillary column does not separate CHR from triphenylene

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Polycyclic Aromatic Hydrocarbons

EU-RL-PAHs-08

EU-RL-PAHs-08 Inter-laboratory comparison on the analysis of
four EU priority PAHs in St John's wort and in a solvent solution

Confirmation of the receipt of the samples

RECEIPT FORM

Surname of Participant	
Name of Participant	
Affiliation	
Lab ID	
Country	

Content of the parcel

a)   One screw cap bottle containing a dry extract of St John's wort

b)   One 5 ml brown glass ampoule with a standard solution of the 15+1 EU priority PAHs in solvent (acetonitrile or toluene) (concentrations <u>unknown</u>)

c)   One 5 ml brown glass ampoule with a standard solution of the 15+1 EU priority PAHs in solvent (acetonitrile or toluene) (concentrations <u>known</u>)

d)   A specification sheet for the item c) content (standard solution)

e)   One material safety data sheet for acetonitrile

f)   One material safety data sheet for toluene

g)   One outline of the study

h)   One paper sheet with the participation key, that is required for submission of results

i)   One inter-laboratory comparison <u>sample receipt form</u> (= this form)

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Polycyclic Aromatic Hydrocarbons

Please ensure that the items listed below have been received undamaged, and then describe the relevant statement:

Date of the receipt of the test materials	
All items have been received undamaged	YES / NO
If NO, please list damaged items according to the letters associated at each item in the list above (in case of samples, please specify the code too) Please write one item per row	
Items are missing	YES / NO
If YES, please list missing items according to the letters associated at each item in the list above Please write one item per row	
Serial number of the St John's wort extract sample you received	
Serial number of the standard solution(s) with unknown concentrations you received	

Signature ....

Store the St John's wort samples at room temperature

and solutions at 4°C in the dark

ATTENTION

Please, submit the filled in form by mail at the following address:

jrc-irmm-crl-pah@ec.europa.eu

or print it and send the printout by fax to the attention of Thomas Wenzl at the following number:

+32 – 14 - 571783

ANNEX 7: Questionnaire on details of analysis method

Comparison for PAHs in St. John's wort extract

Please report the method performance parameters for the determination of PAHs in the St. John's wort material as indicated below. The unit for limit of detection (LOD), and limit of quantitation (LOQ) is μg/kg. The method recovery shall be reported as percentage (%). Please describe also the key elements of the applied analysis procedure. Thank you for your cooperation. The EU-RL Team

Submission Form

1. Which analysis technique did you apply? *

○ 1) HPLC-FLD

○ 2) HPLC-UV-FLD

○ 3) LC-UV

○ 4) LC-MS

○ 5) LC-MS/MS

○ 6) GC-MS

○ 7) GC-MS/MS

○ 8) GC-TOF-MS

○ 9) GC-HRMS

2. How did you quantify the analytes in the test sample? *

○ 1) External calibration

○ 2) Internal standardisation with labelled substances

○ 3) Internal standardisation with unlabelled substances

○ 4) Standard addition method

2.1. In case of internal standardisation, please specify the internal standards applied.

3. What are the major sample preparation elements of the applied analysis method? *

☐ 1) Saponification

☐ 2) Liquid/Liquid partitioning

☐ 3) Gel permeation chromatography

☐ 4) Solid phase extraction

☐ 5) Donor acceptor complex chromatography

☐ 6) Other

3.1. If you selected "other", please specify:

[   ]

4. How was analyte extraction performed? *

☐ A) Pressurised liquid extraction (PLE)

☐ B) Sonication

☐ C) Soxhlet extraction

☐ D) Other

4.1. If you selected "other", please specify:

[   ]

Method performance characteristics

Please provide information on the method perovformance characteristics of the method, which was applied for the analysis of the St. John's wort test sample.

Questions/Response table	LOD (µg/kg)	LOQ (µg/kg)	Recovery (%)
BaA			
BaP			
BbF			
CHR			

ANNEX 8 - Results reported by participants for the food supplements test sample

Table 11: Analysis results reported by the participants for the content of benz[*a*]anthracene (BAA) in the food supplements test material. Values are presented as reported.

Laboratory code	Replicate 1	Replicate 2	Replicate 3	Value for proficiency assessment	Uncertainty	Coverage factor (k)
	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	
3600	3.1	2.9	2.9	3	0.5	2
3602	1.99	1.93	1.78	1.9	0.21	
3603	2.53	2.53	2.46	2.51	0.22	2
3604	1.99	2.09	2.3	2.13	0.71	2
3605	3.01	3	2.71	2.91	0.76	2
3606	3.02	2.88	2.78	2.89	0.64	2
3607	4.56	4.16	4.43	4.38	0.96	2
3608	7.96	6.95	6.51	7.14	1.49	2
3610	1.82	1.92	2.14	1.96	0.39	2
3611	41.4	50.4	48.8	46.9	9.6	2
3612	3	3.4	2.8	3.1	0.6	2
3613	3.2	3.3	3.4	3.3	0.7	2
3614	1.82	1.82	1.91	1.85	0.37	2
3615	3.884	3.822	3.565	3.76	0.752	2
3616	2	2	2	2	0.1	2
3617	3.66	3.7	3.5	3.62	0.46	2
3618	3.2	3.2	3.2	3.2	0.5	2
3619	2.99	3.05	2.97	2.99	0.48	2
3620	3.04	2.94	3.02	3.02	0.45	2
3621	2.9	2.6	2.9	2.8	0.4	2
3622	3.43	3.25	3.18	3.22	0.52	2
3623	3.5	3	3.3	3.3	0.7	2
3624						
3626	3.18	3.1	3.14	3.14	1.26	2

Laboratory code	Replicate 1	Replicate 2	Replicate 3	Value for proficiency assessment	Uncertainty	Coverage factor (k)
	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	
5301	3.1	3	3.2	3.1	0.6	2
5302	3.14	3.19	3.15	3.16	0.63	2
5303	3.102	2.716	2.822	2.876	27	62
5304	2.61	2.63	2.6	2.61	5.7	2
5305	2.3	2.2	2.6	2.4	20	
5306	2.2	2.3	2.3	2.2	1.1	2
5307	4.88	4.61	3.17	4.61		
5308	1.68633	1.68544	1.562025	1.644598		
5309	1.6	1.9	1.6	1.7		
5310	3	3.04	3.11	3.05	0.15	
5311	4.77	4.52	4.48	4.59	15	2
5312	5.06	6.3	4.94	5.43	1.34	2
5313	3.53	3.01	4.04	3.53		
5314	4.31	3.73	3.78	3.94	5	0.96
5315	5.96	5.31	5.1	5.45	1.1	2
5316	2.24	2.31	2.35	2.35	0.22	2
5317	2.16	2.3	2.21	2.22	0.07	2
5318	0.64	0.7		0.67	0.1	2
5319	1.11	1.84	1.46	1.47	0.29	2
5320	5.15	5.25	5.64	5.34	4.8	
5321	2.1					
5322	13	8.7	9	8.7	35	2
5323	3.8	3.3	3.5	3.3	1.6	2

Figure 6: Results of replicate determinations (indicated by triangles) of benz[*a*]anthracene (BAA) in the food supplement test material.

Horizontal blue lines represent the arithmetic mean value of replicate measurements, and blue bars the reported expanded measurement uncertainty (k=2). The assigned value is plotted as dotted line. Red solid lines mark the range of satisfactory performance (|z|≤2). Mean values are given for results outside the displayed data range.
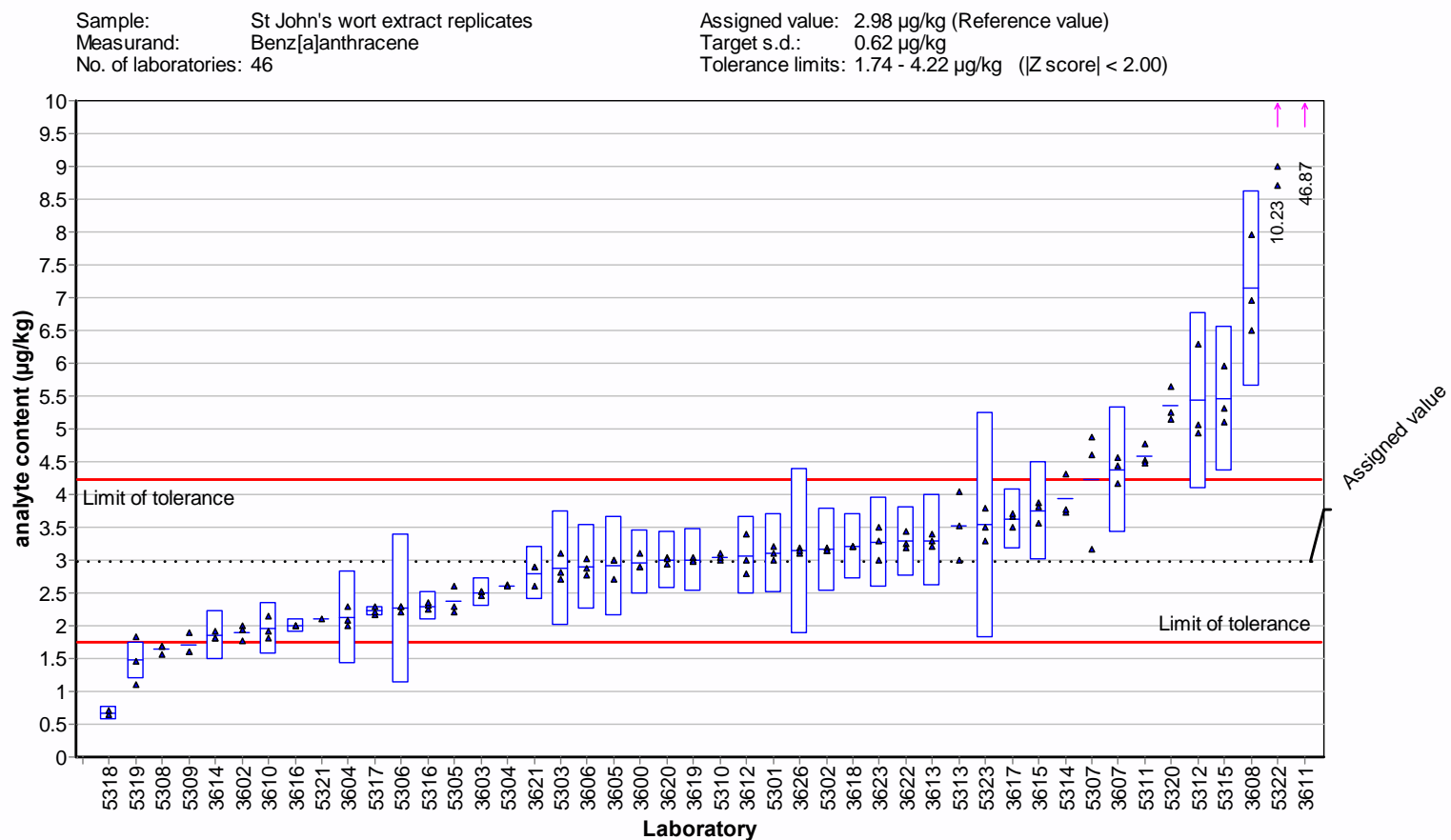
Table 12: Analysis results reported by the participants for the content of benzo[*a*]pyrene (BAP) in the food supplements test material. Values are presented as reported.

