Environmental Sciences Europe

Validation of European horizontal methods for the analysis of PAH, PCB and dioxins in sludge, treated biowaste and soil

Ute Kalbe[1*] , Petra Lehnik-Habrink[1], Nicole Bandow[2] and Andreas Sauer[1]

Abstract

Background: Draft standards for the determination of organic pollutants in the solid matter of environmental matrices such as sludge, treated biowaste and soil have been basically developed in the framework of the European standardisation project HORIZONTAL. A research project financed by the German Federal Environment Agency was initiated to finalise some of these CEN standard drafts, since fully validated standard procedures are crucial for the evaluation of their reliability in the context of implementation in legislation on environmental health.

Approach: Appropriate test materials (< 2 mm particle size) were prepared and homogenised from contaminated soils, sludge and treated biowaste containing polycyclic aromatic hydrocarbons (PAH), polychlorinated biphenyls (PCB), dioxins, furans and dioxin-like-PCB and served, along with reference solutions, as the basis for international interlaboratory comparisons. Performance data of three analytical standard procedures were obtained by the statistical evaluation of results received from 11 to 29 participants per test material.

Results: The overall variation coefficients of reproducibility (between-lab standard deviations) for the sum parameters were roughly between 10 and 35%. The variation coefficients of repeatability (within-lab standard deviations) range between 3 and 8% and show no trend considering the substance groups or matrices. The highest coefficients of reproducibility were found for the analysis of PAH, which were between 26 and 35%, depending on the matrix, whereas 7–17% reproducibility was observed for toxicity equivalents (TEQ) comprising dioxins, furans and dl-PCB.

Conclusions: Overall, the results confirm that the procedures described in the Technical Specifications are fit for purpose for all three matrices and that the feasibility of the HORIZONTAL approach, to cover several matrices with one standard per analyte, was thereby proven.

Keywords: Standardisation, Analytical methods, Organic contaminants, Validation trial, Interlaboratory comparison

Background

An increasing number of countries are establishing threshold values to monitor and evaluate the content of contaminants in soil. These values are subsequently applied to protect the environment and human health by restricting the reuse of soil and soil-like materials or by classifying them into landfill categories. Compliance control requires reliable and reproducible methods of sampling, sample pre-treatment prior to analysis and analytical measurement itself to produce results valid for legal purposes [1, 2]. The development of validated standards for analytical methods can thus be considered as an important part of the implementation of standards and threshold values into related regulations.

Often several standards have been developed for one group of analytes and varying scope as different matrices are considered. Furthermore, analytical standards are available that have been developed in parallel in various countries in the past. Different organisations, such as International Organization for Standardization (ISO) and particularly European Committee for Standardization

*Correspondence: ute.kalbe@bam.de
[1] Federal Institute for Materials Research and Testing–BAM, Unter den Eichen 87, 12059 Berlin, Germany
Full list of author information is available at the end of the article

Kalbe *et al. Environ Sci Eur*   (2019) 31:29

Page 2 of 10

(CEN), are forced to develop harmonised standards, which are accepted on a broader European or international level. The use of harmonised standards in the investigation of environmental compatibility of materials enables stakeholders to demonstrate compliance with relevant EU legislation. Additionally, so-called horizontal methods are preferred in Europe, which means that the standards can be used for a range of different matrices, which is beneficial from an economic point of view.

In the framework of the project HORIZONTAL, financed by the European Commission under the 6th Framework Program, such standards were developed to support EU regulations as the revised version of the Sewage Sludge Directive (91/692/EEC), the Landfill Directive (1999/31/EC), the EU Waste Framework Directive (2008/98/EC, including biological treatment of biodegradable waste) and the initiative on a European framework for soil protection and therefore focuses on the matrices sludge, biowaste and soil [3]. The project included the determination of several hygienic, inorganic and organic parameters in these areas of application. The group of strongly adsorbing non-volatile organic compounds includes hydrophobic organic contaminants that are considered in this validation study: polyaromatic hydrocarbons (PAH), polychlorinated biphenyls (PCB), polychlorinated dibenzo dioxins (PCDD), polychlorinated dibenzo furans (PCDF) and dioxin-like polychlorinated biphenyls (dl-PCB) [4].

All these compounds are well-studied and long-known contaminants that are still relevant today because they are ubiquitously present and may accumulate in soils and soil-like materials [5–7], which can serve as a secondary source of pollution [8].

Polyaromatic hydrocarbons are formed during the incomplete combustion of organic materials and are widely distributed by atmospheric deposition [9]. Another important source of pollution is the use of petroleum products [10]. PAH comprise a large group of several hundred compounds, some of which are carcinogenic, toxic and mutagenic. Often 16 PAH chosen as indicator substances by the Environmental Protection Agency of the United States (16 EPA PAH) are analysed, but the priority PAH may vary depending on the legal context [10]. Although there is a need to update the key substances [11], the 16 EPA priority list is still widely employed [12]. For threshold values, both the concentration of single PAH and summarised concentrations are used, depending on the matrix [13–15].

PCB originate 100% from anthropogenic sources [5]. The technical conditions during the production processes determine the composition of the PCB mixture generated [16]. The group of PCB consists of 209 congeners numbered in accordance with the substituent position of the chlorine atoms by Ballschmiter [17]. Because of the ubiquitous occurrence of PCB, a reasonable approach for the determination of the total PCB content is the quantification of six marker PCB (PCB 28, PCB 52, PCB 101, PCB 138, PCB 153 and PCB 180) and multiplying them by 5 [18, 19]. Many European governmental and regulatory bodies additionally require the determination of PCB 118 as an indicator for the presence of dioxin-like PCB [19].

PCB have been banned worldwide from production and use by the Stockholm Convention on persistent organic pollutants [20]. PCB are toxic, carcinogenic and teratogenic [21]. In the past, PCB mixtures were used in several applications, e.g. as hydraulic fluids and dielectric fluids in transformers and as additives in paints, paper, sealants and plastics [22]. Today, sources of PCB are formed unintentionally in combustion processes, released from PCB-containing sealants and paints still present in buildings or mobilised from contaminated sites [23].

PCDD/F are unintended and undesired by-products mainly of combustion processes in waste incineration, power plants and other industrial activities like metallurgical processes. Emissions from industrial processes have decreased due to better flue gas treatment technologies, resulting in an increase of the relevance of other unregulated sources, such as wood burning [24]. PCDD/F cause adverse health effects including immune suppression, carcinogenicity, tumour promotion and disruption of the endocrine system in laboratory animals and cell cultures [25]. Toxic equivalency factors (TEFs) using 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) as a reference have been introduced to account for the huge difference in toxicity between PCDD/F and dioxin-like PCB TEF [6].

