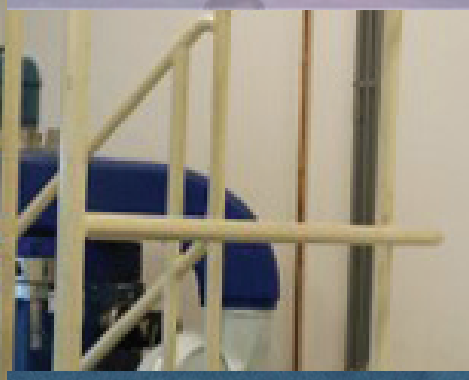

Validation of ISO 16751 Soil Quality -Environmental availability of non-polar organic compounds

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ISO/TC190/Soil Quality has developed a standard to enable measurement of the bioavailability of organic contaminants in soil and soil like materials (ISO/TS16751), To become a full standard a validation is necessary. The validation is based on an intra laboratory validation carried out by the laboratory of Rijkswaterstaat in Lelystad (The Netherlands), and on an international interlaboratory validation study organized by WEPAL, Wageningen. Results are evaluated according ISO 5725. As decided by the responsible ISO-working group, the results support the transfer of ISO/TS 16751 into a full ISO-standard.

Keywords: validation, soil, bioavailability, standard, ISO, Tenax, cyclodextrin

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Summary

ISO/TC190/Soil Quality has developed an international standard to measure the bioavailable fraction of organic chemicals in soil and soil-like materials. The standard 'ISO/TS 16751 Soil Quality - Environmental availability of non-polar organic compounds — Determination of the potentially bioavailable fraction and the non-bioavailable fraction using a strong adsorbent or complexing agent' describes the extraction of the bioavailable fraction, which has to be followed by chemical analysis of the target chemicals. To become a full standard, the method requires validation to demonstrate the repeatability and reproducibility of the method.

During the development of the standard, the Laboratory of Rijkswaterstaat in Lelystad, The Netherlands, performed supporting intra-laboratory experiments. These investigations supplied data on the ratio of soil and extractant that should be used and the size of the soil sample to be analysed. The high costs of Tenax and cyclodextrin and the wish to limit costs of analysis had asked for a limited but still representative size of the sample to be analysed. A sample size of 4 g is prescribed in ISO/TS 16751. Intralaboratory validation in the Rijkswaterstaat Laboratory has shown that repeatability and reproducibility are as can be expected for this kind of methods and ISO/TS 16751 is an adequate method to measure the bioavailable fraction.

The interlaboratory validation was organised by WEPAL (the Wageningen Evaluating Programmes for Analytical Laboratories organisation), which is part of Wageningen University in the Netherlands. Nine laboratories participated in this interlaboratory validation. Four soil samples were selected with different soil properties. Before the validation, WEPAL has tested stability and homogeneity of the samples and all samples were suitable for the validation.

The statistical test for the validation was carried out according to the ISO 5725 Series. Outliers were identified using the Cochran's test and Grubbs' test described in ISO 5725-2. The relative standard variations for repeatability and reproducibility were higher than obtained with more common methods for total analysis of organic contaminants within the SETOC-Program organized by WEPAL. This can be explained by the limited experience with the method. The results obtained reflect the present situation and it is expected that the performance of laboratories using this method will improve in future.

It is the role of the responsible ISO working group to decide if the repeatability and reproducibility calculated according ISO 5725 are sufficient for this method. In its meeting of September 12, 2018 in Brno, Working group TC190/SC7/WG6 'Transfer and mobility of components' discussed the concept version of this report and was satisfied with the results. The group concluded that the results were sufficient and a procedure was started to add the validation results to the standard and to transfer the TS into a full standard.

1 Introduction

ISO/TC190/Soil Quality has developed an international standard to measure the bioavailable fraction of organic chemicals in soil and soil-like materials. The standard 'ISO/TS 16751 Soil Quality - Environmental availability of non-polar organic compounds — Determination of the potentially bioavailable fraction and the non-bioavailable fraction using a strong adsorbent or complexing agent' describes the extraction of the bioavailable fraction, which has to be followed by chemical analysis of the target chemicals. To become a full standard, the method requires validation to demonstrate the repeatability and reproducibility of the method.

WEPAL, (the Wageningen Evaluating Programmes for Analytical Laboratories organisation has been asked to organize this validation. WEPAL, part of Wageningen University organises six large international laboratory-evaluating programs for environmental and agricultural laboratories.

During the development of the standard, the Laboratory of Rijkswaterstaat in Lelystad, The Netherlands, performed supporting intra-laboratory experiments. These investigations supplied data on the ratio of soil and extractant that should be used and the size of the soil sample to be analysed. The high costs of Tenax and cyclodextrin and the wish to limit costs of analysis had asked for a limited but still representative size of the sample to be analysed. A sample size of 4 g is prescribed in ISO/TS 16751.

The interlaboratory validation was organised by WEPAL. Nine laboratories participated in this interlaboratory validation. Four soil samples were selected with different soil properties. Before the validation, WEPAL has tested stability and homogeneity of the samples and all samples were suitable for the validation.

Results of both intra and interlaboratory activities are described in this report.

2 Method

2.1 WEPAL

WEPAL (Wageningen Evaluating Programmes for Analytical Laboratories; www.wepal.nl) is part of Wageningen University in the Netherlands, and organises six large, international, laboratory-evaluating programmes for environmental- and agricultural laboratories. WEPAL organised the validation presented in this report, including the selection, preparation and distribution of samples, instructions for participation and the statistical evaluation.

2.2 Principle of ISO/TS 16751

The International Standard ISO/TS 16751 specifies a method for an estimation of the potential bioavailable and non-bioavailable fractions of organic contaminants, i.e. the amount of the contaminant in the matrix that is potentially exchangeable with the aqueous phase; specifically that, which is strongly adsorbed on/complexed by Tenax®/cyclodextrin.

The extractable and non-bioavailable fraction of the contaminant left in the sample following the action of Tenax®/cyclodextrin can be subsequently measured with an exhaustive/harsh extraction technique (designed to measure the total concentration) and in this way the non-bioavailable fraction of the contaminant is assessed.

Thus, in numerical terms, the total contaminant concentration in a sample is the sum of the bioavailable concentration (established using Tenax®/cyclodextrin) and the non-bioavailable concentration (established using a subsequent harsh extraction method performed on the residue that is left after the matrix has been extracted using a strong sorbent or complexing agent):

$$c_{\text{tot,cont}} = c_{\text{bio}} + c_{\text{non-bio}} \quad (1)$$

Where:

$c_{\text{tot,cont}}$	is the total contaminant concentration;
c_{bio}	is the bioavailable concentration;
$c_{\text{non-bio}}$	is the non-bioavailable concentration.

The soil, soil-like material or sediment sample with particle size <2 mm is extracted with water containing a "receiver phase" for the organic contaminants. This phase is either a complexing agent (cyclodextrin) or a strong adsorbent [Tenax®[1)]]. The solubility of non-polar compounds is limited and in this method, the receiver phase acts as an "infinite sink".

In the following step, the contaminants adsorbed are extracted from the receiver phase and determined by appropriate analytical methods. The measured amount, which is the amount that desorbs from the soil material during 20 h, reflects the fraction of contaminant that can have effects on biotic systems and that can become mobile. The amount of contaminants left in the soil residue, the non-bioavailable fraction, can be measured using a subsequent harsh/exhaustive extraction designed to measure the total concentration. Formula 1 can then be used to determine the total contaminant concentration in the sample (if desired).

2.3 Method development

The original methods were based on a small amount of sample (1-2 g), which can give reliable results in more academic studies where homogeneous soils are investigated. The ISO standard should be applicable for all soil and soil-like materials, including less homogenous samples. In other ISO standards, 10 g is often prescribed to ensure that a representative laboratory sample will be taken. Because further milling will have impact on the bioavailability, particle size reduction is not an option to enable a smaller laboratory sample. There was a necessity to reduce the laboratory sample size, because both Tenax and cyclodextrin are expensive chemicals, and with a smaller laboratory sample size less chemicals are needed. Research on the ratio between the laboratory sample size and the amount of Tenax needed was carried out in the laboratory of Rijkswaterstaat in Lelystad, The Netherlands.

2.4 Preparation of the validation samples

Samples from the SETOC-program were selected with different soil properties (Table 1) and a varying content of organic compounds. Basic properties are given in Table 1.

Table 1 *Consensus values of general soil parameters in the analysed soils*

Parameter	1	2	3	4
Clay (%)	21.2	8.82	31.0	12.9
Organic matter (LOI) (%)	3.49	3.23	6.56	3.13
PAH (sum) (mg/kg)	13.2	80.5	15.2	40
Mineral oil (mg/kg)	682	317	844	505
PCB sum (mg/kg)	0.286	0.172	0.74	0.015

All materials used were homogenized and grinded materials to ensure that all participants received samples with the same composition. They were sieved over 0.5 mm and distributed in pots to contain 100 g of soil. Before sending, it was checked if the soils were homogenously distributed over the pots using previous test results and specific analysis of total concentrations in a random selection of 10 pots. The samples were distributed in June 2016.

2.5 Measurement of bioavailability

The participants originated from Germany, France, Poland and The Netherlands, and carried out the measurement according to ISO/DIS 16751, which is the Draft International Standard version of ISO/TS 16751. Besides some editorial differences, both versions were equal. They were asked to perform the determination in three -fold. Participants submitted their results in an Excel-file supplied by WEPAL (see Annex 1).