Laboratory code	Replicate 1	Replicate 2	Replicate 3	Value for proficiency assessment	Uncertainty	Coverage factor (k)
	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	
3600	1.6	1.7	1.7	1.7	0.3	2
3602	1.18	1.23	1.3	1.24	0.15	
3603	1.65	1.62	1.57	1.61	0.15	2
3604	1.34	1.54	1.39	1.43	0.4	2
3605	1.64	1.66	1.72	1.67	0.57	2
3606	1.81	1.59	1.61	1.67	0.31	2
3607	2.79	2.66	2.79	2.74	0.55	2
3608	5.7	6.87	4.61	5.73	2.26	2
3610	2.26	2.16	1.83	2.06	0.42	2
3611	28.9	32.5	31.1	30.8	3.6	2
3612	2.4	2.2	2	2.2	0.5	2
3613	1.7	1.8	1.9	1.8	0.5	2
3614	0.8	0.8	0.8	0.8	0.16	2
3615	1.545	1.393	1.425	1.45	0.29	2
3616	1.6	1.6	1.4	1.5	0.3	2
3617	1.77	1.71	1.58	1.69	0.23	2
3618	2	2	2	2	0.2	2
3619	2.07	2.07	2.06	2.07	0.75	2
3620	1.65	1.69	1.63	1.66	0.17	2
3621	4.4	4.7	4.9	4.7	0.5	2
3622	2.12	2.08	2.22	2.13	0.38	2
3623	2	2.1	2.2	2.1	0.3	2
3624	1.8	1.78	1.82	1.8	0.31	2
3626	1.72	1.73	1.78	1.74	0.7	2

Laboratory code	Replicate 1	Replicate 2	Replicate 3	Value for proficiency assessment	Uncertainty	Coverage factor (k)
	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	
5301	1.8	1.8	1.8	1.8	0.4	2
5302	1.88	1.9	1.87	1.88	0.38	2
5303	1.691	1.426	1.49	1.535	19	80
5304	1.45	1.42	1.5	1.46	6.9	2
5305	1.5	1.7	1.8	1.6	15	
5306	1.6	1.7	1.7	1.6	0.8	2
5307	1.64	1.57	1.53	1.57		
5308	1.02669	1.09324	1.09035	1.07009		
5309	1	1	1.2	1.1		
5310	1.22	1.22	1.24	1.23	0.06	
5311	1.99	1.84	2.15	2	15	2
5312	2.58	2.48	2.39	2.48	0.5	2
5313	1.99	1.78	2.45	2.07		
5314	1.74	1.71	1.72	1.72	11	0.948
5315	2.53	2.48	2.13	2.38	0.5	2
5316	1.77	1.71	1.73	1.7	0.16	2
5317	2.55	2.77	2.76	2.69	0.12	2
5318	1.14	1.2		1.17	0.2	2
5319	1.9	2.28	2.31	2.16	0.43	2
5320	3.03	3	3.44	3.16	7.8	
5321	1.2	1.2	1.2	1.2		
5322	1.3	1.7	2.4	1.7	35	2
5323	2	2	1.9	2	1	2

Figure 7: Results of replicate determinations (indicated by triangles) of benzo[*a*]pyrene (BAP) in the food supplement test material.

Horizontal blue lines represent the arithmetic mean value of replicate measurements, and blue bars the reported expanded measurement uncertainty (k=2). The assigned value is plotted as dotted line. Red solid lines mark the range of satisfactory performance ($|z| \leq 2$). Mean values are given for results outside the displayed data range.
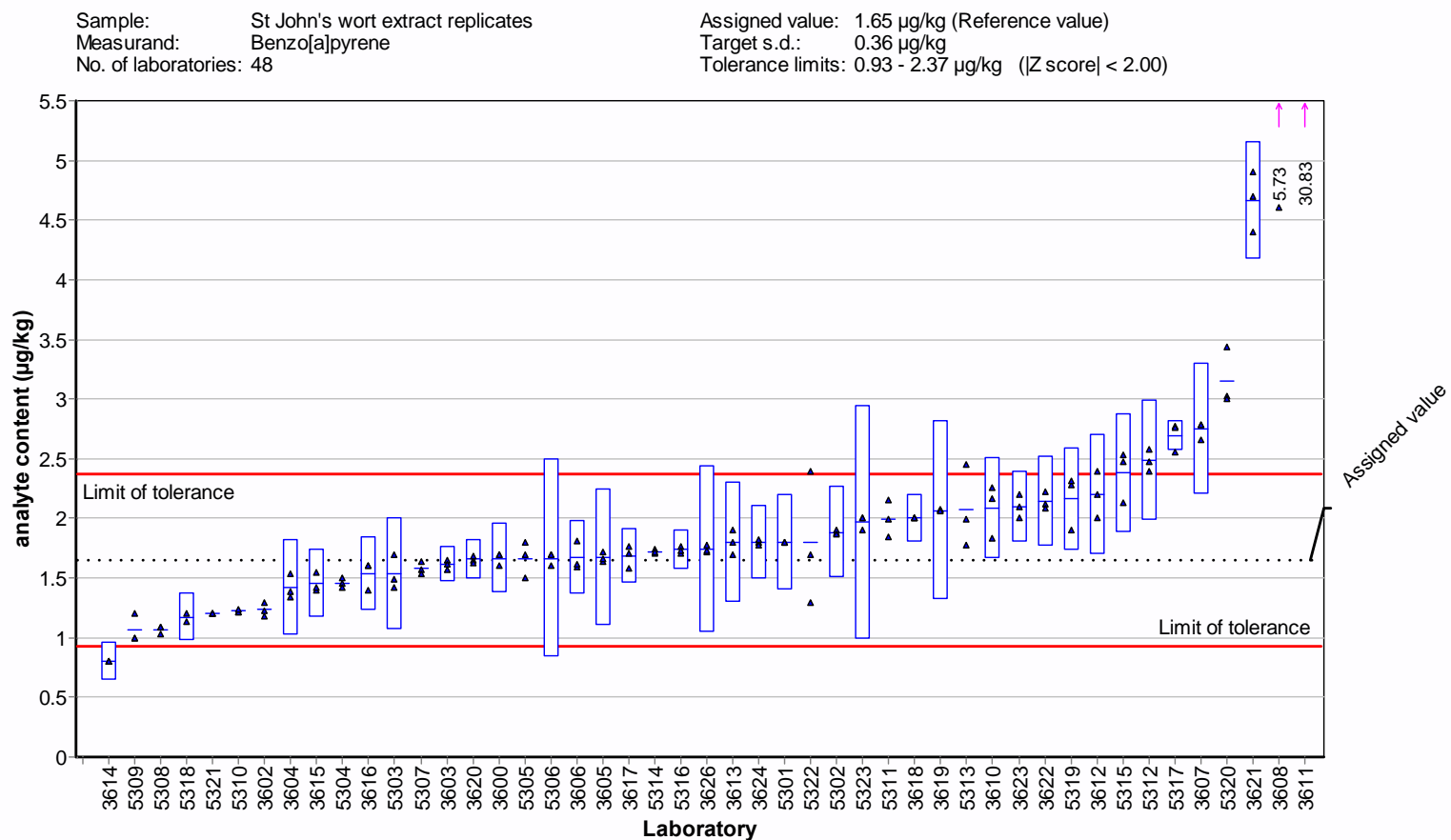
Table 13: Analysis results reported by the participants for the content of benzo[*b*]fluoranthene (BBF) in the food supplements test material. Values are presented as reported.