The aim of this work is to provide performance characteristics for three analytical standards for the determination of PAH, PCB and PCDD/F developed in the framework of the European project HORIZONTAL as the basis of their final validation. The data were obtained by an international round robin trial conveyed by the Federal Institute for Materials and Testing (BAM). The standards allow the application of various extraction procedures prior to analyses. The influence of these options on the results was evaluated to assess their equivalence.

Materials and methods
Processing of test materials
The adsorption and binding of contaminants is a crucial issue that might differ between samples spiked in a laboratory and naturally aged samples, and therefore the latter should be preferred as test materials [26]. However, blending highly contaminated with uncontaminated material or even spiking samples was unavoidable, due to the lack of suitable initial sample materials, especially in the case of treated biowaste.

Kalbe *et al. Environ Sci Eur*   (2019) 31:29

Page 3 of 10

Furthermore, it was desired neither to employ test samples exhibiting high concentration levels nor to take samples with too low concentrations, to avoid unnecessary influences on the performance characteristics of the methods.

Sludge contaminated with PAH originated from a waste-water treatment plant near Berlin, Germany (SL PAH), whereas sludge contaminated with PCB and PCDD/F (SL DIO) was sampled from two former disposal sites in North Rhine-Westphalia, Germany. All sludge samples were freeze-dried and ground using a cross hammer mill (Retsch, Haan, Germany), resulting in a grain size < 500 μm to facilitate homogenisation. The PCB-containing sludge was diluted with sludge from the waste-water treatment plant, due to the high ratio of PCB content (7.8 mg/kg) to SL PCB.

Treated biowaste with a grain size < 15 mm was delivered from a composting plant near Berlin, Germany. The air-dried biowaste, which had a residual water content of 5%, was milled using a cross hammer mill, resulting in a grain size < 250 μm. This material was directly used to produce the test material containing PAH (CO PAH). To spike with PCB, this biowaste was mixed with the sludge containing PCB, sieved to a grain size < 250 μm, homogenised for 2 h using a gyro-wheel mixer and equilibrated for 6 weeks in a cooling chamber at 4 °C (CO PCB). Dioxin-containing biowaste was obtained by blending and equilibrating dioxin-containing soil with the biowaste. Additionally, biowaste spiked with some dioxins was added as a third component to change the contamination pattern in comparison with the soil sample, leading to CO DIO.

The basis for all soil samples was an uncontaminated reference soil (RefeSol 01-A, a dystric cambisol) distributed by Fraunhofer IME (Schmallenberg, Germany) [27]. One aliquot of this soil was blended (diluted) with another soil of similar texture containing PAH sampled at a plant for impregnating railway sleepers in Brandenburg, Germany, and sieved to < 2 mm (SO PAH). Another part of this reference soil was blended with the 63-μm fraction of excavated soil material of a remedial action, which was contaminated with PCB to soil SO PCB. A PCDD/F-containing soil was sieved to < 63 μm and blended with the same reference soil and the aforementioned sludge (SO DIO).

In total, nine test materials were processed as described above. All of them were homogenised on a gyro-wheel mixer and divided into representative subsamples of 100 g (PAH and PCB) or 50 g (PCDD/F) using a cross-riffling scheme [28, 29]. Depending on the amount of starting material, 50–80 subsamples of each test material were obtained.

Content analysis and homogeneity check

8 subsamples of each test material were randomly chosen to determine solid matter content and as the basis for the homogeneity check. This number of subsamples fulfils the ASTM-E826-14 [30] criterion of a minimum amount of 8% of subsamples. From every subsample, two replicates were analysed in accordance with the correspondent standards: PCB [18] and dioxins [31]. Analyses of dioxin content were performed by a commercial analytical laboratory as subcontractor. Diverging from the standards, soxhlet extraction was carried out with *n*-hexane before dioxin analysis. PAH content was analysed following a method published by LANUV NRW (state office for nature and environment North Rhine-Westphalia, Germany) [32]. Methodological details are given in Additional file 1. Homogeneity was evaluated using an *F* test in accordance with DIN 53803-1 [33]. All processed materials passed the homogeneity tests and could be used for the round robin test.

Round robin trial

The final validation step during the standardisation of an analytical method is the evaluation of performance through an interlaboratory ring test to demonstrate that the method is fit for purpose and to determine the performance characteristics of the method (i.e. mainly standard deviations of repeatability and reproducibility for relevant analytes).

Interlaboratory comparisons were conducted based on the given draft standard methods: FprCEN/TS 16181 for PAH [34], EN 16167 for PCB [18] and CEN/TS 16190 for PCDD/F and dl-PCB [31].

The participants in the interlaboratory trial were requested to strictly follow the given standards. This is important, since in contrast to round robin tests for proficiency testing, validation trials aim at the evaluation of the performance of a procedure and not at the evaluation of the analytical abilities of the participating laboratories. Unfortunately, laboratories frequently tend to deviate from the specified procedure to rank their own method. Furthermore, there are several options within the draft standard, e.g. for extraction, clean-up and analytical method, which influences the final measurement result of individual labs. This aspect was evaluated additionally.

The validation trial was internationally announced via standardisation bodies and other networks dealing with environmental analysis to acquire a sufficient number of participating laboratories and to ensure a large number of individual test results for the evaluation, even after outlier rejection and the representation of both analytical methods for the determination of PAH (HPLC and GC-MS) and PCB (GC-MS and GC-ECD).

Kalbe *et al. Environ Sci Eur*   (2019) 31:29

Page 4 of 10

Applications were received from 34 participating laboratories from 11 European and 2 Asian countries, whereby the number of participants varied depending on the analyte and the matrix (17 PAH GC-MS, 9 PAH HPLC, 17 PCB GC-MS, 9 PCB GC-ECD, 12 PCDD/ PCDF/dl-PCB). All participants received one sample of sludge, biowaste and soil for each of the analytes they had registered for. As a quality control measure, every set of samples was accompanied by control samples for each analyte group produced from commercially available standard solutions by BAM. The control samples were stored in CERTAN® vials (Promochem, Wesel, Germany) to minimise evaporation. Details on preparation of the control samples can be found in Additional file 1.