2.6 Statistical evaluation

2.6.1 ISO 5725 model

Statistical testing of the validation was carried out according to the ISO 5725 Series. Outliers were identified using the Cochran's test and Grubbs' test described in ISO 5725-2. ISO 5725 assumes that only small differences exist in the intra laboratory variance between laboratories. Cochran's test, as described in ISO 5725-2, was used to test the validity of this assumption. Results with too large intra laboratory variance were identified as outliers and excluded from further evaluation.

Next, Grubbs' test was applied to identify outliers with an extreme mean.

After eliminating the outliers, the following parameters are calculated and reported:

x mean value in mg/kg;
S_r repeatability standard deviation in mg/kg;
VC_r relative repeatability standard deviation, in percent;
S_R reproducibility standard deviation, in mg/kg;
VC_R relative reproducibility standard deviation, in percent.

It is the role of the responsible ISO working group to decide if the repeatability and reproducibility calculated according to ISO 5725 are sufficient for this method. In its meeting of September 12, 2018 in Brno, Working group TC190/SC7/WG6 'Transfer and mobility of components' discussed the concept version of this report.

3 Results and discussion

3.1 Method development

3.1.1 Introduction

In the original papers on the Tenax and cyclodextrin method, 1.5 g soil or sediment has been used as a laboratory sample. This is sufficient for homogeneous samples as used in academic studies. A larger laboratory sample is necessary to make sure that the laboratory sample is representative. Multiplying all masses and volumes of reagents in the method with the factor 10/1.5 would lead to an amount of 10 g of Tenax, which increase is not desirable (Costs and difficulties with handling). In this chapter on method development, results are described of an investigation on the amount of Tenax to be used. In addition, results are described on the amount of petroleumether necessary to extract the Tenax. The last part of method development was on testing of the inner-laboratory performance.

3.1.2 Results and Discussion

During first discussions of the method, the following questions raised.

- The method is already in use in several laboratories. Hexane is used to extract the bioavailable compounds from Tenax or cyclodextrin; is it possible to replace hexane which is neurotoxic by more safe petroleumether (PE)?
- Some laboratories used ethanol as first solvent to make the Tenax accessible for PE. After extraction, ethanol was removed again with water. Is this necessary?;
- Is there an effect on bioavailability by using a dried instead of a wet sample?

In an experiment a wet and dry sample having the same origin were extracted with hexane, PE and first with ethanol followed by PE. Ethanol was removed using water. Results of the experiments are given in Table 2 and 3. Triplo sampes were analysed (1. 2 and 3).

Table 2 *Effect of different solvents to extract individual bioavailable PAHs from Tenax and the use of a wet or dry sample. Results in mg/kg d.m. Triplo samples are analysed*

	fluorene	fenantrene	anthracene	fluoranthe-ne	pyrene	benz(a)an-thracene	chryseen	benz(b)fluorant henen	benz(k)fluorant hene	benz(a)py-rene	benzo(ghi)-perylene	indeno(1,2,3cd) pyrene
dry n-hexane 1	0.07	0.25	0.14	1.48	1.22	0.60	0.61	0.42	0.15	0.14	0.13	0.06
dry n-hexane 2	0.05	0.23	0.11	1.42	1.15	0.56	0.55	0.39	0.14	0.13	0.13	0.06
dry n-hexane 3	0.07	0.25	0.11	1.56	1.10	0.58	0.58	0.39	0.14	0.13	0.10	0.06
dry PE 1	0.04	0.23	0.12	1.56	1.02	0.53	0.52	0.35	0.13	0.11	0.10	0.06
dry PE 2	0.04	0.23	0.12	1.32	1.04	0.54	0.54	0.36	0.13	0.12	0.10	0.06
dry PE 3	0.05	0.23	0.12	1.49	1.03	0.53	0.52	0.34	0.12	0.11	0.09	0.05
dry ethanol-PE-water 1	0.06	0.24	0.15	1.43	1.20	0.58	0.58	0.38	0.14	0.13	0.12	0.06
dry ethanol-PE-water 2	0.06	0.25	0.12	1.50	1.11	0.55	0.50	0.37	0.14	0.12	0.10	0.06
dry ethanol-PE-water 3	0.06	0.25	0.13	1.42	1.09	0.58	0.57	0.38	0.14	0.13	0.11	0.07
median	**0.06**	**0.24**	**0.12**	**1.48**	**1.10**	**0.56**	**0.55**	**0.38**	**0.14**	**0.13**	**0.10**	**0.06**
wet n-hexane 1	0.01	0.11	0.18	1.45	1.29	0.74	0.59	0.43	0.17	0.28	0.12	0.06
wet n-hexane 2	0.02	0.11	0.16	1.78	1.31	0.75	0.61	0.44	0.17	0.28	0.13	0.06
wet n-hexane 3	0.04	0.13	0.17	1.64	1.36	0.78	0.62	0.45	0.18	0.29	0.13	0.06
wet PE 1	0.01	0.09	0.17	1.59	1.33	0.72	0.58	0.39	0.16	0.25	0.13	0.05
wet PE 2	0.02	0.08	0.13	1.44	1.28	0.70	0.55	0.40	0.16	0.26	0.15	0.05
wet PE 3	0.01	0.09	0.16	1.40	1.26	0.72	0.58	0.40	0.16	0.25	0.12	0.05
wet ethanol-PE-water 1	0.01	0.07	0.14	1.54	1.32	0.70	0.57	0.37	0.15	0.25	0.12	0.05
wet ethanol-PE-water 2	0.01	0.11	0.14	1.48	1.32	0.71	0.55	0.40	0.17	0.26	0.12	0.05
wet ethanol-PE-water 3	0.03	0.10	0.13	1.50	1.29	0.68	0.46	0.40	0.16	0.25	0.11	0.05
median	**0.01**	**0.10**	**0.16**	**1.50**	**1.31**	**0.72**	**0.58**	**0.40**	**0.16**	**0.26**	**0.12**	0.05

Table 3 *Effect of different solvents to extract individual bioavailable chlorinated compounds from Tenax and the use of a wet or dry sample. Results in µg/kg d.m.*

	HCB	PCB 28	PCB 52	PCB 101	p,p-DDE	o,p-DDD	PCB 118	p,p-DDD	PCB 153	p,p-DDT	PCB 138	PCB 180
dry n-hexane 1	1.84	11.0	22.7	23.6	7.82	2.53	10.4	12.2	20.5	3.60	14.3	6.93
dry n-hexane 2	1.60	8.6	18.7	21.1	6.57	1.84	9.5	10.8	18.6	3.11	12.3	5.89
dry n-hexane 3	2.06	10.6	21.7	23.5	7.17	2.13	11.2	11.2	20.9	3.71	13.3	6.42
dry PE 1	1.79	9.0	19.7	22.3	7.03	2.15	10.5	11.1	20.9	3.78	13.6	6.73
dry PE 2	1.74	9.2	19.6	22.7	7.12	2.09	10.3	10.5	20.1	3.65	13.4	6.59
dry PE 3	1.82	10.1	21.5	23.1	6.99	2.09	10.1	10.6	19.4	3.51	13.0	6.27
dry ethanol-PE-water 1	1.63	10.0	20.2	24.3	7.67	2.40	10.0	11.2	20.5	3.54	14.6	6.81
dry ethanol-PE-water 2	1.68	8.7	19.1	21.9	6.92	1.91	10.1	11.1	20.0	3.55	13.1	6.42
dry ethanol-PE-water 3	2.21	11.0	22.1	25.6	8.03	2.26	11.0	12.0	21.3	3.87	14.8	7.12
median	**1.79**	**10.0**	**20.2**	**23.1**	**7.12**	**2.13**	**10.3**	**11.1**	**20.5**	**3.60**	**13.4**	**6.59**
wet n-hexane 1	0.65	6.5	18.9	23.5	7.63	2.14	10.3	12.5	22.8	3.78	14.2	6.17
wet n-hexane 2	0.69	6.7	19.5	24.0	7.74	2.23	10.7	12.9	22.0	3.63	14.5	6.29
wet n-hexane 3	0.74	7.1	20.9	24.8	8.51	2.28	11.8	12.8	23.7	4.06	15.2	6.72
wet PE 1	0.40	7.4	19.8	25.3	8.61	2.77	10.2	14.3	23.0	3.68	14.7	6.46
wet PE 2	0.54	5.9	19.0	23.6	8.03	2.21	10.7	13.2	23.1	3.82	14.6	6.49
wet PE 3	0.64	6.1	19.6	23.7	7.94	2.23	11.2	13.4	23.6	4.15	14.7	6.50
wet ethanol-PE-water 1	0.54	6.4	19.4	25.3	8.24	2.51	11.4	14.4	23.4	3.78	15.2	6.72
wet ethanol-PE-water 2	0.73	7.2	21.4	25.1	8.43	2.23	11.3	13.1	23.5	3.83	14.8	6.46
wet ethanol-PE-water 3	0.70	6.6	20.2	25.0	8.39	2.42	11.4	14.0	23.1	4.10	15.1	6.59
median	**0.65**	**6.6**	**19.6**	**24.8**	**8.24**	**2.23**	**11.2**	**13.2**	**23.1**	**3.82**	**14.7**	**6.49**

From the values in Table 2 and Table 3 it can be concluded that extraction of the bioavailable PAHs from Tenax can be done with all solvents applied. It is therefore possible to replace hexane by PE. Use of ethanol is also possible. Using wet or dry samples gives comparable results (median value) for most of the analysed compounds (white columns) and not comparable for some compounds (green columns). This can also be caused by differences in composition in the dry and wet sample. The wet samples was splitted and a part was dried. It is difficult to split a large wet sample in two portions with the same composition.