Laboratory code	Replicate 1	Replicate 2	Replicate 3	Value for proficiency assessment	Uncertainty	Coverage factor (k)	Laboratory code	Replicate 1	Replicate 2	Replicate 3	Value for proficiency assessment	Uncertainty	Coverage factor (k)
	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg			µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	
3600	3.2	3	3	3.1	0.9	2	5301	2.9	3	2.9	2.9	0.6	2
3602	1.73	1.75	1.6	1.7	0.17		5302	2.97	3	2.96	2.98	0.6	2
3603	2.65	2.52	2.5	2.56	0.25	2	5303	2.705	2.444	2.608	2.586	22	78
3604	1.16	1.08	1.05	1.1	0.28	2	5304	2.45	2.54	2.6	2.53	8.5	2
3605	2.86	2.25	2.32	2.47	0.74	2	5305	2.3	2.3	2.6	2.4	15	
3606	2.94	2.73	2.61	2.76	0.46	2	5306	4.2	4.3	4	4.2	2.1	2
3607	5.79	5.79	5.44	5.67	1.13	2	5307	2.21	2.16	2.41	2.21		
3608	10.3	12.01	8.76	10.36	3.26	2	5308	2.32169	2.46654	2.40722	2.29848		
3610	1.73	1.96	2.04	1.91	0.38	2	5309	1.9	2.2	1.9	2	0	
3611	54.6	61.8	56.5	57.6	7.5	2	5310	3	3.15	3.25	3.13	0.16	
3612	3	2.8	2.8	2.9	0.6	2	5311	2.62	2.77	3.04	2.81	15	2
3613	2.8	3.1	3.2	3.1	0.6	2	5312	2.7	2.89	3.37	2.99	0.75	2
3614	1.35	1.11	1.35	1.27	0.22	2	5313	3.21	2.84	3.78	3.28		
3615	4.187	3.678	4.111	4	0.8	2	5314	4.23	4.04	3.85	4.04	34	0.83
3616	2	2	2	2	0.1	2	5315	4.2	5.06	4.16	4.44	0.9	2
3617	3.3	2.7		2.99	0.35	2	5316	2.65	2.67	2.77	2.73	0.18	2
3618	3.1	3.1	3.1	3.1	0.5	2	5317	1.85	1.97	1.95	1.92	0.06	2
3619	3.26	3.24	3.12	3.26	0.64	2	5318	2.76	2.81		2.79	0.4	2
3620	2.77	2.8	2.72	2.76	0.69	2	5319	3.96	3.89	4.03	3.96	0.79	2
3621	2.6	2.5	2.3	2.5	0.4	2	5320	6.23	6.18	6.35	6.25	1.4	
3622	3.46	3.36	3.33	3.4	0.52	2	5321	3.1					
3623	2.6	2.8	2.9	2.8	0.2	2	5322	2.4	3.3	4.9	3.3	35	2
3624							5323	3.8	4.5	3.5	4.5	2.7	2
3626	2.88	2.8	3.07	2.92	1.17	2							

Figure 8: Results of replicate determinations (indicated by triangles) of benzo[*b*]fluoranthene (BBF) in the food supplement test material.

Horizontal blue lines represent the arithmetic mean value of replicate measurements, and blue bars the reported expanded measurement uncertainty (k=2). The assigned value is plotted as dotted line. Red solid lines mark the range of satisfactory performance (|z|≤2). Mean values are given for results outside the displayed data range.

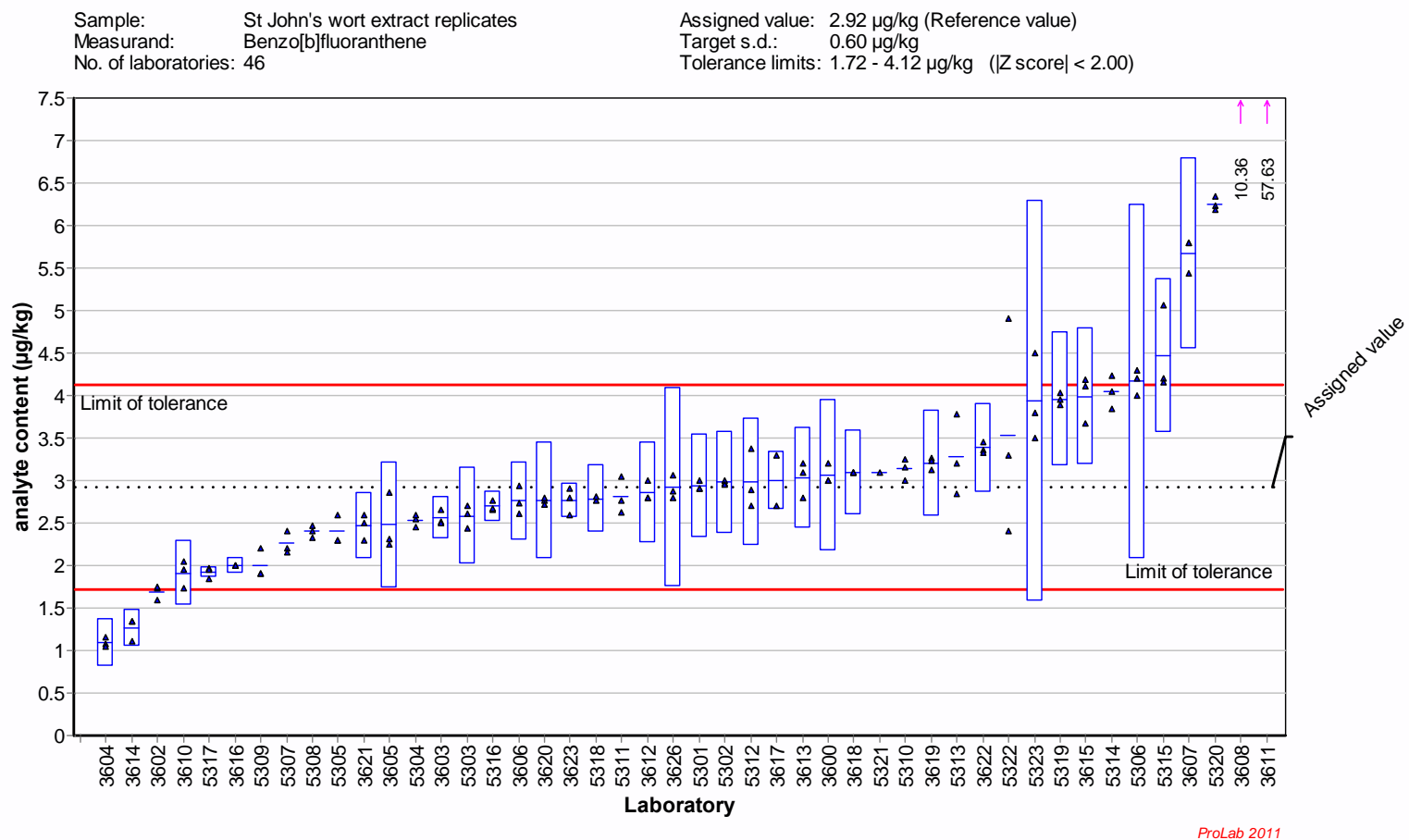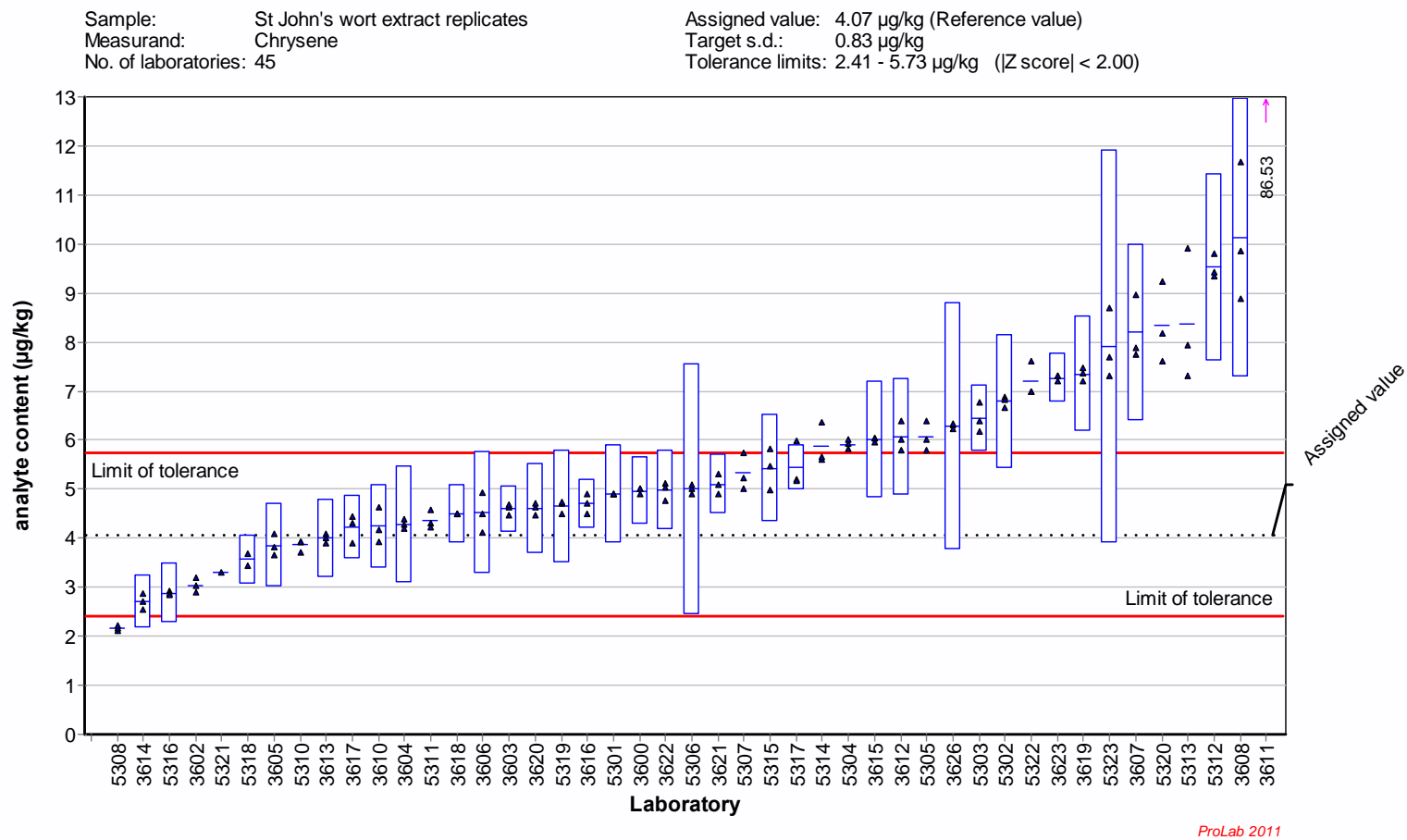**Table 14: Analysis results reported by the participants for the content of chrysene (CHR) in the food supplements test material. Values are presented as reported.**