Data evaluation

The participants reported the results to BAM using the prepared reporting sheets, which could be directly read and processed by a commercially available software [35]. Standard deviations and variation coefficients were calculated for the reproducibility and repeatability of each of the analytes and matrices.

Values below the limit of quantification (LOQ) were not considered for further evaluation, because this would have resulted in a distortion of the evaluations. Thus, for some analytes, the number of results was actually lower. PAH analysis using HPLC with fluorescence detection does not allow the detection of one of the 16 EPA PAH (acenaphthylene). Thus, a sum parameter of 15 EPA PAH was calculated for laboratories using this method. This compound was also excluded for the calculation of the sum parameter for GC-MS measurements, enabling the comparison of the two methods.

The results of the quality control solution were not used as a criterion for exclusion of participants, but to evaluate the contribution of the analysis to the variance of the statistical results.

Sample preparation methods that are allowed optionally in the draft standard for PAH and PCB were grouped in accordance with Table 1 to facilitate the evaluation of the influence of the chosen test options by the laboratories.

The performance characteristics were calculated using the software PROLab Plus (QuoData GmbH, Dresden, Germany) [35] in accordance with DIN ISO 5725-2 [36]. The following outliers were removed from the data set: type A) deviating single value within the repetitions of a certain laboratory (Grubbs Test); type B), a differing laboratory mean (Grubbs-Test) and type C), excessive standard deviation (low repeatability) of a single laboratory (Cochran Test).

Table 1 Grouping of sample preparation methods for analysis of PAH and PCB according to extraction method and polarity of solvent

Code	Extraction	Solvent
1	Agitation	Mixture polar/non-polar
2	Agitation	Non-polar
3	Pressurised liquid extraction	Non-polar
4	Pressurised liquid extraction	Mixture polar/non-polar
5	Soxhlet	Non-polar
6	Soxhlet	Mixture polar/non-polar
7	Ultrasonic bath	Polar
8	Agitation and ultrasonic bath	Non-polar
9	Ultrasonic bath	Mixture polar/non-polar

Results and discussion

Mandel's statistic and outliers

Graphic plots of Mandel's statistics revealing differences between laboratories [37] are shown in Additional file 1. A comparison of diagrams for reference solutions and extracts of solid samples does not indicate that certain laboratories show constantly high relative deviations from the mean value (*h* value) or insufficient precision compared with the standard deviation among all the laboratories (*k* value). Thus, the results of the reference solution were not used to exclude laboratories from further data evaluation. Evaluation shows that few outliers appear for most analytes and matrices, but for individual compounds such as acenaphthylene and dibenzo[ah] anthracene in the SOPAH sample and several PCDD/F in the RSDIO and SODIO samples, the number of valid results was clearly lower. However, the minimum number of participation laboratories as required for a successful validation [38] was achieved.

Repeatability and reproducibility

A summary of the sum parameters of the substance groups is shown in Table 2. Detailed results for individual compounds of the interlaboratory comparisons are given as tables in Additional file 1 (reference solutions: Tables S2, S4 and S6, matrix samples: Tables S3, S5 and S7) or in the final report [39].

It must be considered that the uncertainty both of sample preparation and of the chemical analysis itself contributes to the overall variation. The amount of this contribution could be estimated by comparing the results of the quality control solutions and the samples themselves. The variation coefficient for PCB and some of the HCDD/F and dl-PCB is in the same order of magnitude for all samples, depending on the matrix, while for PAH the variation is usually higher for samples containing a larger amount of organic matter.

The variation coefficients of repeatability CV_r of the sum parameters range between 3.1 and 7.6% for all

Kalbe *et al. Environ Sci Eur*   (2019) 31:29

Page 5 of 10

Table 2 Performance characteristics for the analysis of PAH following FprCEN/TS 16181 (sum parameters), of PCB following EN 16167 (sum parameters) and of dioxins, furans and dl-PCB following CEN/TS 16190 (sum parameters)

Parameter	Matrix	l_o	l	n_o	n	$x;^-$ (µg/ml)	S_R (µg/ml)	CV_R (%)	S_r (µg/ml)	CV_r (%)
Sum 15 EPA PAH	RS HPLC	9	9	21	21	8.93	0.894	7.5	0.283	2.9
	RS GC-MS	18	18	47	47	5.84	1.153	19.8	0.234	4.0
Sum 7 PCB	RS PCB	27	26	78	77	2.91	0.666	22.89	0.178	6.13
Parameter	**Matrix**	l_o	l	n_o	n	$x;^-$ (mg/kg)	S_R (mg/kg)	CV_R (%)	S_r (mg/kg)	CV_r (%)
Sum 15 EPA PAH	SOPAH	29	27	87	81	2.24	0.672	29.99	0.160	7.16
	COPAH	29	27	87	81	2.78	0.723	25.98	0.138	4.96
	SLPAH	29	28	87	84	2.73	0.950	34.84	0.163	5.98
Sum 7 PCB	SOPCB	27	27	81	81	3.14	0.641	20.41	0.185	5.89
	COPCB	27	25	81	75	2.81	0.545	19.42	0.120	4.28
	SLPCB	27	26	81	78	2.91	0.666	22.89	0.178	6.13
Parameter	**Matrix**	l_o	l	n_o	n	$x;^-$ (ng/kg)	S_R (ng/kg)	CV_R (%)	S_r (ng/kg)	CV_r (%)
NATO/CCMS-TEQ	SODIO	12	12	35	35	480.0	74.51	15.52	36.31	7.56
	CODIO	12	12	35	35	1321	142.9	10.82	91.99	6.97
	SLDIO	12	12	35	35	166.6	28.45	17.08	9.34	5.60
WHO-TEQ 2005	SODIO	11	11	32	32	382.0	63.83	16.72	27.05	7.08
	CODIO	11	11	32	32	1162	116.1	10	72.72	6.26
	SLDIO	11	10	31	29	533	39.11	7.34	16.59	3.11

l_0 number of participating laboratories, l number of participating laboratories after elimination of outliers, n_0 number of individual values, n number of individual values after elimination of outliers; $x;^-$ total mean after elimination of outliers, S_R standard deviation of reproducibility, CV_R variation coefficient of reproducibility, S_r standard deviation of repeatability, CV_r variation coefficient of repeatability

parameters and show no trend considering the substance groups or matrices (Table 2). The CV_r for the individual substances are listed in Additional file 1: Tables S3, S5 and S7.