Ratio sample versus Tenax, first experiment

In the first experiment, we used different amounts of sample (1-10 g dry) matter and used the original amount of Tenax (1.5 g). The sample used contained a high amount of PAHs (approx. 800 mg/kg d.m. EPA-PAHs) and mineral oil (12,000 mg/kg d.m.). The bioavailability of the PAHs was high (approx. 70% of the PAHs were available). The origin of the sample was the Petroleum Harbour in Amsterdam and the sample was considered a difficult sample in relation to the amount of Tenax to be used. Saturation of Tenax by the high amounts of PAHs and mineral oil could occur,

Results are presented in Figure 1. Not all PAHs are presented because for some PAHs the Tenax extract was diluted too far for a reliable result. The blue results were obtained from the wet sample, orange from a freeze-dried sample. The dark colour represents the amount in the first PE extract of 20 ml and the light colour represents the amount in the second extract of 20 ml.

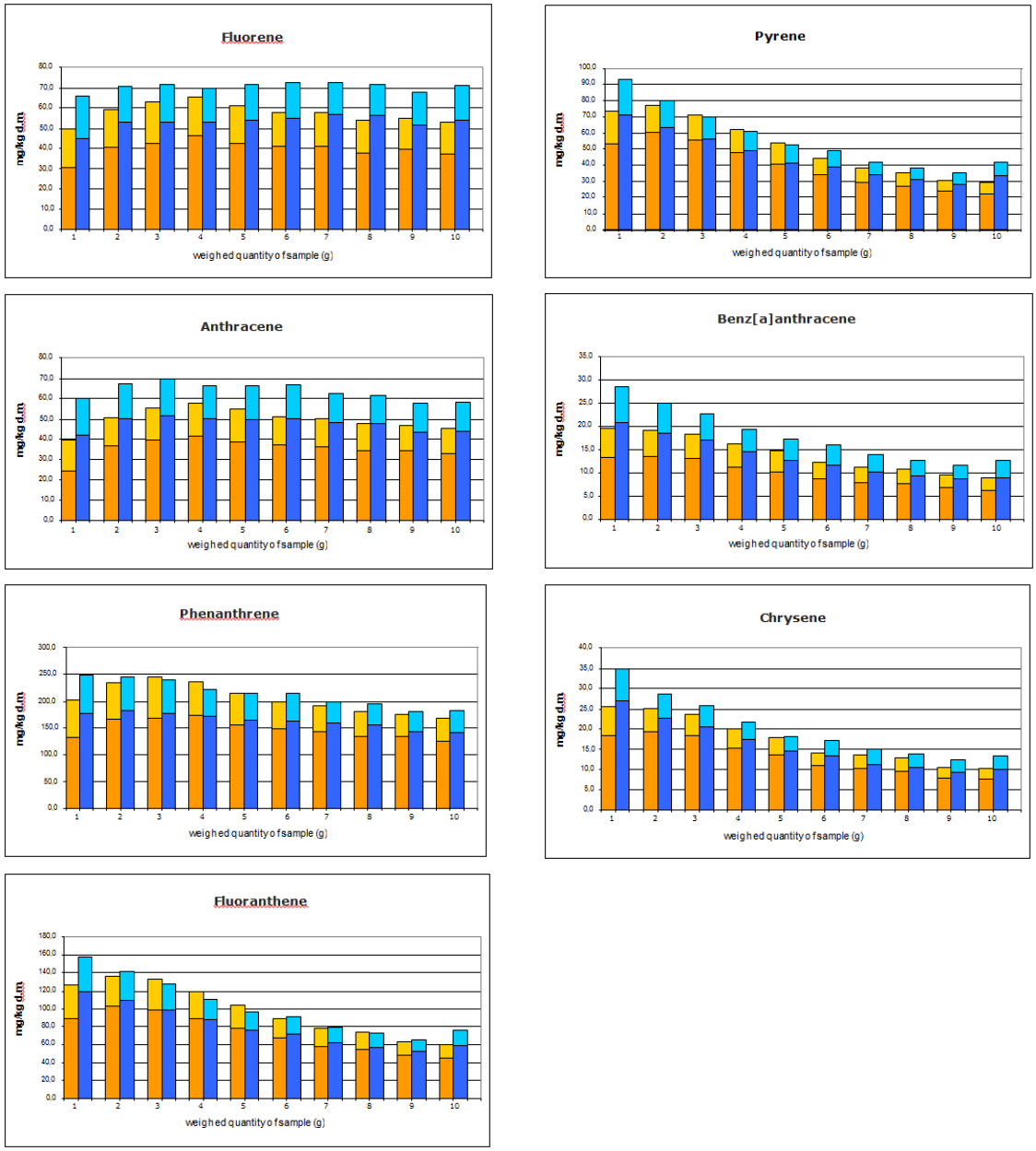


Figure 1 *The environmental available concentration of different PAHs measured with Tenax in mg/kg d.m. as a function of the size of the laboratory sample (g).*
Blue, wet sample: Orange, freeze-dried sample; dark colour, amount in the first PE extract of 20 ml and Light colour, amount in the second extract of 20 ml

From Figure 1 it can be concluded that for some compounds there is a difference between the results of wet and dry samples. The difference can be explained by the inhomogeneity of the sample as explained before. This experiment was not designed to investigate the difference between the pretreatment procedure and a difference in one wet and one freeze-dried portion taken from a large amount is to be expected. Table 2 and 3 show that results of wet and dry samples can also be comparable.

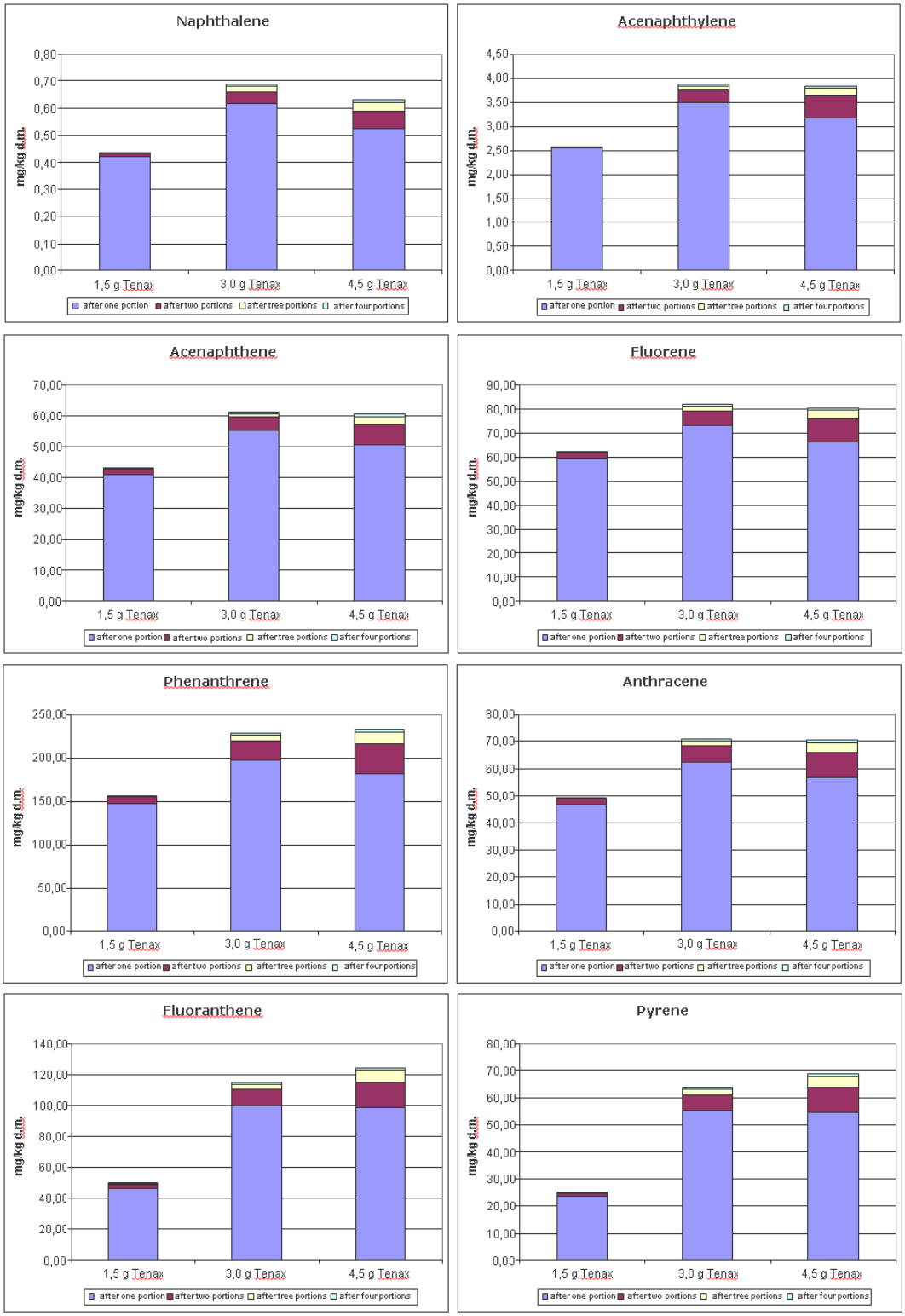
Figure 1 shows that 20 ml of PE extract most of the PAH but is insufficient to extract all. With 40 ml most will be extracted and the results are therefore suitable to evaluate the ratio Tenax/soil.