Laboratory code	Replicate 1	Replicate 2	Replicate 3	Value for proficiency assessment	Uncertainty	Coverage factor (k)	Laboratory code	Replicate 1	Replicate 2	Replicate 3	Value for proficiency assessment	Uncertainty	Coverage factor (k)
	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg			µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	
3600	5	4.9	5	5	0.7	2	5301	4.9	4.9	4.9	4.9	1	2
3602	3.2	3.04	2.9	3.04	0.36		5302	6.87	6.83	6.66	6.79	1.36	2
3603	4.62	4.69	4.47	4.59	0.48	2	5303	6.777	6.181	6.382	6.441	24	70
3604	4.38	4.28	4.2	4.29	1.19	2	5304	5.92	5.81	6	5.91	8.5	2
3605	3.65	4.1	3.81	3.86	0.85	2	5305	6	5.8	6.4	6.1	20	
3606	4.94	4.11	4.5	4.51	1.24	2	5306	4.9	5	5.1	4.9	2.5	2
3607	8.97	7.74	7.87	8.19	1.8	2	5307	5.24	5	5.74	5.24		
3608	9.86	8.87	11.68	10.14	2.84	2	5308	2.11488	2.16782	2.21146	2.13243		
3610	4.18	3.92	4.62	4.24	0.85	2	5309						
3611	83.3	89.3	87	86.5	6.1	2	5310	3.72	3.93	3.94	3.86	0.19	
3612	6	6.4	5.8	6.1	1.2	2	5311	4.3	4.59	4.22	4.37	15	2
3613	4.1	3.9	4	4	0.8	2	5312	9.34	9.43	9.81	9.53	1.91	2
3614	2.72	2.54	2.88	2.71	0.54	2	5313	7.93	7.3	9.9	8.38		
3615	6.05	6.028	5.964	6.01	1.2	2	5314	6.36	5.61	5.65	5.88	18	0.818
3616	4.7	4.5	4.9	4.7	0.5	2	5315	5.47	5.83	4.99	5.42	1.1	2
3617	4.45	4.31	3.89	4.22	0.65	2	5316	2.92	2.86	2.85	2.82	0.6	2
3618	4.5	4.5	4.5	4.5	0.6	2	5317	5.2	5.98	5.17	5.45	0.46	2
3619	7.38	7.47	7.2	7.38	1.18	2	5318	3.68	3.45		3.57	0.5	2
3620	4.63	4.72	4.46	4.6	0.92	2	5319	4.49	4.71	4.74	4.65	1.16	2
3621	5.3	4.9	5.1	5.1	0.6	2	5320	7.6	9.24	8.18	8.34	9.9	
3622	4.78	5.13	5.05	4.88	0.8	2	5321	3.3					
3623	7.3	7.3	7.2	7.2	0.5	2	5322	7	7	7.6	7	35	2
3624							5323	8.7	7.3	7.7	7.3	3.7	2
3626	6.3	6.22	6.34	6.28	2.51	2							

Figure 9: Results of replicate determinations (indicated by triangles) of chrysene (CHR) in the food supplement test material.

Horizontal blue lines represent the arithmetic mean value of replicate measurements, and blue bars the reported expanded measurement uncertainty (k=2). The assigned value is plotted as dotted line. Red solid lines mark the range of satisfactory performance ($|z| \leq 2$). Mean values are given for results outside the displayed data range.

Table 15: Analysis results reported by the participants for the sum of the four marker PAHs in the food supplements test material. Values are presented as reported.

Laboratory code	Value for proficiency assessment	Uncertainty	Coverage factor (k)
	µg/kg	µg/kg	
3600	12.8	2.4	2
3602	7.9	1.26	
3603	11.27	0.96	2
3604	8.93	2.58	2
3605	10.91	3.05	2
3606	11.84	2.38	2
3607	20.98	4.2	2
3608	33.36	3.25	2
3610	11.38	1.7	2
3611	221.8	25.9	2
3612	14.3	2.9	2
3613	12.2	2	2
3614	6.63	1.29	2
3615	15.2	3.04	2
3616	10.2	0.3	2
3617	12.52	1.69	2
3618	12.8	1.8	2
3619	15.7	3.05	2
3620	12.04	1.2	2
3621	15.1	1.9	2
3622	13.61	1.15	2
3623	15.4	0.9	2
3624			
3626	14.09	5.6	2

Laboratory code	Value for proficiency assessment	Uncertainty	Coverage factor (k)
	µg/kg	µg/kg	
5301	12.6		
5302	3.7	0.74	2
5303	13.438	92	
5304	12.51	8.5	2
5305	12.5	20	
5306	12.9	6.5	2
5307	13.63		
5308	7.245598		
5309			
5310	11.27	0.56	
5311	13.67	15	2
5312	20.42	4.08	2
5313	17.26		
5314	15.58		
5315	17.7	3.5	2
5316	9.6	0.73	2
5317	12.29	0.66	2
5318	8.2	0.5	2
5319	12.25	2.67	2
5320	23.09	4.1	
5321			
5322	20.7	35	2
5323	17.1	10.3	2

ANNEX 9: Results reported for the standard solutions with undisclosed analyte content

Table 16: Analysis results reported by the participants for the four marker PAHs in the <u>standard solution in toluene</u> with undisclosed analyte content.

Values are presented as reported, U: measurement uncertainty; k: coverage factor

Laboratory code	Unit	benz[a]anthracene			benzo[a]pyrene			benzo[b]fluoranthene			chrysene		
		Result	U	k	Result	U	k	Result	U	k	Result	U	k
3600	µg/l	38	6.7	2	25	4.5	2	21.1	6.8	2	27.5	3.8	2
3605	µg/l	36.7	1.84	2	24.82	1.24	2	25.92	1.3	2	23.5	1.18	2
3606	µg/kg	37.63	1.76	2	26.09	1.42	2	27.21	1.18	2	25.39	1.34	2
3607	µg/kg	37.06	8.15	2	28.24	5.65	2	25.91	5.18	2	25.87	5.69	2
3612	µg/l	38	0.8	2	23.7	1.1	2	24.4	1.8	2	25.7	0.2	2
3614	µg/l	32.4	6.5	2	20.9	3.8	2	21.5	3.7	2	21	4.2	2
3616	µg/kg	31.8	0.7	2	22.4	1.4	2	23.1	2.6	2	21.6	1	2
3618	µg/kg	41.7	1.7	2	28.4	0.8	2	29.2	0.6	2	29.7	0.3	2
3619	µg/kg	47	7.42	2	27.4	4.68	2	22.8	3.87	2	27.4	4.34	2
3620	µg/l	49.5	7.4	2	29.4	2.9	2	32.1	8	2	32.1	6.4	2
3621	µg/l	24.1	2.2	2	14.3	1.3	2	18.8	1.6	2	14.5	1.4	2
3623	µg/kg	47.6	2.5	2	31.1	5.3	2	26.9	3	2	29.7	0.3	2
3626	µg/l	35.9	3.8	2	23.5	2.3	2	23.8	2.7	2	24	1.8	2
5301	µg/kg	42.6			29			29.9			28.9		
5302	µg/kg	43.6	8.7	2	29.6	5.9	2	29.6	5.9	2	28.9	5.8	2
5303	µg/l	37.21	27	102	22.838	19	99	23.756	22	99	25.486	24	98
5304	µg/kg	38	2.9	2	22.5	2.6	2	25.7	3.7	2	25.8	5.2	2
5305	µg/kg	30.5			22.5			24.5			29		
5306	µg/kg	31.6	6.3	2	24.7	4.9	2	65	13	2	22	4.4	2
5307	µg/kg	36.58			25.59			24.1			24.14		
5308	µg/kg	1.6854			1.09324			2.46654			2.16782		
5311	µg/l	37.4	20	2	27.9	15	2	26.8	15	2	27.2	15	2
5313	µg/l	34.08			21.65			22.75			23.73		
5314	µg/kg	39.5	5		28.75	11		36.14	34		29.05	18	
5320	µg/l	32.67	3.68		19.86	5.71		28	6.83		24.27	5.12	
5322	µg/l	28.2	35	2	22.5	35	2	24.1	35	2	21	35	2

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Figure 10: Youden plots of the percent deviation of the reported values from the assigned values for the food supplement test sample and the standard solution in toluene with undisclosed content.