The concentrations of the individual substances varied by several orders of magnitude. Usually the variation coefficients for substances with low concentrations are higher [40]. Thus, their contribution to the sum parameter may be small and the sum parameter may exhibit a better variation coefficient than the individual substances, as was already observed in other studies, e.g. [41].

The two reference solutions for PAH measurement (Table 2) show that the variation coefficient for repeatability is smaller for the HPLC (2.9%) method than for the measurement using GC-MS (4.01%). The differences become even more visible if individual PAH are evaluated (Additional file 1: Table S3): anthracene (1.52% HPLC and 7.31% GC-MS) and benzo[a]anthracene (1.73% HPLC and 6.78% GC-MS) are the most prominent examples. The same diagnostic ions are always used to analyse anthracene and phenanthrene, as well as benzo[a]anthracene and chrysene, and chromatographic separation depends mainly on the length of the chromatographic column. Shorter columns hamper a reliable peak integration. PAH with higher molecular weight

have broader peaks due to the enhanced retention time and have a lower response factor in mass spectrometry, resulting in higher variance in results. Incomplete separation between dibenzo[ah]anthracene and indeno[123-cd]pyrene further increases observed variance. This general chromatographic phenomenon also influences the performance of samples containing matrix. Thus, it can be concluded that, at least for samples in the concentration range used in the validation trial, the HPLC method seems to be more robust. Gratz et al. [42] found that GC-MS methods are more robust for the analysis of PAH, but due to a different level of concentration (approx. 100-fold higher than in this study), comparability with our study is not given.

The repeatability of the measurements of PCB is slightly higher (2.49–8.62%) and does not differ much for individual PCB congeners. The repeatability for PCDD/F and dl-PCB is higher (6.42–19.79%) than for PAH and PCB, which are below 10% with the exception of 1,2,3,7,8,9-HxCDF (19.79%) and 2,3,7,8-TCDF (10.05%). This is probably caused by the high chromatographic requirements for this group of substances and the development of specific chromatographic phases [43]. The repeatability of the matrix samples is higher, but still less than 10% for three-fourths of the samples. Despite the higher variation for HCDD/F and

Kalbe *et al. Environ Sci Eur*   (2019) 31:29

Page 6 of 10

dl-PCB, the results are satisfying, taking into account that the clean-up for the analysis of HCDD/F and dl-PCB is challenging [44].

The variation coefficient of reproducibility CV_R for the three substance groups and three matrices demonstrates higher differences between the substance groups than between the matrices (sewage sludge, treated biowaste and soil). The greatest CV_R was found for the analysis of 15 EPA PAH which was between 26 and 35%, depending on the matrix. For the sum of the 7 key PCB, the CV_R ranged between 19 and 23%. The lowest CV_R—7 to 17%—were obtained for the calculated toxicity equivalents based on selected PCDD/F and dl-PCB.

The higher value for CV_R for the analysis of PAH in sludge meets the expectations, as this matrix requires a laborious clean-up procedure. The same chromatographic issues as discussed above concerning repeatability lead to higher values for individual PAH. Additionally, the separation between benzo[b]fluoranthene and benzo[k]fluoranthene is often insufficient and the sum of both analytes is used. Benzo[j]fluoranthene, which is not part of the EPA PAH, elutes between the two analytes and can lead to higher concentrations than expected if it is not sufficiently excluded from the sum of the two benzofluoranthenes [45]. Reproducibility is, in general, concentration-dependent and is higher for lower analyte content. For an analyte content < 0.05 mg/kg, reproducibility is > 50%. Especially PAH with low molecular weight are affected, due to higher volatility.

In the case of PCB, the sludge has also the greatest reproducibility (34.92%), while reproducibility is lower for treated biowaste (25.26%) and soil (22.68%). In contrast to the PAH, the values for individual congeners do not vary so much, which is probably caused by a more equal concentration pattern and a better chromatographic separation of individual compounds. PCDD/F and dl-PCB show similar values for the reference solution and the samples with matrix except for individual extreme values. The concentration of the individual compounds comprises a wide range (1–106 ng/kg), and their reproducibility shows a clear dependence on the concentration [40]. Especially the high values for 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD and 1,2,3,4,7,8-HxCDD can be explained by this relation. Co-elution of 1,2,3,7,8,9-HxCDF and PCB may cause higher values for these compounds, but the wide variety of chromatographic columns used by participants means that general conclusions cannot be drawn [43].

Overall, the CV_R were in the usual range for the analysis of organic substances 25–50% [41, 46–48]. The higher variation for PAH and PCB might be attributed to the different analytical methods admitted in each case. This factor probably increased the variance of measured values.

Repeatability (within-lab variation) CV_r is always better (lower values) than reproducibility (between-lab variation) CV_R. Typically, reproducibility ranges between 2 and 4 times repeatability, as found in other studies, e.g. [49].