For the smallest 2-and 3-ring systems (fluorene, anthracene and phenathrene), 1.5 g of Tenax is sufficient to extract the available PAHs in 10 g of sample (based on dry mass). Going to more complex ring structures, which adsorb more strongly to soil and are less soluble in water, 1.5 g of Tenax is able to extract 3-5 g of Petroleum harbour sediment. Based on the adsorption properties and solubility, the results are logical, strong adsorbing PAHs (low solubility) are extracted to a smaller extent with an increasing amount of laboratory sample in a constant volume of extractant.

Ratio sample versus Tenax, second experiment

The first experiment had shown that about 3-5 g of Tenax will be necessary to extract the environmental available fraction in 10 g of Petroleum Harbour sediment (based on dry matter). In the second experiment, it was investigated if it was possible to reduce the amount of Tenax from 10 g to an amount of approx. 3 g.

As mentioned before, the amount of sample used was 10 g (based on dry matter) and we used 1.5, 3.0 and 4.5 gram of Tenax in combination with 140 ml of water. The extraction time of 20 hours was used. The Tenax was extracted with 60 ml of acetone followed by 80 ml of petroleum ether in portions of 20 ml, which were analysed separately. Results are presented in Figure 2.



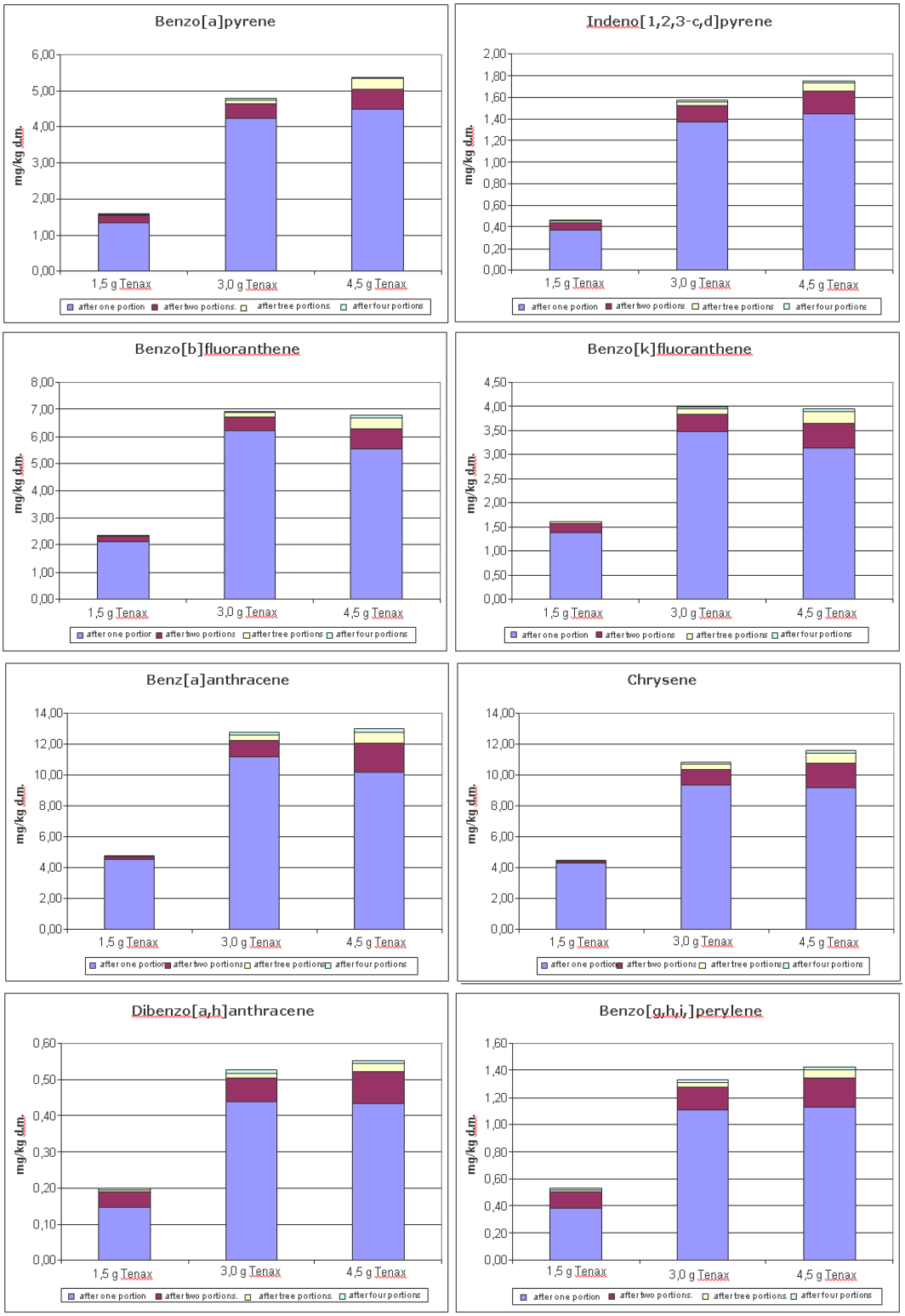


Figure 2 *The environmental available concentration of different PAHs in mg/kg d.m. measured with three different amounts of Tenax. Laboratory sample size 10 g.*

Figure 2 shows that 1.5 g Tenax is insufficient to extract 10 g of sample and 3-4.5 g will be necessary. The difference between 3 and 4.5 g is very small. Taken into account that Petroleum harbour sediment will be a difficult sample it is assumed that 3 g Tenax will be sufficient. This will be tested with other samples.

Figure 2 also shows that 20 ml PE is not sufficient to extract the Tenax. After two portions of 20 ml most is extracted and the following two portions do not add much. It is therefore concluded that 60 ml will be sufficient. It is proposed to use 30 ml followed by two portions of 15 ml.

3.2 Inner laboratory performance of the method

In a first experiment, the repeatability of the method was established by analysing eight different samples in duplicate. A sample size of 10 g was used and the samples were extracted with Tenax. The samples, all wet sediments, originated from different sites in The Netherlands.

- Harbour of Wemeldinge, province Zeeland;
- Petroleum Harbour, Amsterdam ;
- Sludge Depot Ketelmeer, unknown location;
- River Vecht in province of Utrecht;
- Willemsvaart, province of Overijssel;
- Channel from Maarssen to Breukelen, province Utrecht;
- Sediment originating from the province of Limburg;
- Jan van Riebeek Harbour, Amsterdam.

The results are presented in Table 4. Results of repeatability are considered to be sufficient for the method.

Table 4 *Results of calculated repeatability (VC_r%)*

	CAS number	%VC_r	N
PCB 52	35693-99-3	15.7	7
PCB 101	37680-73-2	10.4	7
PCB 118	31508-00-6	20.7	7
PCB 153	35065-27-1	17.5	8
PCB 138	35065-28-2	17.3	8
PCB 180	35065-29-3	13.1	8
QCB	608-93-5	25.1	6
HCB	118-74-1	8.1	5
Heptachlor-exo-epoxide	1024-57-3	29.1	6
Endrin	72-20-8	30.2	4
p,p'-DDD	72-54-8	7.2	6
Acenaphylene	208-96-8	17.8	3
Fluorenene	86-73-7	27.4	3
Fenantreen	85-01-8	20.0	8
Antracene	120-12-7	12.9	8
Fluoranthene	206-44-0	23.8	8
Pyrene	12900-00-0	21.7	8
Benz(a)anthracene	56-55-3	28.7	8
Chrysene	218-01-9	17.2	8
Benz(b)fluoranthene	20599-99-2	14.2	8
Benz(k)fluoranthene	207-08-9	30.5	8
Indeno(1,2,3cd)pyreen	193-39-5	24.3	8

Based on the results shown above, the wish to take a representative sample, but also the costs of chemicals to be used, the ISO working group decided to use a laboratory sample of 4 g combined with an amount of 1.5 g of Tenax. In line with this, it was decided to use 40 ml of cyclodextrin extraction solution.

Just before the decision on the sample size, two samples were analysed in the laboratory of Rijkswaterstaat.

1. Fenelab sample which is a freeze-dried sample, used as a reference sample in The Netherlands. It is a well-homogenized sample. We used 3 g of sample instead of the 10 g prescribed in the working draft version of ISO16751
2. Sample 7 is a wet sediment originating from the province of Limburg. We used 10 g of sample.

In a period of 8 weeks, the samples were analysed 8 times in duplicate. From these results repeatability (VC_r%) and reproducibility (VC_{rw}%) were calculated. The results are presented in Table 5 and Table 6. Only values above the limit of detection are reported. In the last column the total content measured in the sample is presented.