(The identity of the respective measurand is given above the figure. Results exceeding a difference of more than 100 % were omitted.)
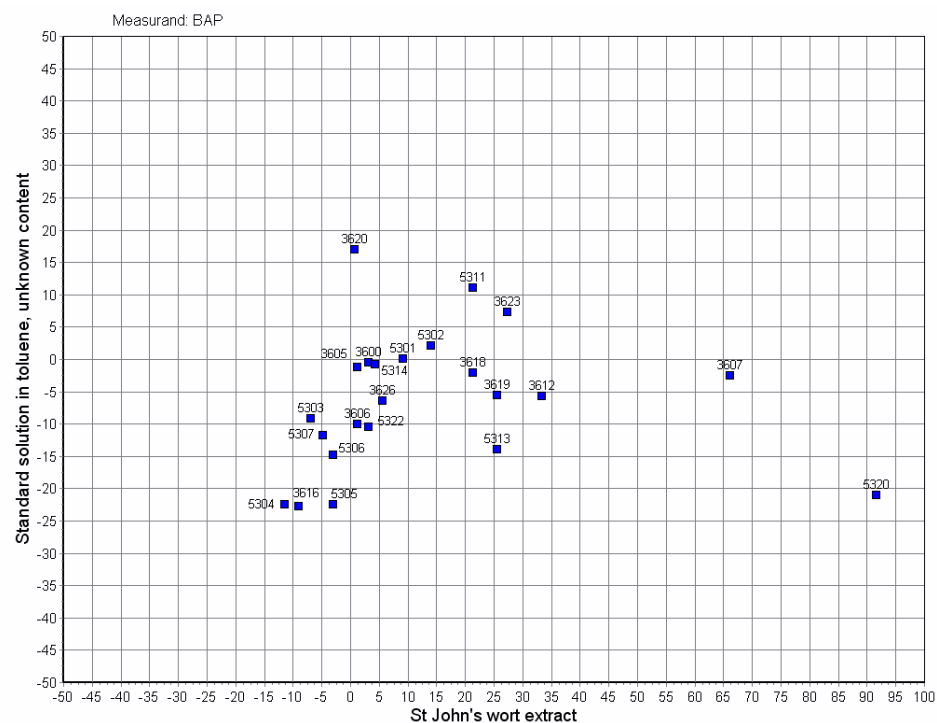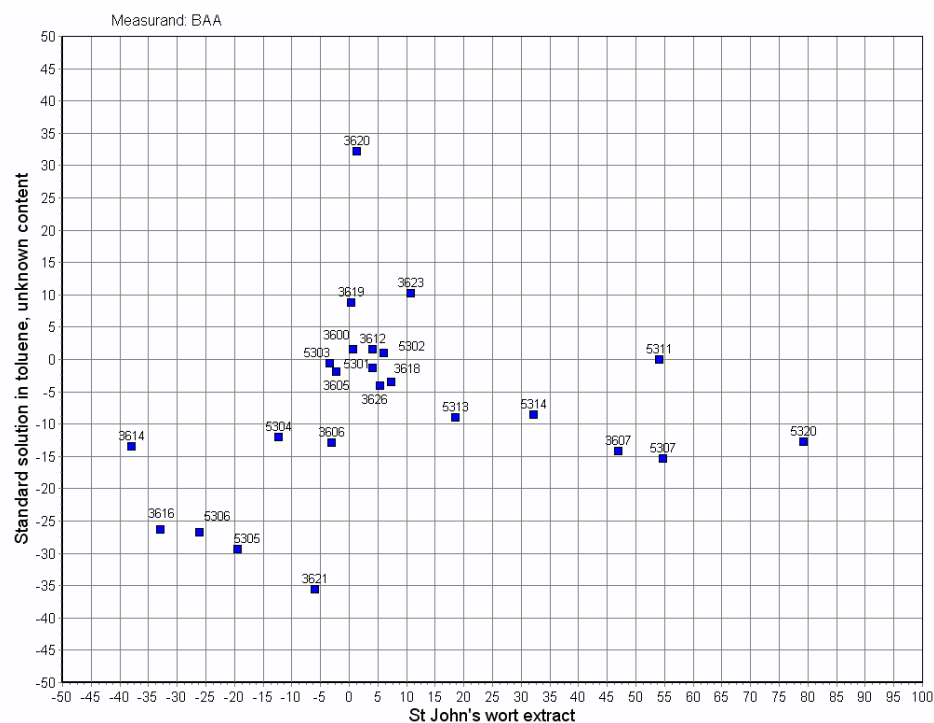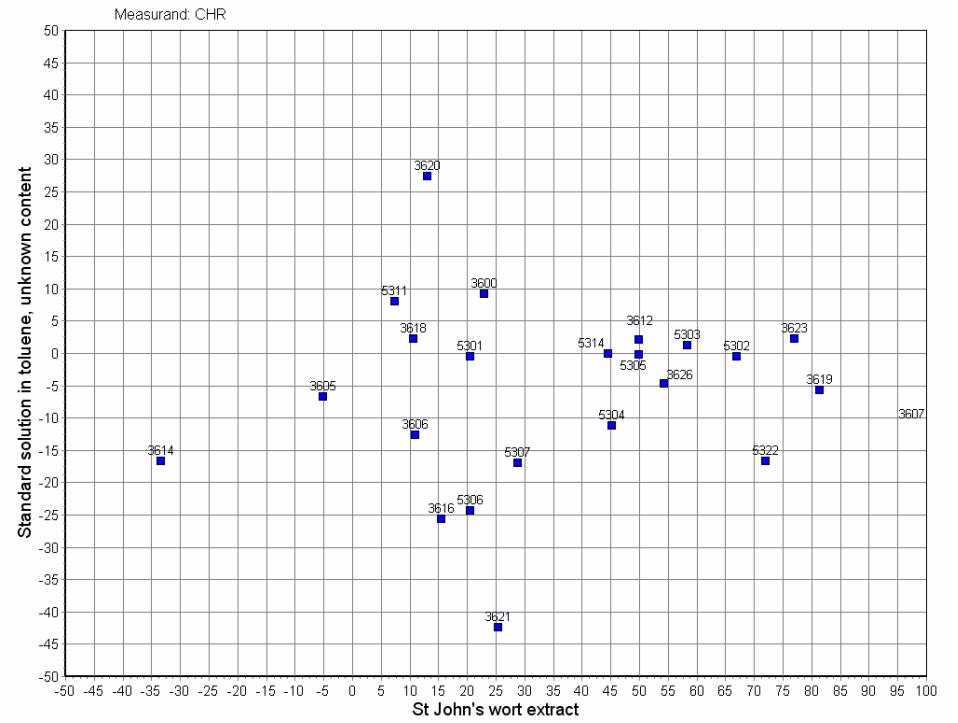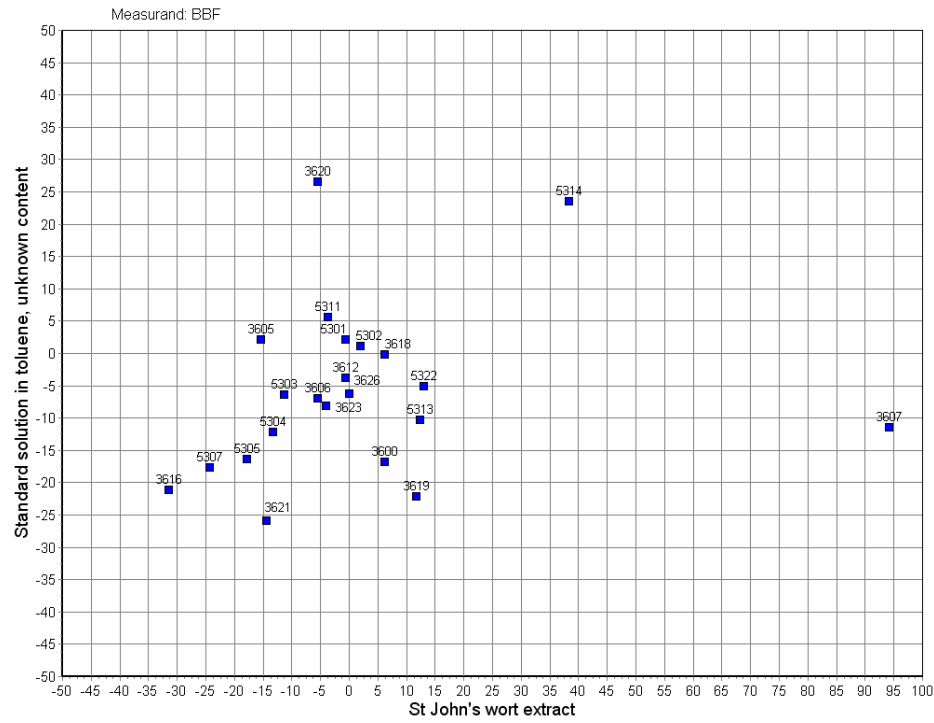
Figure 10 - continued

Table 17: Analysis results reported by the participants for the four marker PAHs in the <u>standard solution in acetonitrile</u> with undisclosed analyte content.