Overall, the results confirm that the procedures described in the draft standards are comparably applicable for all three matrices and thereby confirm the feasibility of the HORIZONTAL approach.
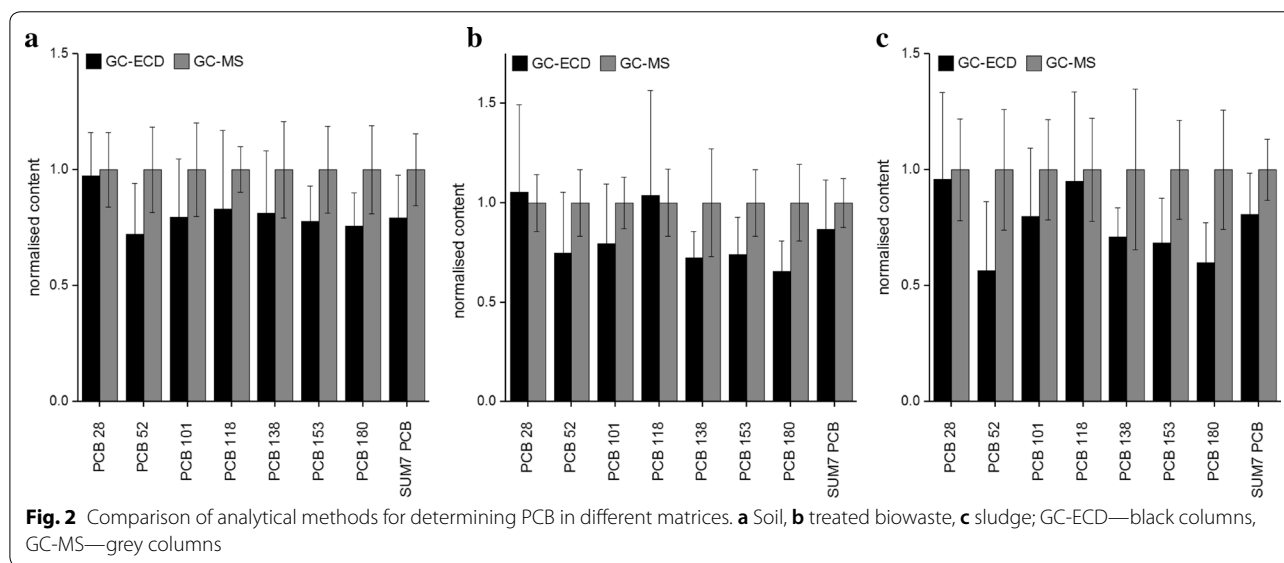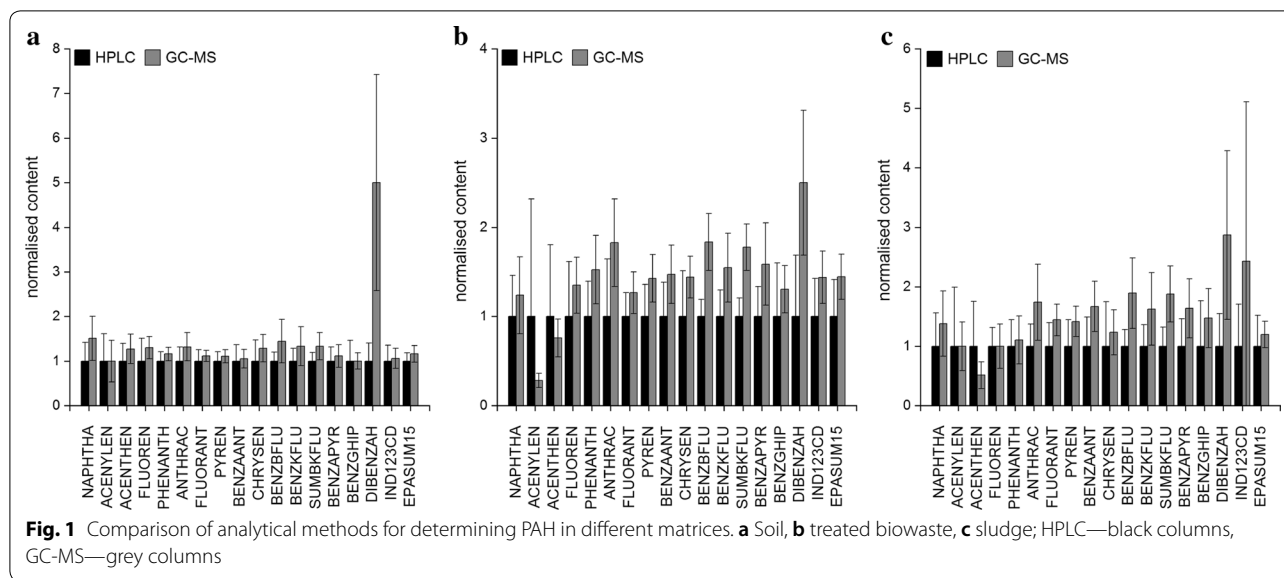
Comparison of analytical methods and sample pre-treatment procedures for PAH and PCB

Results of the reference solution for PAH cannot be evaluated for this purpose, as two reference solutions with different solvents were used. The results for the different matrices shown in Figs. 1 and 2 are normalised to one of the analytical methods (PAH to HPLC and PCB to GC-MS) and indicate that the concentration determined using GC-MS is usually higher than for the HPLC (PAH) and GC-ECD (PCB). However, it must be considered that extraction and clean-up have a significant influence on the results. For both groups of analytes, four extraction and four (PAH) or eleven (PCB) clean-up methods are included in the standard. This large number of possible treatments results in a data set that cannot be statically evaluated, as not only the measurement method, but also the sample preparation may vary between laboratories.

Figure 3 shows data for selected analytes in more detail; for results of all analytes, refer to the report [39]. The examples of fluoranthene, benzo[a]pyrene and PCB 180 show no trend for the sample preparation method. For the analytical method, a slight trend for higher concentration values for GC-MS analysis is observed for benzo[a]pyrene and PCB 180, while the analytical method does not influence the results for the other two examples. Aside from the trend to higher concentrations when GC-MS is used, there is no statistical justification to prefer a particular analytical method.