Table 5 *Inner laboratory performance of Fenelab sample*

	S_r (µg/kg d.m.)	VC_r %	S_{Rw} (µg/kg d.m.)	VC_{Rw} %	$x_{gem, Availability}$ (µg/kg d.m.)	Availability (%)	total (µg/kg d.m.)
Fenantrene	4.27	5.8	10.6	12.4	86.0	8.5	1110
Fluoranthene	13.6	6.2	29.0	13.4	223	12.3	2000
Pyrene	8.63	5.6	28.4	17.4	153		n.m.
benz(a)anthracene	5.28	7.0	3.77	5.2	75.2	7.5	1100
Chrysene	8.09	6.9	5.45	4.6	117	10.9	1190
Benz(b)fluoranthene	7.60	6.4	6.24	5.3	119		n.m.
Benz(k)fluoranthene	3.40	6.9	3.76	7.7	49.5	7.7	708
Benz(a)pyrene	2.81	8.2	10.1	24.1	33.84	3.5	1070
Dibenz(ah)anthracene	0.52	7.5	0.50	7.1	7.00		n.m.
Benzo(ghi)perylene	4.75	10.0	5.16	10.8	47.2	5.3	984
Indeno(1,2,3cd)pyrene	3.49	8.0	4.11	9.2	44.5	4.5	1090
HCB	1.32	8.8	2.67	17.6	14.2	9.4	166
PCB 28	0.13	9.8	0.28	22.6	1.19	5.5	24.1
PCB 52	0.53	8.9	1.01	17.3	5.64	18.0	34.5
PCB 101	1.14	9.9	1.80	14.0	11.9	24.8	52.7
PCB 118	0.53	9.8	0.54	9.9	5.46	18.2	33.0
PCB 153	1.24	8.3	1.82	11.6	15.3	20.9	80.2
PCB 138	1.40	8.2	2.34	13.2	17.4	24.3	78.9
PCB 180	1.28	12.4	1.90	18.7	8.69	19.4	49.2
p,p-DDD	0.56	8.8	2.83	36.6	7.25	47.7	16.7
p,p-DDE	0.62	12.9	0.47	10.2	4.78	44.6	11.8
o,p-DDD	0.43	13.0	3.99	57.7	4.75	85.1	6.14

n.m. = not measured

Table 6 *Inner laboratory performance of wet sediment sample 7*

	S_r (µg/kg dm)	VC_r %	S_{Rw} (µg/kg dm)	VC_{Rw} %	$x_{gem, Availability}$ (µg/kg dm)
Pyreen	9.56	16.0	5.73	11.1	57.0
Benz(a)anthraceen	5.67	25.8	1.77	12.6	16.6
Benz(k)fluorantheen	3.95	24.1	1.32	11.7	12.5
Benz(a)pyreen	8.28	23.6	6.17	26.2	23.1
Benzo(ghi)peryleen	3.17	22.1	1.32	14.0	10.5
PCB 28	0.73	12.1	0.86	14.1	6.23
PCB 52	0.92	9.1	1.26	12.4	10.2
PCB 101	1.28	9.0	1.71	12.2	14.5
PCB 118	0.60	8.8	0.74	10.8	6.86
PCB 153	1.41	8.4	1.79	10.8	17.0
PCB 138	1.30	8.9	1.62	11.4	14.8
PCB 180	0.89	8.9	1.17	12.0	10.0
p,p-DDT	7.61	74.1	12.0	61.8	15.8
p,p-DDE	0.93	50.7	0.27	6.9	3.64

The repeatability and reproducibility for the Tenax-method in the Fenelab sample (Table 5) are comparable with values for the total analysis of organic contaminants in this sample found in earlier WEPAL proficiency tests (except DDT/DDE/DDD). They are better than values obtained in less homogenized samples. The values of sample 7 in Table 6 are an example of such a less homogenized sample. In such a sample, the difference in daily subsamples can even be larger than the differences in averages of different days of analysis. The values for repeatability and reproducibility presented in Table 5 can be considered as best achievable. The values in Table 6 should be considered as 'normal' values.
The values also show that in a homogenized sample 3 g of sample is sufficient for a proper analysis. It is not allowed to conclude that it is necessary to homogenize the samples before analysis. This may have effect on the availability.
The values for DDT/DDE and DDD are relatively high. This is caused by the instability of the chromatographic system for these compounds. The calibration of the two column GC/ECD was difficult. The values are therefore less suitable for validation. They are presented to show that also for bioavailability measurements it is important to use a properly functioning instrument.
The available fraction in Table 5 is as expected. For PAHs, the availability is lower than the availability of the PCBs. This is caused by the biodegradability of the PAHs. Part of the available PAHs have probably been degraded during storage, drying and pre-treatment. The availability of DDT/DDE/DDD is much higher than the availability of the PCBs. This is not expected, which shows again that in measurement of bioavailability the instrumental analytical method should be without any doubt.

3.3 Suitability of samples for intra laboratory validation

Before the validation samples were distributed it was tested if an experienced lab was able to produce repeatable data to make sure that the samples distributed were homogeneous regarding the size of the bioavailable fractions. Data were supplied by the laboratory of Rijkswaterstaat. Results are presented in Table 7 to 10.

Table 7 *Results of the repeatability in Sample 1*

	Tenax		Residue		Sum	Tenax as % of sum	Sum as fraction of consensus value
	Average n=3	RSD	Average n=3	RSD			
	µg/kg d.m.	(%)	µg/kg d.m.	(%)	µg/kg d.m.	(%)	(%)
PCB 28	5.8	23.4	16.5	16.0	22.3	26	
PCB 52	11.9	18.7	12.8	14.3	24.7	48	59
PCB 101	12.7	17.2	16.4	9.7	29.1	44	56
PCB 118	6.6	18.8	12.5	5.2	19.0	34	58
PCB 153	8.2	16.7	18.0	3.5	26.2	31	59
PCB 138	4.6	11.2	13.3	14.7	17.8	26	
PCB 180	2.2	20.7	9.2	3.4	11.5	19	72
Naphtalene	15	15.2	238	34	253.3	6	76
Fluorene	13	15.5	85	10.3	98	13	44
Phenantrene	169	15.9	646	8.9	815	21	75
Fluoranthene	404	15.2	1260	6.0	1664	24	79
Pyrene	333	15.6	902	12.3	1235	27	74
Benz(a)anthracene	157	15.1	458	15.4	615	26	60
Chrysene	200	14.7	465	4.5	665	30	60
Benz(b)fluoranthene	93	13.5	795	7.3	888	10	70
Benz(k)fluoranthene	35	13.4	370	5.0	405	9	73
Benz(a)pyrene	31	10.7	624	5.3	655	5	73
Dibenz(ah)anthracene	3	12.6	78	7.2	81	4	47
Benzo(ghi)perylene	20	10.6	213	7.0	234	9	34
Indeno(1,2,3cd)pyrene	14	6.5	600	26.1	613	2	87

Table 8 *Results of the repeatability in Sample 2*

	Tenax		Residue		Sum	Tenax as % of sum	Sum as fraction of consensus value
	Average n=3	RSD	Average n=3	RSD			
	µg/kg d.m.	(%)	µg/kg d.m.	(%)	µg/kg d.m.	(%)	(%)
PCB 28	2.8	11.9	7.6	3.1	10.4	27	45
PCB 52	9.6	11.3	20.0	3.6	29.7	33	60
PCB 101	3.7	14.5	11.6	1.3	15.3	24	68
PCB 118	3.7	20.3	10.6	1.5	14.3	26	84
PCB 153	1.7	15.4	7.8	15.9	9.5	18	70
PCB 138	1.1	25.3	7.8	22.5	8.9	13	69
PCB 180	6.1	31.1	4.3	37.3	10.4	59	110
Naphtalene	76	1.7	463	4.9	539	14	51
Fluorene	151	14.7	267	10.4	418	36	41
Phenantrene	2917	4.9	3487	4.7	6404	46	72
Fluoranthene	5088	9.6	8406	4.1	13493	38	86
Chrysene	1472	10.5	3324	5.2	4796	31	66
Benz(b)fluoranthene	849	8.9	5448	8.1	6298	13	82
Benz(k)fluoranthene	368	7.9	2342	6.2	2711	14	77
Benz(a)pyrene	204	7.4	3670	6.5	3874	5	71
Dibenz(ah)anthracene	40	6.3	615	10.2	655	6	59
Benzo(ghi)perylene	91	6.3	3394	10.2	3485	3	82
Indeno(1,2,3cd)pyrene	169	5.3	4719	7.5	4888	3	104

Table 9 *Results of the repeatability in Sample 3*

	Tenax		Residue		Sum	Tenax as % of sum	Sum as fraction of consensus value
	Average n=3	RSD	Average n=3	RSD			
	µg/kg d.m.	(%)	µg/kg d.m.	(%)	µg/kg d.m.	(%)	(%)
PCB 28	13.5	20.1	57.8	8.1	71	19	42
PCB 52	16.8	64.0	37.1	9.7	54	31	47
PCB 101	21.1	24.4	46.5	6.6	68	31	57
PCB 118	7.9	48.2	31.7	13.1	40	20	59
PCB 153	14.4	28.7	53.7	7.9	68	21	61
PCB 138	9.7	24.8	34.0	4.4	44	22	67
PCB 180	1.9	32.0	22.4	11.8	24	8	62
Naphtalene	28	14.4	561	8.9	589	5	81
Fluorene	9	10.3	157	52.5	166	6	67
Phenantrene	525	76.9	952	25.5	1478	36	122
Anthracene	47	14.7	596	8.8	643	7	47
Fluoranthene	352	9.4	1261	43.0	1613	22	88
Pyrene	295	9.0	782	15.4	1078	27	70
Benz(a)anthracene	146	14.0	471	9.3	616	24	60
Chrysene	215	11.4	578	9.5	793	27	65
Benz(b)fluoranthene	100	13.5	1096	8.9	1196	8	89
Benz(k)fluoranthene	41	13.6	422	8.8	463	9	79
Benz(a)pyrene	23	13.3	615	8.9	638	4	87
Dibenz(ah)anthracene	7	83.4	77	19.2	84	8	45
Benzo(ghi)perylene	18	15.5	214	10.8	233	8	35
Indeno(1,2,3cd)pyrene	13	14.0	591	38.3	605	2	86