Values are presented as reported, U: measurement uncertainty; k: coverage factor

Laboratory code	Unit	benz[*a*]anthracene			benzo[*a*]pyrene			benzo[*b*]fluoranthene			chrysene		
		Result	U	k	Result	U	k	Result	U	k	Result	U	k
3602	µg/kg	36.75			24.7			24.7			24.4		
3603	µg/kg	35.8	0.86	2	23.6	0.28	2	23.5	0.28	2	23.6	0.57	2
3604	µg/l	47.5	2.9	2	19	1.2	2	19.4	1.3	2	25.8	1.2	2
3608	µg/l	36.23	0.78	2	23.85	0.23	2	23.43	1.8	2	22.96	0.92	2
3610	µg/kg	36.9	1.85	2	25	1.25	2	25.1	1.26	2	24.4	1.22	2
3611	µg/kg	48	0.2	2	32.3	0.2	2	32.8	0.4	2	31.7	0.3	2
3613	µg/l	33.2	3.1	2	26.1	3	2	24.9	2.5	2	23.9	2.4	2
3615	µg/l	41.5			26.35			27.29			26.89		
3617	µg/kg	37	0.9	2	24.4	0.6	2	28.4	0.7	2	23.9	0.6	2
3622	µg/l	37	1.2	2	25	2.3	2	25.7	2.1	2	23.8	1	2
3624	µg/kg	40.2	1.04	2	25.5	0.84	2	26.5	0.96	2	25.3	0.7	2
5306	µg/kg	56.3	11.3	2	37.1	7.4	2	35.9	7.2	2	33.3	6.7	2
5308	µg/kg	1.68633			1.02669			2.32169			2.11488		
5309	µg/l	40.2			26.8			27					
5310	µg/l	31.5	1.58		25	1.25		25	1.25		28.8	1.44	
5312	µg/l	42.8	0.43	2	28.2	0.28	2	27.2	0.27	2	27.5	0.28	2
5315	µg/kg	3.87	0.8	2	2.56	0.5	2	2.51	0.5	2	2.53	0.5	2
5316	µg/l	38.39	3.42	2	24.85	1.91	2	25.59	1.68	2	23.9	5.1	2
5317	µg/l	24.66	0.43	2	22.12	0.34	2	24.72	0.16	2	21.46	0.68	2
5319	µg/l	41.4	4.1	2	27.9	2.8	2	26.8	2.7	2	26	3.1	2
5321	µg/kg	47.6			31.6			32.6			32.8		
5322	µg/l				25.47	35	2						
5323	µg/kg	40.5	20.3	2	23.9	11.9	2	25.5	15.3	2	20.1	11	2

Figure 11: Youden plots of the percent deviation of the reported values from the assigned values for the food supplement test sample and the standard solution in acetonitrile with undisclosed content.

(The identity of the respective measurand is given above the figure. Results exceeding a difference of more than 100 % were omitted.)
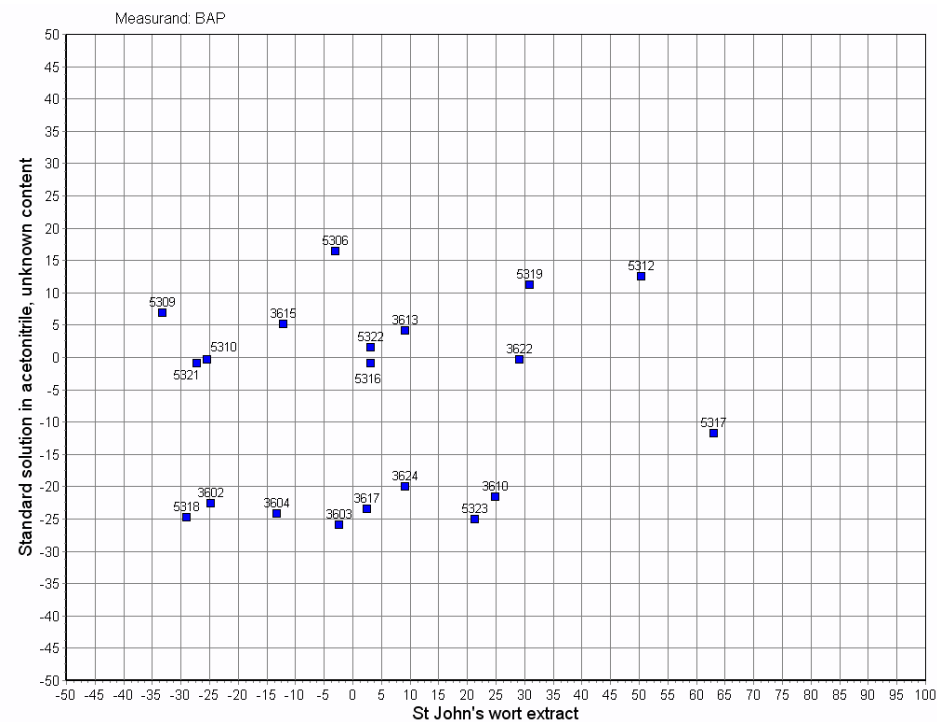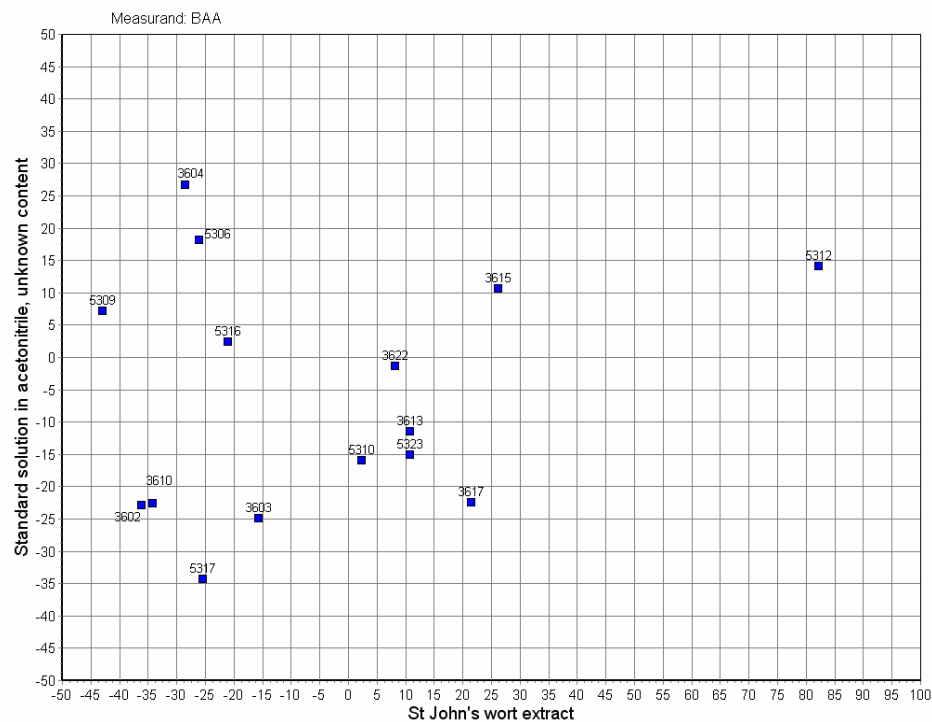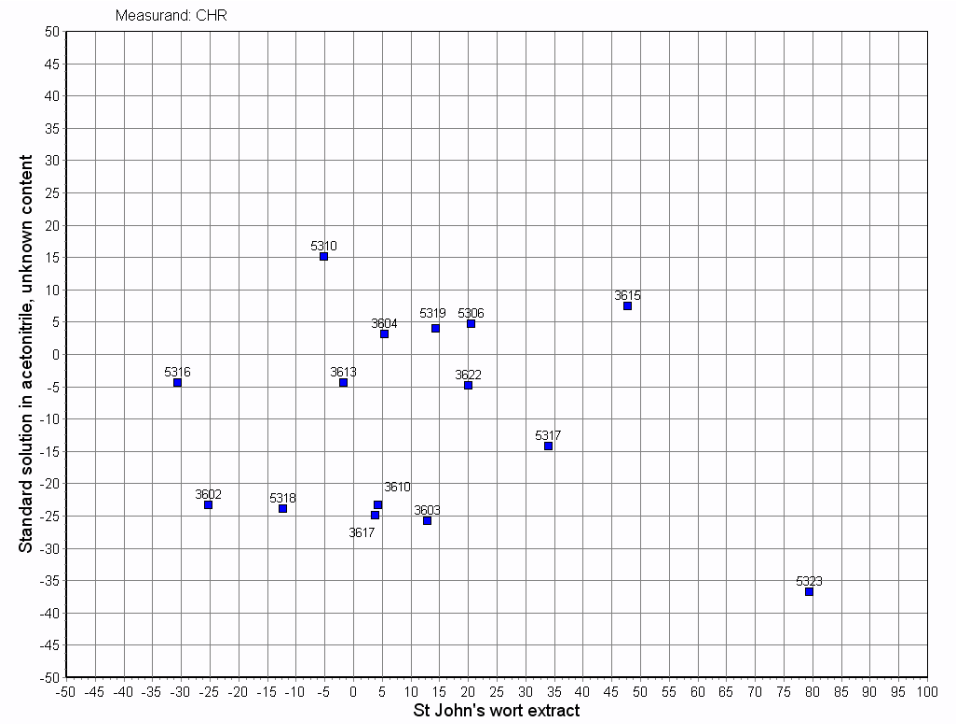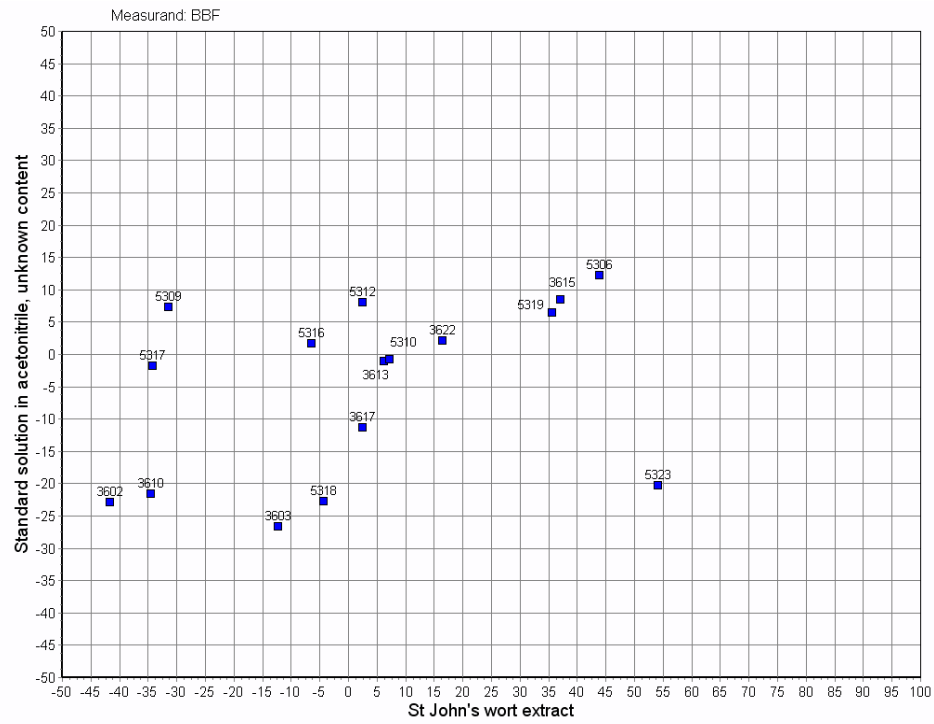
Figure 11 - continued