Conclusions

This study considered the analytic standards for environmentally relevant organic contaminants in sludge, treated biowaste and soil that were drafted in the framework of the EU project HORIZONTAL but were not accepted as European Standards due to missing validation data. This aim could be reached to provide validation data for all matrices and selected substance groups taking into account CEN/TS 16190:2012 "Sludge, treated biowaste and soil—determination of dioxins and furans and dioxin-like polychlorinated biphenyls by gas chromatography with high-resolution mass selective detection (HR GC-MS)" [31], FprCEN/TS 16181:2012 "Sludge, treated biowaste and soil—determination of polycyclic aromatic
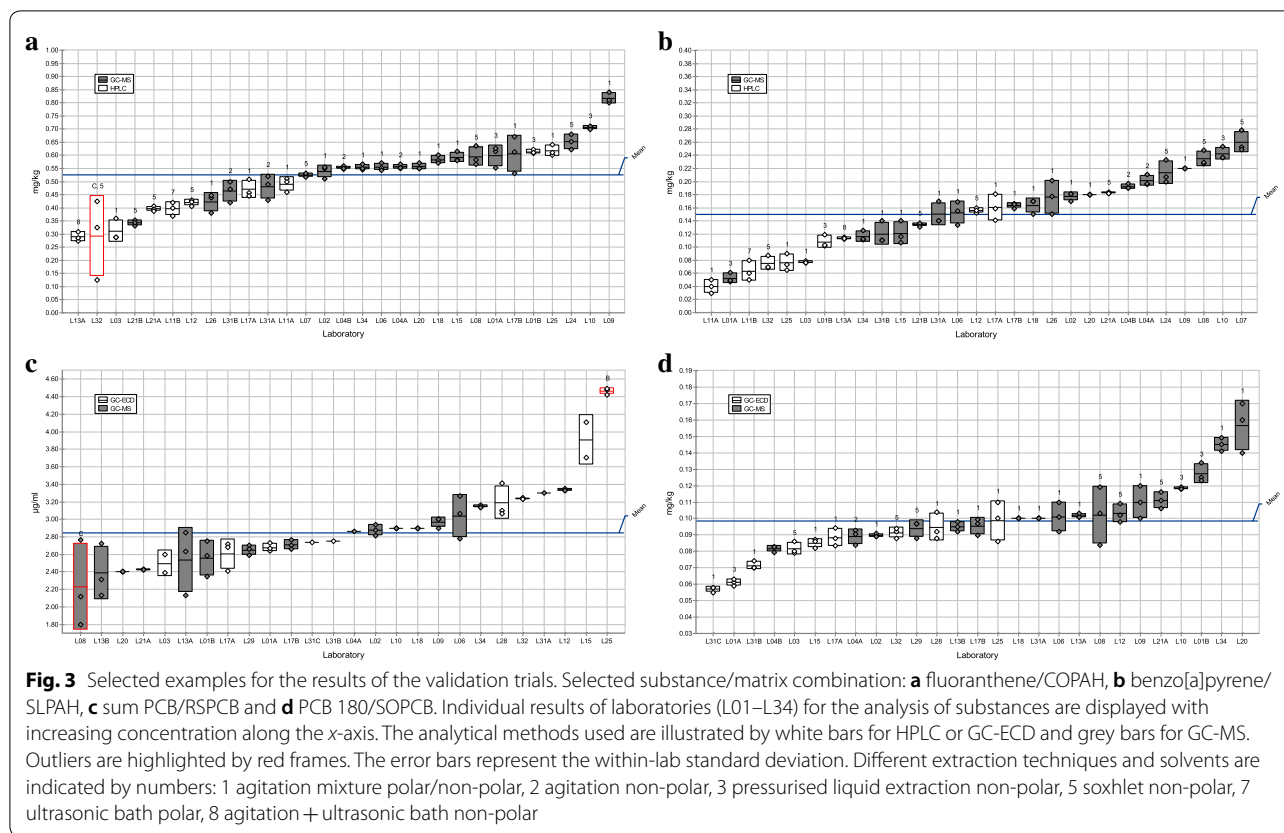
Kalbe *et al. Environ Sci Eur*   (2019) 31:29

Page 7 of 10

Fig. 1 Comparison of analytical methods for determining PAH in different matrices. **a** Soil, **b** treated biowaste, **c** sludge; HPLC—black columns, GC-MS—grey columns

Fig. 2 Comparison of analytical methods for determining PCB in different matrices. **a** Soil, **b** treated biowaste, **c** sludge; GC-ECD—black columns, GC-MS—grey columns

hydrocarbons (PAH) by gas chromatography (GC) and high-performance liquid chromatography (HPLC)" [34] and EN 16167:2012 "Sludge, treated biowaste and soil—determination of polychlorinated biphenyls (PCB) by gas chromatography with mass selective detection (GC-MS) and gas chromatography with electron-capture detection (GC-ECD)" [18, 19].

Specifications for the analysis of the selected organic substance groups comprise instructions on the sample preparation steps (extraction and clean-up of the extract), as well as on the analytic measurement. There are several options available to choose for both the

extraction and the clean-up of the extract. Within the validation trial, the number of participants was too low to cover and evaluate all possible combinations of extraction, clean-up and measurement methods.

The obtained results of the interlaboratory comparison confirm that the technical specifications considered in this study are appropriate for the analysis of the differing matrices sludge, treated biowaste and soil. It can be assumed that the results of the interlaboratory comparison give a realistic impression of the currently feasible performance of the analytical protocols evaluated. This applies also to the substances where the performance data are less sufficient.

Kalbe *et al. Environ Sci Eur*   (2019) 31:29

Page 8 of 10

Fig. 3 Selected examples for the results of the validation trials. Selected substance/matrix combination: **a** fluoranthene/COPAH, **b** benzo[a]pyrene/
SLPAH, **c** sum PCB/RSPCB and **d** PCB 180/SOPCB. Individual results of laboratories (L01–L34) for the analysis of substances are displayed with
increasing concentration along the *x*-axis. The analytical methods used are illustrated by white bars for HPLC or GC-ECD and grey bars for GC-MS.
Outliers are highlighted by red frames. The error bars represent the within-lab standard deviation. Different extraction techniques and solvents are
indicated by numbers: 1 agitation mixture polar/non-polar, 2 agitation non-polar, 3 pressurised liquid extraction non-polar, 5 soxhlet non-polar, 7
ultrasonic bath polar, 8 agitation + ultrasonic bath non-polar

Outlook regarding the implementation of results in standardisation

The obtained performance data have been accepted
by the Technical Committee 444 "Test methods for
environmental characterisation of solid matrices" and
will be published in the following three standards: EN
16190, EN 16181 and EN 16167. In addition, the Tech-
nical Committee is working on harmonising these vali-
dated standards for PAH and PCB applicable for sludge,
treated biowaste and soil with standards for different
types of waste, aiming at reducing the number of very
similar standards and enhancing the efficiency of labo-
ratory work. The first result is the publication of prEN
17322 "Environmental Solid Matrices—Determination
of polychlorinated biphenyls (PCB) by gas chromatog-
raphy—mass selective detection (GC-MS) or electron-
capture detection (GC-ECD)" [50] applicable for the
determination of PCB in sludge, soil, biowaste and
waste. A corresponding standard for PAHs is currently
under revision and will be published by the end of 2019.

For a subsequent adoption of the EN standards as ISO
standards, a project registration will be submitted to ISO
TC 190 SC 3 "Chemical and physical characterization".