Table 10 *Results of the repeatability in Sample 4*

	Tenax		Residue		Sum	Tenax as % of sum	Sum as fraction of consensus value
	Average n=3	RSD	Average n=3	RSD			
	µg/kg d.m.	(%)	µg/kg d.m.	(%)	µg/kg d.m.	(%)	(%)
PCB 28	0.1	57	0.5	8.6	0.6	18	116
PCB 52	0.6	39	1.5	55.3	2.1	26	
PCB 101	0.3	44	1.2	40.1	1.4	19	96
PCB 118	0.8	95	0.6	72.4	1.4	57	155
PCB 153	0.5	46	1.5	85.1	2.0	26	57
PCB 138	0.7	47	1.7	85.0	2.3	28	63
Naphtalene	20	45	267	14	288	7	63
Acenaphtene	5	41	87	29	92	5	47
Fluorene	9	42	204	69	213	4	98
Phenantrene	728	55	2028	18	2756	26	100
Fluoranthene	872	103	5699	7	6572	13	93
Pyrene	293	44	3885	3	4178	7	77
Benz(a)anthracene	174	43	2013	14	2186	8	59
Chrysene	509	102	1908	1	2417	21	62
Benz(b)fluoranthene	132	43	3114	2	3246	4	76
Benz(k)fluoranthene	68	43	1386	3	1454	5	75
Benz(a)pyrene	37	44	2376	87	2412	2	68
Dibenz(ah)anthracene	7	42	397	5	404	2	63
Benzo(ghi)perylene	47	43	2053	2	2100	2	78
Indeno(1,2,3cd)pyrene	32	45	2827	4	2859	1	104

The relative standard deviations (RSD) presented in Table 7-10 are mostly in an expected range. In sample 4 higher RSD's are obtained, but can be explained by the low concentrations measured in this sample. For the laboratory, the deviation from the consensus values was higher than normal. It was decided to use the data as obtained and not to re-analyse, because a better calibration should not affect the RSD's, which was the purpose of the experiment. It was decided to use all samples for the validation.

3.4 Statistical evaluation according to ISO 5725

3.4.1 Introduction

The validation of ISO/DIS 16751 was organized by WEPAL in Wageningen. Samples were distributed among 12 laboratories. Unfortunately, not all potential participants have provided WEPAL with their results. A few laboratories needed more time and this has been accepted by WEPAL in order to have sufficient data. Data for Anthracene, Benz(a)anthracene, Benz(k)fluoranthene, Benz(a)pyrene, PCB52, PCB101,PCB 138 and PCB180 are presented in this document.

Four homogeneous samples were distributed. The participants were asked to measure the bioavailable fraction of PAHs and PCBs using ISO/DIS 16751 and the concentration in the residual soil fractions. Results have been statistical evaluated according to the ISO 5725 series.

The standard allows applying Tenax or cyclodextrin to extract the bioavailable fraction. Because of the limited number of participants, no differentiation was made (see also 3.4.2). In the individual results (Annex 1 and Annex 2) it is mentioned which of the two extractants was used. The results were statistical evaluated according ISO 5725. Outliers were identified using the Cochran's test and Grubb's test described in ISO 5725-2.

We distinguished:

- The available amount as extracted according ISO/DIS 16751;
- The amount present in the soil after extraction with Tenax or cyclodextrin;
- The available amount calculated as percentage of the total: 100*available/(available+residue).

3.4.2 Used extraction methods

Two extractants, cyclodextrin and Tenax, are allowed in ISO/DIS 16751 to extract the bioavailable fraction. Nine laboratories participated, three of them applied cyclodextrin, and the other six used Tenax. These numbers are too low to establish if there is a difference between the extractants, especially if the variation in the results is high as was observed in this study (Figure 3).

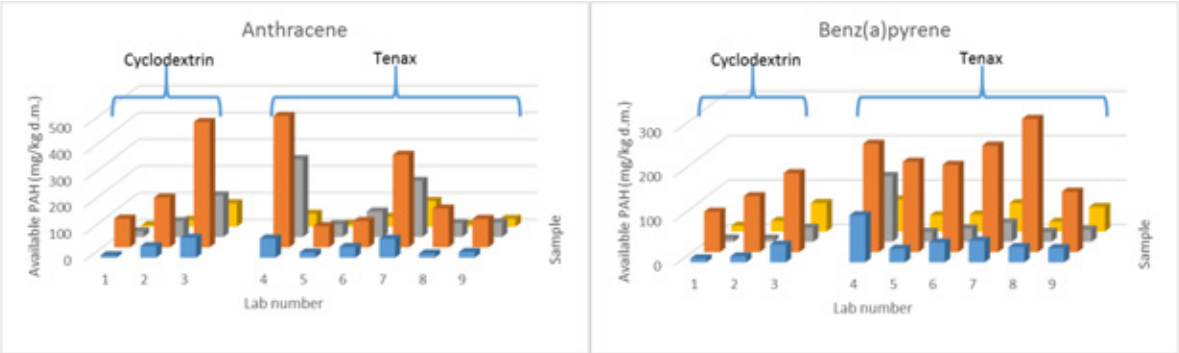


Figure 3 *Bioavailability of Anthracene and Benz(a)pyrene. Results of cyclodextrin and Tenax extraction of participating laboratories in four samples*

Figure 3 shows the results of the measured bioavailability for two PAHs, which were analysed by all laboratories. Comparable results were obtained for other analysed parameters. The first three labs in Figure 3 used cyclodextrin and the other six used Tenax. As mentioned, the variation was high and results of both extraction methods are part of the same population. Thus, for the overall statistical evaluation no differentiation was made between the two extraction methods.

3.4.3 Results of PAHs

Not all participants reported values for all PAHs. We evaluated results of PAHs with the highest number of participants and having different ring systems. Results of the statistical evaluation for the PAHs Anthracene, Benz(a)anthracene, Benz(k)fluoranthene, Benz(a)pyrene, are presented in Table 11-13.

Table 11 *PAHs - available fraction. Results in µg/kg*

Sample	element	L	n	%outliers	x-average	S_R	S_r	VC_R	VC_r
1	Ant A	9	27	0.0	40.4	27.0	7.47	66.7%	18.5%
2	Ant A	9	26	0.0	221	172	53.2	77.9%	24.1%
3	Ant A	9	26	0.0	102	93.8	51.5	91.5%	50.2%
4	Ant A	9	26	0.0	36.5	32.2	8.56	88.3%	23.5%
1	BaA A	9	27	0.0	142	56.5	28.5	39.7%	20.0%
2	BaA A	9	26	0.0	1302	480	224	36.8%	17.2%
3	BaA A	9	26	0.0	126	59.5	42.1	47.2%	33.5%
4	BaA A	9	27	0.0	191	86.2	36.0	45.2%	18.8%
1	BkF A	9	27	0.0	47.6	27.2	10.0	57.2%	21.0%
2	BkF A	9	27	11.1	368	109	47.1	29.5%	12.8%
3	BkF A	9	26	22.2	43.9	20.7	5.39	47.3%	12.3%
4	BkF A	9	27	0.0	78.5	33.3	15.2	24.4%	19.3%
1	BaP A	9	27	0.0	40.0	29.1	9.12	72.7%	22.8%
2	BaP A	9	27	11.1	174	56.0	18.2	32.2%	10.5%
3	BaP A	9	26	11.1	24.0	12.9	5.25	53.7%	21.8%
4	BaP A	9	27	0.0	43.3	22.8	9.55	52.6%	22.1%

Table 12 *PAHs – residual fraction. Results in µg/kg*

Sample	element	L	n	%outliers	x-average	S_R	S_r	VC_R	VC_r
1	Ant Res.	7	21	14.3	336	121	32.8	36.0%	9.8%
2	Ant Res.	6	18	0.0	844	416	121	49.3%	14.3%
3	Ant Res.	7	20	14.3	1018	469	127	46.1%	12.5%
4	Ant Res.	7	21	0.0	416	221	77.4	53.1%	18.6%
1	BaA Res.	7	21	0.0	751	352	67.5	46.9%	9.0%
2	BaA Res.	7	20	0.0	4576	2587	469	56.5%	10.3%
3	BaA Res.	7	20	0.0	748	444	160	59.4%	21.4%
4	BaA Res.	7	20	14.3	2737	943	244	34.5%	8.9%
1	BkF Res.	7	21	14.3	405	107	19.5	26.5%	4.9%
2	BkF Res.	7	21	0.0	2740	1031	226	37.6%	8.2%
3	BkF Res.	7	20	0.0	464	198	76.5	42.6%	16.5%
4	BkF Res.	7	20	0.0	1321	619	174	46.9%	13.2%
1	BaP Res.	7	21	0.0	582	278	87.2	47.8%	15.0%
2	BaP Res.	7	21	0.0	3998	1232	361	30.8%	9.0%
3	BaP Res.	7	20	0.0	603	210	101	34.9%	16.7%
4	BaP Res.	7	20	28.6	2613	1612	117	61.7%	4.5%