ANNEX 10:

Table 18: Method performance parameters reported by the participants

Laboratory code	Analyte	LOD	LOQ	Recovery		Analyte	LOD	LOQ	Recovery
		µg/kg	µg/kg	%			µg/kg	µg/kg	%
3600	BaA	0.3	0.6	97.8		BaP	0.3	0.7	94.8
3602	BaA	0.07	0.21	92.6		BaP	0.05	0.15	99
3603	BaA	0.011	0.5	98.7		BaP	0.005	0.51	95.5
3604	BaA	0.2	0.4	86		BaP	0.2	0.4	88
3605	BaA	0.025	0.05	118		BaP	0.025	0.05	108
3606	BaA	0.01	0.03	65		BaP	0.01	0.03	70
3607	BaA	0.21	0.71	74		BaP	0.27	0.89	94
3608	BaA	0.16	0.48	82.13		BaP	0.15	0.46	83.68
3610	BaA	0.2	0.5	85		BaP	0.05	0.2	85
3611	BaA					BaP			
3612	BaA		1	117		BaP		1	120
3613	BaA	0.1	0.3	90		BaP	0.1	0.3	90
3614	BaA	0.2	0.5	96		BaP	0.2	0.5	93
3615	BaA	0.05	0.15	110		BaP	0.05	0.15	105
3616	BaA	0.1	0.5	108		BaP	0.1	0.5	75
3617	BaA	0.18	0.37	40.5		BaP	0.09	0.18	38.7
3618	BaA	0.1	0.3	63		BaP	0.1	0.3	59
3619	BaA	0.05	0.05	59		BaP	0.33	0.33	54
3620	BaA	0.009	0.018	64		BaP	0.005	0.01	55
3621	BaA	0.07	0.2	84		BaP	0.07	0.2	92
3622	BaA	0.1	0.3	110.1		BaP	0.1	0.3	98.5
3623	BaA	0.2	0.8	105		BaP	0.2	0.8	101
3624	BaA					BaP	0.1	0.33	85.1
3625	BaA					BaP			
3626	BaA					BaP			
5301	BaA	0.03	0.1	102		BaP	0.03	0.1	100
5302	BaA	0.1	0.3	78		BaP	0.1	0.3	86
5303	BaA	0.03	0.1	62		BaP	0.03	0.1	80
5304	BaA	1	1	70		BaP	1	1	95
5305	BaA	0.2	0.5	85		BaP	0.2	0.5	85
5306	BaA	0.3	1	69.9		BaP	0.3	1	90
5307	BaA			65.37		BaP			81.16
5308	BaA	0.088	0.265	99		BaP	0.0262	0.0785	100
5309	BaA	0.1	0.8	92		BaP	0.1	0.8	92
5310	BaA	0.1	0.3			BaP	0.1	0.3	90
5311	BaA	0.02	0.05	80		BaP	0.02	0.04	91
5312	BaA	0.3	0.9	105		BaP	0.3	0.9	105
5313	BaA	0.4	0.8			BaP	0.4	0.8	
5314	BaA	0.01	0.02	96		BaP	0.01	0.02	94.8
5315	BaA	0.1	0.3	50 - 100		BaP	0.1	0.3	50 - 100
5316	BaA	0.02	0.11	82.7		BaP	0.04	0.19	104.4
5317	BaA	0.5	1	76		BaP	0.5	1	75
5318	BaA					BaP			
5319	BaA	0.09	0.29	95.1		BaP	0.11	0.41	99
5320	BaA	1.2	1.8	94.6		BaP	0.6	1	83.4
5321	BaA	0.3	0.5			BaP	0.5	1	
5322	BaA	1	2	100		BaP	1	2	100
5323	BaA			26		BaP			26

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ANNEX 10:

Table 18 - continued

Laboratory code	Analyte	LOD µg/kg	LOQ µg/kg	Recovery %	Analyte	LOD µg/kg	LOQ µg/kg	Recovery %
3600	BbF	0.3	0.5	96	CHR	0.2	0.3	101.5
3602	BbF	0.15	0.45	94.7	CHR	0.03	0.09	91.9
3603	BbF	0.014	0.5	101.2	CHR	0.004	0.5	100.3
3604	BbF	0.2	0.4	85	CHR	0.2	0.4	87
3605	BbF	0.05	0.1	94	CHR	0.025	0.05	86
3606	BbF	0.01	0.03	70	CHR	0.01	0.03	65
3607	BbF	0.18	0.6	100	CHR	0.14	0.28	94
3608	BbF	0.15	0.46	79.79	CHR	0.15	0.45	71.25
3610	BbF	0.05	0.2	85	CHR	0.2	0.5	85
3611	BbF				CHR			
3612	BbF		1	118	CHR		1	117
3613	BbF	0.1	0.3	90	CHR	0.1	0.3	90
3614	BbF	0.1	0.4	96	CHR	0.2	0.5	95
3615	BbF	0.08	0.25	109	CHR	0.03	0.1	108
3616	BbF	0.1	0.5	78	CHR	0.1	0.5	72
3617	BbF	0.35	0.7	38	CHR	0.12	0.24	41.6
3618	BbF	0.1	0.3	62	CHR	0.1	0.3	62
3619	BbF	0.16	0.16	63	CHR	0.1	0.1	59
3620	BbF	0.003	0.006	55	CHR	0.01	0.02	65
3621	BbF	0.07	0.2	103	CHR	0.07	0.2	89
3622	BbF	0.2	0.6	96.3	CHR	0.3	0.9	104
3623	BbF	0.2	0.8	106	CHR	0.2	0.8	119
3624	BbF				CHR			
3625	BbF				CHR			
3626	BbF				CHR			
5301	BbF	0.03	0.1	96	CHR	0.03	0.1	114
5302	BbF	0.1	0.3	84	CHR	0.1	0.3	83
5303	BbF	0.03	0.1	78	CHR	0.03	0.1	70
5304	BbF	1	1	86	CHR	1	1	70
5305	BbF	0.2	0.5	90	CHR	0.2	0.5	95
5306	BbF	0.3	1	143.5	CHR	0.3	1	118
5307	BbF			81.16	CHR			65.37
5308	BbF	0.079	0.238	99	CHR	0.09	0.26	100
5309	BbF	0.1	0.8	92	CHR			
5310	BbF	0.7	2		CHR	0.1	0.3	
5311	BbF	0.02	0.04	95	CHR	0.02	0.04	89
5312	BbF	0.3	0.9	105	CHR	0.3	0.9	105
5313	BbF	0.4	0.8		CHR	0.4	0.8	
5314	BbF	0.01	0.02	83	CHR	0.01	0.02	81.8
5315	BbF	0.2	0.5	50 -100	CHR	0.2	0.5	50 - 100
5316	BbF	0.05	0.24	81.4	CHR	0.01	0.07	95.9
5317	BbF	0.5	1	73	CHR	0.5	1	78
5318	BbF				CHR			
5319	BbF	0.11	0.38	94.6	CHR	0.13	0.43	96.7
5320	BbF	0.7	0.9	119.4	CHR	2.3	3.2	104.5
5321	BbF	0.5	1		CHR	0.5	1	
5322	BbF	1	2	100	CHR	1	2	100
5323	BbF			26	CHR			26

ANNEX 11

Table 19: Details of analysis method reported by the participants
Data are presented as reported