Additional file

Abbreviations
ACENYLEN: acenaphthylene; ACENTHEN: acenaphthene; ANTHRAC: anthra-
cene; BENZAANT: benzo[a]anthracene; BENZAPYR: benzo[a]pyrene; BENZBFLU:
benzo[b]fluoranthene; BENZGHIP: benzo[ghi]perylene; BENZKFLU: benzo[k]
fluoranthene; SUMBKFLU: sum benzo[b+k]fluoranthene; CEN: European
Committee for Standardization (Comité Européen de Normalisation); CODIO:
biowaste contaminated with PCDD/PCDF and dl-PCB; COPAH: biowaste
contaminated with PAH; COPCB: biowaste contaminated with PCB; CHRYSEN:
chrysene; DIBENZAH: dibenzo[ah]anthracene; DIN: German Standardiza-
tion Organization; dl-PCB: dioxin-like polychlorinated biphenyls; FLUORANT:
fluoranthene; FLUOREN: fluorene; GC-ECD: gas chromatography with
electron-capture detection; GC-MS: gas chromatography with mass selective
detection; HPLC: high-performance liquid chromatography; GC-HRMS: gas
chromatography with high-resolution mass selective detection; IND123CD:
indeno[1,2,3-cd]pyrene; ISO: International Standardization Organization; LOQ:
limit of quantification; NAPHTHA: naphthalene; PAH: polycyclic aromatic
hydrocarbons; 16 EPA PAH: sum of the 16 PAH substances selected by US EPA
as priority pollutants; 15 EPA PAH: sum 16 EPA PAH excluding acenaphthyl-
ene; PCB: polychlorinated biphenyls; PCB 28: 2,4,4′-trichlorobiphenyl; PCB 52:
2,2′,5,5′-tetrachlorobiphenyl; PCB 101: 2,2′,4,5,5′-pentachlorobiphenyl; PCB
118: 2,3′,4,4′,5-pentachlorobiphenyl; PCB 138: 2,2′,3,4,4′,5′-hexachlorobiphenyl;

Kalbe *et al. Environ Sci Eur*   (2019) 31:29

Page 9 of 10

Author details
[1] Federal Institute for Materials Research and Testing–BAM, Unter den Eichen 87, 12059 Berlin, Germany. [2] Federal Environmental Agency, Bismarkplatz 1, 14193 Berlin, Germany.

References
1. Lambkin D, Nortcliff S, White T (2004) The importance of precision in sampling sludges, biowastes and treated soils in a regulatory framework. TrAC Trends Anal Chem 23:704–715
2. Meyer VR (2007) Measurement uncertainty. J Chromatogr A 1158:15–24
3. European Commission (2003) Standardisation Mandate to CEN for the development of horizontal standards in the fields of slufdge, biowaste and soil. http://ec.europa.eu/growth/tools-databases/mandates/index.cfm?fuseaction=search.detail&id=63#. Accessed 07 Mar 2019
4. Gawlik BM, van der Sloot HA, Ulberth F, Nortcliff S, Simonart T, Cooper B, Leschber R, Andersen K, Wichman K (2004) On the development of horizontal CEN standards supporting the implementation of EU Directives on Sludge, Soil and Biowaste—Project HORIZONTAL. TrAC Trends Anal Chem 23:686–703
5. Desaules A, Ammann S, Blum F, Brandli RC, Bucheli TD, Keller A (2008) PAH and PCB in soils of Switzerland—status and critical review. J Environ Monit 10:1265–1277
6. Van den Berg M, Birnbaum LS, Denison M, De Vito M, Farland W, Feeley M, Fiedler H, Hakansson H, Hanberg A, Haws L, Rose M, Safe S, Schrenk D, Tohyama C, Tritscher A, Tuomisto J, Tysklind M, Walker N, Peterson RE (2006) The 2005 World Health Organization reevaluation of human and mammalian toxic equivalency factors for dioxins and dioxin-like compounds. Toxicol Sci 93:223–241
7. Achten C, Andersson JT (2015) Overview of polycyclic aromatic compounds (PAC). Polycyclic Aromat Compd 35:177–186
8. Di Guardo A, Terzaghi E, Raspa G, Borin S, Mapelli F, Chouaia B, Zanardini E, Morosini C, Colombo A, Fattore E, Davoli E, Armiraglio S, Sale VM, Anelli S, Nastasio P (2017) Differentiating current and past PCB and PCDD/F sources: the role of a large contaminated soil site in an industrialized city area. Environ Pollut 223:367–375
9. Harvey RG (1991) Polyaromatic hydrocarbons—chemistry and carcinogenicity. Cambridge University Press, Cambridge
10. Abbas I, Badran G, Verdin A, Ledoux F, Roumié M, Courcot D, Garçon G (2018) Polycyclic aromatic hydrocarbon derivatives in airborne particulate matter: sources, analysis and toxicity. Environ Chem Lett 16:439–475
11. Andersson JT, Achten C (2015) Time to say goodbye to the 16 EPA PAHs? Toward an up-to-date use of PACs for environmental purposes. Polycyclic Aromat Compd 35:330–354
12. Wise SA, Sander LC, Schantz MM (2015) Analytical methods for determination of polycyclic aromatic hydrocarbons (PAHs)—a historical perspective on the 16 U.S. EPA priority pollutant PAHs. Polycyclic Aromat Compd 35:187–247
13. Kuppusamy S, Thavamani P, Venkateswarlu K, Lee YB, Naidu R, Megharaj M (2017) Remediation approaches for polycyclic aromatic hydrocarbons (PAHs) contaminated soils: technological constraints, emerging trends and future directions. Chemosphere 168:944–968
14. German Federal Environmental Agency (2016) Polycyclic aromatic hydrocarbons: harmful to the environment! toxic! inevitable? https://www.umweltbundesamt.de/en/publikationen/polycyclic-aromatic-hydrocarbons. Accessed 07 Mar 2019
15. Keith LH (2015) The source of U.S. EPA's sixteen PAH priority pollutants. Polycyclic Aromat Compd 35:147–160
16. Breivik K, Sweetman A, Pacyna JM, Jones KC (2002) Towards a global historical emission inventory for selected PCB congeners—a mass balance approach: 1. Global production and consumption. Sci Total Environ 290:181–198
17. Ballschmiter K, Zell M (1980) Analysis of polychlorinated biphenyls (PCB) by glass capillary gas chromatography. Fresenius' Zeitschrift für analytische Chemie 302:20–31
18. EN 16167: 2012-08 Sludge, treated biowaste and soil—determination of polychlorinated biphenyls (PCB) by gas chromatography with mass selective detection (GC-MS) and gas chromatography with electron-capture detection (GC-ECD)
19. prEN 17322: 09-2018 Environmental solid matrices—determination of polychlorinated biphenyls (PCB) by gas chromatography—mass selective detection (GC-MS) or electron-capture detection (GC-ECD)
20. Stockholm Convention on Persistent Organic Pollutants (POPs) (2004, last revision in 2017) http://chm.pops.int/TheConvention/Overview/TextoftheConvention/tabid/2232/Default.aspx. Accessed 07 Mar 2019
21. Hooper SW, Pettigrew CA, Sayler GS (1990) Ecological fate, effects and prospects for the elimination of environmental polychlorinated biphenyls (PCBs). Environ Toxicol Chem 9:655–667
22. Erickson MD, Kaley RG (2011) Applications of polychlorinated biphenyls. Environ Sci Pollut Res 18:135–151
23. Weber R, Herold C, Hollert H, Kamphues J, Ungemach L, Blepp M, Ballschmiter K (2018) Life cycle of PCBs and contamination of the environment and of food products from animal origin. Environ Sci Pollut Res 25:16325–16343
24. Cheruiyot NK, Lee WJ, Yan P, Mwangi JK, Wang LC, Gao X, Lin NH, Chang-Chien GP (2016) An overview of PCDD/F inventories and emission factors from stationary and mobile sources: what we know and what is missing. Aerosol Air Qual Res 16:2965–2988
25. Safe S (1996) Human toxicology of chlorinated organic micropollutants. In: Hester RE, Harrison RM (eds) chlorinated organic micropollutants. The Royal Society of Chemistry, Cambridge, pp 73–88