Table 13 *PAHs - available fraction in % of total*

Sample	element	L	n	%outliers	x-average	S_R	S_r	VC_R	VC_r
1	ANT %A	7	21	14.3	8.24	4.99	1.79	60.6%	21.7%
2	ANT %A	6	18	0.0	17.9	9.24	6.78	51.5%	37.8%
3	ANT %A	7	20	0.0	9.16	10.3	7.26	113%	79.2%
4	ANT %A	7	20	28.6	4.81	3.66	1.47	76.2%	30.7%
1	BaA %A	7	21	0.0	16.8	9.17	3.75	54.7%	22.4%
2	BaA %A	7	20	0.0	32.5	31.2	3.94	95.9%	12.1%
3	BaA %A	7	20	14.3	13.9	8.88	2.46	63.9%	17.7%
4	BaA %A	7	20	0.0	6.59	4.36	1.88	66.2%	28.6%
1	BkF %A	7	21	0.0	9.66	5.66	2.27	58.6%	23.5%
2	BkF %A	7	21	14.3	11.59	4.22	2.17	36.5%	18.7%
3	BkF %A	7	20	14.3	7.57	4.16	1.77	55.0%	23.4%
4	BkF %A	7	20	0.0	6.06	3.71	1.57	61.2%	26.3%
1	BaP %A.	7	21	0.0	7.49	5.13	1.31	68.5%	17.5%
2	BaP %A.	7	21	14.3	4.35	1.96	0.84	44.9%	19.4%
3	BaP %A.	7	20	28.6	2.69	1.67	0.44	62.1%	16.5%
4	BaP %A.	7	20	14.3	1.49	1.26	0.32	84.2%	21.3%

Explanation of symbols

L is the number of laboratories after elimination of outliers;

n is the number of results;

x is the mean value;

S_R is the reproducibility standard deviation;

S_r is the repeatability standard deviation;

VC_r is the relative repeatability standard deviation, in percent;

VC_R is the relative reproducibility standard deviation, in percent.

Repeatability values of the available fraction are higher than the values obtained in an experienced laboratory (chapter 3.2 and 3.3). Repeatability of PAHs in the residual fraction is better. Laboratories are more experienced in analysing soil and less in analysing Tenax or cyclodextrin.

The variation coefficients for reproducibility VC_R are high. Calculation of the bioavailability as percentage of the total concentration had no effect on reproducibility (Table 13). Reproducibility of the available concentration is the most important factor.

Because we used WEPAL-samples, it is possible to have an indication of the truthfulness of the results. In Table 14 the total values (available + residual) are compared with the consensus values (NDA mean) obtained after several analyses of the samples in the WEPAL proficiency tests (PT). Results are comparable, but the individual results (Annexes 1 and 2) indicate that some of the laboratories can improve. This is also indicated by the VC_R. This value is around 25% in most WEPAL tests. Higher values are found for the analyses of the residual fraction in this investigation.

In this investigation, the measured percentages available are relatively low.

Table 14 *Comparing WEPAL PT consensus value (NDA_{mean}) and total concentration of individual PAH measured during validation. Recovery = x-av as percentage of NDA mean*

Sample ID	element	NDA mean (mg/kg)	NDA s.d. (mg/kg)	NDA CV (%)	x-av. Tot (mg/kg)	s.d. (mg/kg)	CV (%)	Recovery (%)
1	Ant. Tot.	447	124	28	376	124	33	84.1
2	Ant. Tot.	1238	349	28	1064	450	42	86.0
3	Ant. Tot.	1374	325	24	1020	478	43	81.5
4	Ant. Tot.	670	152	23	452	223	49	67.5
1	BaA Tot.	992	182	18	894	357	40	90.1
2	BaA Tot.	6750	1053	16	5878	2631	45	87.1
3	BaA Tot.	1010	219	22	874	448	51	86.5
4	BaA Tot.	3590	466	13	2928	947	32	81.6
1	BkF Tot.	546	79.4	15	453	111	25	82.9
2	BkF Tot.	3440	717	21	3108	1036	33	90.3
3	BkF Tot.	585	119	20	508	199	39	86.9
4	BkF Tot.	1940	263	14	1400	620	44	72.1
1	BaP Tot.	826	115	14	622	278	45	75.3
2	BaP Tot.	4630	790	17	4172	1232	30	90.1
3	BaP Tot.	672	110	16	627	210	34	93.3
4	BaP Tot.	3457	367	11	2657	1612	61	76.8

3.4.4 Results of PCBs

We selected PCBs with the highest number of participants. PCBs 52, 101, 138 and 180 are presented in Table 15-17. Individual results are presented in Annex 2.

Table 15 *PCBs – available fraction. Results in µg/kg*

Sample	element	L	n	%outliers	x-average	S_R	S_r	VC_R	VC_r
1	PCB52A	7	21	0.0	20.4	9.50	4.92	46.6%	24.1%
2	PCB52A	8	22	0.0	25.2	21.1	5.54	84.0%	22.0%
3	PCB52A	8	22	25.0	42.3	24.2	6.23	57.2%	14.7%
4	PCB52A	7	16	14.3	0.93	1.20	1.03	129%	111%
1	PCB101A	7	21	0.0	15.3	6.38	3.17	41.8%	20.7%
2	PCB101A	7	20	0.0	5.17	2.31	2.21	44.6%	42.7%
3	PCB101A	7	18	14.3	25.6	12.85	3.40	50.2%	13.3%
4	PCB101A	6	17	16.7	0.35	0.30	0.20	83.2%	55.6%
1	PCB138A	7	21	0.0	7.19	3.21	1.54	44.7%	21.4%
2	PCB138A	7	21	0.0	2.63	1.58	0.85	60.3%	32.4%
3	PCB138A	7	20	0.0	10.3	6.51	3.35	63.0%	32.5%
4	PCB138A	6	17	0.0	1.45	1.66	1.02	114%	70.6%
1	PCB180A	7	21	0.0	1.84	1.06	0.46	57.4%	25.3%
2	PCB180A	7	21	14.3	1.09	0.74	0.28	68.4%	25.6%
3	PCB180A	7	19	0.0	2.28	1.61	1.52	70.6%	66.8%
4	PCB180A	6	18	0.0	0.99	1.26	1.02	127%	103%

Table 16 *PCBs residual fraction. Results in µg/kg*

Sample	element	L	N	%outliers	x-average	S_R	S_r	VC_R	VC_r
1	PCB52R	6	18	0.0	26.8	8.16	3.88	30.5%	14.5%
2	PCB52R	6	18	0.0	48.8	31.4	6.84	64.3%	14.0%
3	PCB52R	5	15	0.0	86.5	38.3	7.63	44.3%	8.83%
4	PCB52R	5	15	20.0	2.97	3.01	2.54	101	85.5%
1	PCB101R	5	15	0.0	31.9	11.7	3.83	36.5%	12.0%
2	PCB101R	5	15	0.0	19.7	7.11	2.40	36.1%	12.2%
3	PCB101R	5	15	0.0	89.7	41.0	5.44	45.7%	6.07%
4	PCB101R	4	12	0.0	1.74	1.19	1.06	68.2%	60.7%
1	PCB138R	6	18	16.7	21.6	9.23	1.08	42.7%	4.99%
2	PCB138R	5	14	0.0	12.2	2.89	1.19	23.7%	9.76%
3	PCB138R	6	18	16.7	52.1	4.12	4.71	7.91%	9.05%
4	PCB138R	4	12	0.0	2.68	1.27	0.97	47.2%	36.2%
1	PCB180R	5	15	0.0	17.4	7.31	3.05	42.1%	17.6%
2	PCB180R	5	14	0.0	10.2	4.95	3.00	48.4%	29.3%
3	PCB180R	6	18	0.0	39.8	13.4	4.50	33.6%	11.3%
4	PCB180R	4	12	25.0	4.91	1.57	1.23	32.0%	25.0%

Table 17 *PCBs available fraction in % of total*

Sample	element	L	N	%outliers	x-average	S_R	S_r	VC_R	VC_r
1	PCB52 %A	5	15	0.0	38.9	12.0	6.42	30.7%	16.5%
2	PCB52 %A	6	18	0.0	30.5	9.33	7.96	30.6%	26.1%
3	PCB52 %A	6	17	16.7	44.2	35.1	2.83	79.4%	6.4%
4	PCB52 %A	5	15	0.0	17.1	9.90	3.85	58.0%	22.5%
1	PCB101 %A	5	15	0.0	34.3	9.48	6.41	27.6%	18.7%
2	PCB101 %A	5	15	20.0	21.6	6.00	4.07	27.8%	18.8%
3	PCB101 %A	5	14	0.0	24.1	11.1	3.47	44.1%	13.8%
4	PCB101 %A	4	11	0.0	13.8	11.3	10.02	81.7%	72.7%
1	PCB138 %A	5	15	0.0	22.3	7.33	3.56	32.8%	16.0%
2	PCB138 %A	5	14	0.0	13.6	7.70	5.05	56.4%	37.0%
3	PCB138 %A	5	14	0.0	14.0	6.32	3.48	45.1%	24.9%
4	PCB138 %A	4	12	25.0	17.4	14.6	16.6	84.2%	95.7%
1	PCB180 %A	5	15	20.0	7.22	4.02	2.22	55.7%	30.8%
2	PCB180 %A	5	14	20.0	5.94	4.03	1.88	67.9%	31.7%
3	PCB180 %A	5	14	20.0	4.71	2.53	0.85	53.6%	18.0%
4	PCB180 %A	4	12	20.0	7.64	5.75	2.97	75.3%	38.8%

The results of the PCBs are comparable with those of PAHs. Sample 4 is an exception, but concentrations in this sample are very low. Calculation of the bioavailable fraction as a percentage has a positive effect on reproducibility (Table 17).