Laboratory code	Analysis technique	Instrument calibration	Internal standards applied	Sample clean up	Details of sample clean up	Extraction technique	Details of sample extraction
3600	6) GC-MS	2) Internal standardisation with labelled substances	Deuterated PAH mix 9 (Ehrenstorfer)	1) Saponification, 2) Liquid/Liquid partitioning		D) Other	Liquid/liquid partitioning
3602	2) HPLC-UV-FLD	1) External calibration		3) Gel permeation chromatography		A) Pressurised liquid extraction (PLE)	
3603	1) HPLC-FLD	1) External calibration		3) Gel permeation chromatography		D) Other	1 minute with dichloromethane in vortex
3604	2) HPLC-UV-FLD	2) Internal standardisation with labelled substances	DiP D14	5) Donor acceptor complex chromatography		D) Other	L/L
3605	1) HPLC-FLD	1) External calibration		3) Gel permeation chromatography		C) Soxhlet extraction	
3606	7) GC-MS/MS	2) Internal standardisation with labelled substances	BaP 13C4 ; CHR 13C6 ; BaA 13C6 ; BbF 13C6	4) Solid phase extraction		A) Pressurised liquid extraction (PLE)	
3607	6) GC-MS	2) Internal standardisation with labelled substances	BaA-d12, CHR-d12, BbF-d12, BkF-d12, BaP-d12, ICP-d12, BgP-d12, DiP-d14	1) Saponification		D) Other	solvent partitioning
3608	1) HPLC-FLD	1) External calibration		6) Other	Liquid extraction	D) Other	Extraction in a rotary agitator and centrifugation
3610	2) HPLC-UV-FLD	1) External calibration		4) Solid phase extraction		B) Sonication	
3611							
3612	6) GC-MS	2) Internal standardisation with labelled substances	chrysene-d12, dibenzo(a,h)anthracene-d14, dibenzo(a,i)pyrene-d14	4) Solid phase extraction		B) Sonication	
3613	5) LC-MS/MS	3) Internal standardisation with unlabelled substances	I use Chrysene-D12	3) Gel permeation chromatography		D) Other	extraction with hexan

Table 19 - continued

Laboratory code	Analysis technique	Instrument calibration	Internal standards applied	Sample clean up	Details of sample clean up	Extraction technique	Details of sample extraction
3614	6) GC-MS	1) External calibration		3) Gel permeation chromatography		B) Sonication	
3615	1) HPLC-FLD	1) External calibration		6) Other	PLE / GPC	A) Pressurised liquid extraction (PLE)	
3616	6) GC-MS	2) Internal standardisation with labelled substances	Benzo(a)pyrene-13C4; Benzo(a)anthracene-13C6; Benzo(b)fluoranthene-13C6; Chrysene-13C6	2) Liquid/Liquid partitioning, 4) Solid phase extraction		B) Sonication	
3617	1) HPLC-FLD	1) External calibration		4) Solid phase extraction		C) Soxhlet extraction	
3618	6) GC-MS	2) Internal standardisation with labelled substances	deuterated standards	1) Saponification, 4) Solid phase extraction		D) Other	Handshaken with cyclohexane
3619	6) GC-MS	2) Internal standardisation with labelled substances	C13 Labelled US EPA 16	1) Saponification, 2) Liquid/Liquid partitioning, 4) Solid phase extraction		D) Other	Homegenisation & saponiofication
3620	9) GC-HRMS	2) Internal standardisation with labelled substances	mix of deuterated PAHs (BAA-D12, CHR-D12, BAP-D12, BBF-D12)	3) Gel permeation chromatography, 4) Solid phase extraction		A) Pressurised liquid extraction (PLE)	
3621	6) GC-MS	2) Internal standardisation with labelled substances	Chrysene D12; Benzo(a)pyrene D12; Benzo(b)fluoranthene D12; Benzo(a)anthracene	2) Liquid/Liquid partitioning, 4) Solid phase extraction		B) Sonication	
3622	1) HPLC-FLD	4) Standard addition method		4) Solid phase extraction		D) Other	Extraction with cyclohexane by heating under reflux
3623	7) GC-MS/MS	2) Internal standardisation with labelled substances	C13	4) Solid phase extraction		A) Pressurised liquid extraction (PLE)	
3624	2) HPLC-UV-FLD	1) External calibration		2) Liquid/Liquid partitioning		D) Other	hot solvent extraction
3626							

Table 19 - continued

Laboratory code	Analysis technique	Instrument calibration	Internal standards applied	Sample clean up	Details of sample clean up	Extraction technique	Details of sample extraction
5301	7) GC-MS/MS	2) Internal standardisation with labelled substances	EACH SUBSTANCE C13	4) Solid phase extraction		A) Pressurised liquid extraction (PLE)	
5302	7) GC-MS/MS	2) Internal standardisation with labelled substances	Isotope labelled PAHs 13C for each analyte	4) Solid phase extraction		C) Soxhlet extraction	
5303	7) GC-MS/MS	3) Internal standardisation with unlabelled substances	HAP C13	4) Solid phase extraction		D) Other	automate ASE200 DIONEX
5304	7) GC-MS/MS	2) Internal standardisation with labelled substances	with C13 for each HAP	2) Liquid/Liquid partitioning, 4) Solid phase extraction		A) Pressurised liquid extraction (PLE)	
5305	7) GC-MS/MS	2) Internal standardisation with labelled substances	C13 isotopiques internal standards	4) Solid phase extraction		A) Pressurised liquid extraction (PLE)	
5306	7) GC-MS/MS	2) Internal standardisation with labelled substances	C13	4) Solid phase extraction		A) Pressurised liquid extraction (PLE)	
5307	6) GC-MS	2) Internal standardisation with labelled substances		1) Saponification, 2) Liquid/Liquid partitioning		D) Other	solvent extraction
5308	1) HPLC-FLD	1) External calibration		4) Solid phase extraction		B) Sonication	
5309	1) HPLC-FLD	1) External calibration		4) Solid phase extraction		D) Other	only SPE
5310	1) HPLC-FLD	1) External calibration		1) Saponification, 2) Liquid/Liquid partitioning, 3) Gel permeation chromatography		C) Soxhlet extraction	
5311	6) GC-MS	2) Internal standardisation with labelled substances	13 C Chrysene, 13 C Benz(a)pyrene	1) Saponification, 2) Liquid/Liquid partitioning, 4) Solid phase extraction		B) Sonication	
5312	1) HPLC-FLD	3) Internal standardisation with unlabelled substances	Benzo(b)chrysen	2) Liquid/Liquid partitioning, 4) Solid phase extraction	Quechers	D) Other	Quechers

Table 19 - continued

Laboratory code	Analysis technique	Instrument calibration	Internal standards applied	Sample clean up	Details of sample clean up	Extraction technique	Details of sample extraction
5313	6) GC-MS	2) Internal standardisation with labelled substances	Benz(a)anthracene D12, Benzo(b)fluranthene D12, Benzo(a)pyrene D12, Chrysene D12	1) Saponification, 2) Liquid/Liquid partitioning, 3) Gel permeation chromatography		A) Pressurised liquid extraction (PLE)	
5314	6) GC-MS	2) Internal standardisation with labelled substances	Benzo(a)pyren d12	2) Liquid/Liquid partitioning		D) Other	Extraction with
5315	1) HPLC-FLD	3) Internal standardisation with unlabelled substances	Benz(b)chrysen	1) Saponification, 2) Liquid/Liquid partitioning		D) Other	shaker
5316	1) HPLC-FLD	1) External calibration		1) Saponification, 2) Liquid/Liquid partitioning, 4) Solid phase extraction		D) Other	Liquid/Liquid extraction with cyclohexane and dimethylformamide
5317	7) GC-MS/MS	1) External calibration		3) Gel permeation chromatography, 6) Other	silica gel	D) Other	VDLUFA online method
5318							
5319	1) HPLC-FLD	3) Internal standardisation with unlabelled substances	Benzo(b)chrysene	3) Gel permeation chromatography		A) Pressurised liquid extraction (PLE)	
5320	6) GC-MS	3) Internal standardisation with unlabelled substances	2,2'-binaphthyl	1) Saponification, 2) Liquid/Liquid partitioning, 4) Solid phase extraction		D) Other	3 hours refluxing
5321	1) HPLC-FLD	2) Internal standardisation with labelled substances	6-méthylchrysène	1) Saponification, 2) Liquid/Liquid partitioning		B) Sonication	
5322	6) GC-MS	2) Internal standardisation with labelled substances	naftalene-d8, acenaphtalene-d10, phenanthrene-d10, chrysene-d12, perylene-d12, benzo(e)anthracene-d12	1) Saponification, 2) Liquid/Liquid partitioning, 6) Other	clean-up with silica packed column	C) Soxhlet extraction	
5323	1) HPLC-FLD	1) External calibration		4) Solid phase extraction, 6) Other	Accelerated Solvent Extraction	A) Pressurised liquid extraction (PLE)	

European Commission

Abstract
The proficiency test here reported concerned the determination of the contents of four marker polycyclic aromatic hydrocarbons (PAHs), and of their sum in an food supplements test sample. The set of marker PAHs consists of benz[*a*]anthacene, benzo[*a*]pyrene, benzo[*b*]fluoranthene, and chrysene. Participants to these PT were National Reference Laboratories for PAHs (NRLs-PAHs) and EU official food control laboratories. The number of participants was in total 48.

The PT was organised according ISO Standard 17043:2010.

The test material used was a commercial dry extract of St. John's wort. Participants also received a solution of the same PAHs either in acetonitrile or in toluene for checking their instrument calibration.

The results from participants were rated with z-scores and zeta-scores. About 68 % of the reported results were attributed with z-scores with an absolute value of below two, which is the threshold for satisfactory performance

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