Kalbe *et al. Environ Sci Eur*   (2019) 31:29

Page 10 of 10

26. Northcott GL, Jones KC (2000) Spiking hydrophobic organic compounds into soil and sediment: a review and critique of adopted procedures. Environ Toxicol Chem 19:2418–2430
27. Fraunhofer-Institut für Molekularbiologie und Angewandte Oekologie IME. http://www.refesol.de/english/boden01a.shtml. Accessed 07 Mar 2019
28. van der Veen AMH, Nater DAG (1993) Sample preparation from bulk samples: an overview. Fuel Process Technol 36:1–7
29. DIN 19747: 2009-07 Untersuchung von Feststoffen—Probenvorbehandlung, -vorbereitung und -aufarbeitung für chemische, biologische und physikalische Untersuchungen. (Investigation of solids—Pre-treatment, preparation and processing of samples for chemical, biological and physical investigations)
30. ASTM E826-14 (2014) Standard practice for testing homogeneity of a metal lot or batch in solid form by spark atomic emission spectrometry. American Society for Testing and Materials
31. CEN/TS 16190: 2011-12 Sludge, treated biowaste and soil—determination of dioxins and furans and dioxin-like polychlorinated biphenyls by gas chromatography with high resolution mass selective detection (HR GC-MS)
32. Landesumweltamt Nordrhein-Westfalen (2000) Bestimmung von polyzyklischen aromatischen Kohlenwasserstoffen (PAK) in Bodenproben. Merkblätter Nr. 1
33. DIN 53803-1: 1991-03 Probenahme; Statistische Grundlagen der Probenahme bei einfacher Aufteilung (Sampling; statistical basis, one-way layout)
34. FprCEN/TS 16181: 2012-03 Sludge, treated biowaste and soil—determination of polycyclic aromatic hydrocarbons (PAH) by gas chromatography (GC) and high performance liquid chromatography (HPLC)
35. QuoData (2011) PROLab™ Plus, Software for PT programs and collaborative studies. Gesellschaft für Qualitätsmanagement und Statistik mbH, Dresden
36. DIN ISO 5725-2: 2002-12 Genauigkeit (Richtigkeit und Präzision) von Messverfahren und Messergebnissen—Teil 2: Grundlegende Methode für Ermittlung der Wiederhol- und Vergleichpräzision eines vereinheitlichten Messverfahrens (ISO 5725-2:1994 einschließlich Technisches Korrigendum 1:2002) (Accuracy (trueness and precision) of measurement methods and results—Part 2: basic method for the determination of repeatability and reproducibility of a standard measurement method (ISO 5725-2:1994 including Technical Corrigendum 1:2002))
37. Mandel J (1991) The validation of measurement through interlaboratory studies. Chemom Intell Lab Syst 11:109–119
38. CEN/TS 16800: 12-2015 Guideline for the validation of physico-chemical analytical methods
39. Scharf H, Lück D, Kalbe U, Lehnik-Habrink P, Bandow N, Berger W, Buchholz A (2015) Validierung von Analyseverfahren, die im Rahmen des Projektes HORIZONTAL entwickelt, jedoch nicht validiert wurden. Dessau. https://www.umweltbundesamt.de/en/publikationen/validierung-von-analyseverfahren-die-im-rahmen-des. Accessed 07 Mar 2019
40. Horwitz W (1982) Evaluation of analytical methods used for regulation of foods and drugs. Anal Chem 54:67A–76A
41. Harner T, Kucklick J (2003) Interlaboratory study for the polychlorinated naphthalenes (PCNs): phase 1 results. Chemosphere 51:555–562
42. Gratz LD, Bagley ST, Leddy DG, Johnson JH, Chiu C, Stommel P (2000) Interlaboratory comparison of HPLC-fluorescence detection and GC/MS: analysis of PAH compounds present in diesel exhaust. J Hazard Mater 74:37–46
43. Reiner EJ (2010) The analysis of dioxins and related compounds. Mass Spectrom Rev 29:526–559
44. Reiner EJ, Clement RE, Okey AB, Marvin CH (2006) Advances in analytical techniques for polychlorinated dibenzo-*p*-dioxins, polychlorinated dibenzofurans and dioxin-like PCBs. Anal Bioanal Chem 386:791–806
45. Balzamo S, de Zorzi P, Barbizzi S, Calabretta E, Potalivo M, Rosamilia S (2009) Measurement of PAHs in environmental matrices: results from an interlaboratory comparison on the different steps of the measurement procedure. Accred Qual Assur 14:487–495
46. de Boer J, Cofino WP (2002) First world-wide interlaboratory study on polybrominated diphenylethers (PBDEs). Chemosphere 46:625–633
47. Becker R, Koch M, Lehnik-Habrink P, Nehls I (2006) Quantification of polychlorinated biphenyls in plastic granulates from the recycling of cables—reference materials and interlaboratory comparison. Chemosphere 65:1652–1659
48. Hartonen K, Bøwadt S, Dybdahl HP, Nylund K, Sporring S, Lund H, Oreld F (2002) Nordic laboratory intercomparison of supercritical fluid extraction for the determination of total petroleum hydrocarbon, polychlorinated biphenyls and polycyclic aromatic hydrocarbons in soil. J Chromatogr A 958:239–248
49. Geurts R, Spooren J, Quaghebeur M, Broos K, Kenis C, Debaene L (2016) Round robin testing of a percolation column leaching procedure. Waste Manag 55:31–37
50. prEN 17322: 2018-11 Environmental solid matrices—determination of polychlorinated biphenyls (PCB) by gas chromatography—mass selective detection (GC-MS) or electron-capture detection (GC-ECD)

Publisher's Note