In Table 18, the results of the individual PCBs are compared with the WEPAL PT consensus values. Most of the results are above 100%. The recovery of PCB52 in sample 4 is extremely high and this result is not reliable. Coefficients of variation in the validation study are higher than obtained in the WEPAL proficiency tests, showing that also for these parameters the laboratory can improve.

Table 18 *Comparing WEPAL PT consensus value (NDA_{mean}) and total concentration of the individual PCBs measured during validation.*

Sample ID	element	NDA mean (mg/kg)	NDA s.d. (mg/kg)	NDA CV (%)	x-av. Tot (mg/kg)	s.d. (mg/kg)	CV (%)	Recovery (%)
1	PCB52	45.1	10.7	24	47.1	12.5	27	105
2	PCB52	48.3	12.5	26	73.9	37.8	51	153
3	PCB52	123	27.6	22	129	45.3	35	105
4	PCB52	1.02	0.95	94	3.91	3.24	83	383
1	PCB101	52.9	7.87	15	47.2	13.3	28	89
2	PCB101	23.0	5.72	25	24.8	7.47	30	108
3	PCB101	122	23.8	20	115	42.9	27	95
4	PCB101	1.56	0.44	28	2.10	1.22	58	134
1	PCB138	29.1	8.32	29	28.8	9.77	34	99
2	PCB138	13.9	3.79	27	14.8	3.30	22	107
3	PCB138	62.6	18.4	29	62.4	7.70	12	100
4	PCB138	3.65	0.95	26	4.13	2.09	51	113
1	PCB180	16.2	2.13	13	19.2	7.38	39	119
2	PCB180	9.75	2.20	23	11.3	5.01	44	116
3	PCB180	39.9	7.55	19	42.0	13.5	32	105
4	PCB180	3.69	0.71	19	5.90	2.01	34	160

4 Conclusions and recommendations

This report presents the validation of ISO/TS 16751. The first part of the validation, carried out in a laboratory having a large experience with using Tenax, has shown that repeatability and reproducibility are as can be expected for this kind of methods. Potentially the method described in ISO/TS 16751 is an adequate method to measure the bioavailable fraction. As expected, both repeatability and reproducibility standard deviations in the interlaboratory validation are higher,. Reproducibility is also higher than obtained within the SETOC-program of WEPAL for total analysis of organic contaminants.

It is the role of the ISO-standardization committee to decide if the presented results are of sufficient quality to make the method a full standard. In its meeting of September 12, 2018 in Brno, Working group TC190/SC7/WG6 'Transfer and mobility of components' discussed the concept version of this report and was satisfied with the results. The group concluded that the results were sufficient and a procedure was started to add the validation results to the standard and to transfer the TS into a full standard.

Because the reproducibility was less than obtained in other WEPAL proficiency tests, we think that improvement is still possible. To obtain this improvement the following might be helpful:

- In the present laboratory practise, laboratories have a large experience in measuring total concentrations. Good results are obtained in intra- and interlaboratory investigations. With new methods, it takes some time to obtain parameters with the same quality. It is the experience of WEPAL that also new laboratories may need several proficiency tests (=time) to analyse on the same level as the experienced laboratories.

- WEPAL/QUASIMEME has organized 'Developing exercises' to increase the quality of participating laboratories. In such an exercise, samples, extracts and standard solutions are distributed in proficiency tests. Results are reported and discussed in workshops, making it possible for laboratories to improve their performance.

- ISO/TS 16751 is a new method and most of the participating laboratories had no, or only limited experience with the method. Higher values of VC_R and VC_r are thus to be expected. The reported results reflect the present international situation and have to be accepted. However, the performance of the laboratories is expected to improve in future.

- The quality parameters as presented in this report are not expected to change with a validation with more participants. We recommend building up international experience with the method, finding a way to exchange the experience between laboratories and then revalidate it after a few years.

References

Cofino, W.P., Molenaar, J. and Torfs, P. Evaluating Proficiency tests with Robust Statistics. Wiley StatsRef: Statistics Reference Online. In press.

ISO 5725-2:1994 – 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 11465:2005 – Soil Quality – Determination of dry matter and water content on a mass basis – Gravimetric method.

ISO 13528:2015 (Cor. 2016-10) – Statistical methods for use in proficiency testing by interlaboratory comparisons.

Annex 1 Results PAHs of individual laboratories

Orange: Outliers, not used in evaluation
Yellow: stragglers

Anthracene		**Available**					**Residu**					**% available**				
Lab no	extr. Liquid	Repl.1	Repl.2	Repl.3	average	stdev	Repl.1	Repl.2	Repl.3	average	stdev	Repl.1	Repl.2	Repl.3	average	stdev
		Sample 1														
1	Cyclodextrine	7.47	8.14	9.92	8.5	1.3	354	470	354	393	67.0	2.1	1.7	2.7	2.2	0.5
2	Cyclodextrine	57.32	35.93	34.79	42.7	12.7	443	464	465	457	12.7	11.5	7.2	7.0	8.5	2.5
3	Cyclodextrine	74.10	75.40	74.15	74.6	0.7										
4	TENAX	76.69	77.51	60.47	71.6	9.6	374	411	392	392	18.5	17.0	15.9	13.4	15.4	1.9
5	TENAX	20.19	16.14	21.19	19.2	2.7	208	209	169	195	22.7	8.8	7.2	11.1	9.1	2.0
6	TENAX	26.54	49.14	44.12	39.9	11.9	272	71	81	141	113	8.9	40.8	35.4	28.4	17.1
7	TENAX	71.04	72.36	68.60	70.7	1.9										
8	TENAX	17.74	23.54	5.63	15.6	9.1	368	409	414	397	25.3	4.6	5.4	1.3	3.8	2.2
9	TENAX	17.57	21.11	24.54	21.1	3.5	162	177	198	179	17.9	9.8	10.6	11.0	10.5	0.6
		Sample 2														
1	Cyclodextrine	99.8	110.2	111.2	107.1	6.3	1271	1468	1238	1325	125	7.3	7.0	8.2	7.5	0.7
2	Cyclodextrine	164.6	231.7	161.8	186.0	39.6	1070	1003	1073	1049	40	13.3	18.8	13.1	15.1	3.2
3	Cyclodextrine	442.2	457.2	505.4	468.3	33.0										
4	TENAX	548.3	368.0	557.3	491.2	106.8	995	1276	1168	1147	141	35.5	22.4	32.3	30.1	6.8
5	TENAX	76.4	78.1	83.2	79.2	3.5	385	343	259	329	64	16.6	18.5	24.3	19.8	4.0
6	TENAX	47.7	156.8	89.6	98.1	55.0	n.d.	n.d.	n.d.							
7	TENAX	331.3	360.8	3136	346.0	20.8										
8	TENAX	153.5	105.9	175.2	144.8	35.4	1006	786	596	796	205	13.2	11.9	22.7	15.9	5.9
9	TENAX	153.0	21.0	144.2	106.1	73.8	356	403	489	416	68	30.1	4.9	22.8	19.3	12.9
		Sample 3														
1	Cyclodextrine	23.07	19.20	22.10	21.5	2.0	1451	1219	1440	1370	130.7	1.6	1.6	1.5	1.5	0.0
2	Cyclodextrine	48.12	54.39	75.10	59.2	14.1	1506	1500	1479	1495	14.1	3.1	3.5	4.8	3.8	0.9
3	Cyclodextrine	164.42	132.31	169.65	155.5	20.2										
4	TENAX	381.34	200.48		290.9	127.9	602	1073	941	837	333	38.8	15.7		27.3	16.3

5	TENAX	40.37	54.30	47.20	47.3	7.0	638	537	612	596	52.2	6.0	9.2	7.2	7.4	1.6
6	TENAX	100.77	94.99	88.95	94.9	5.9	1190	233	n.d.	712	677	7.8	29.0		18.4	15.0
7	TENAX	334.19	158.47	135.84	209.5	108.6										
8	TENAX	51.48	44.50	59.81	51.9	7.7	1349	1252	1350	1317	56.5	3.7	3.4	4.2	3.8	0.4
9	TENAX	102.43	22.20	38.21	54.3	42.5	397	348	547	430	103.6	20.5	6.0	6.5	11.0	8.2
		Sample 4														
1	Cyclodextrine	5.36	7.50	4.42	5.76	1.58	752.0	722.3	652.4	708.9	51.1	0.7	1.0	0.7	0.8	0.2
2	Cyclodextrine	29.54	35.63	11.08	25.42	12.78	468.5	462.4	486.9	472.6	12.8	5.9	7.2	2.2	5.1	2.6
3	Cyclodextrine	78.34	104.39	77.97	86.90	15.15										
4	TENAX	48.71	52.70	42.93	48.11	4.91	528.4	467.7	526.2	507.4	34.4	8.4	10.1	7.5	8.7	1.3
5	TENAX	6.76	14.95	10.21	10.64	4.11	139.0	163.6	29.9	110.8	71.2	4.6	8.4	25.5	12.8	11.1
6	TENAX	31.97	39.53	39.52	37.01	4.36	510.5	425.7	433.4	456.5	46.9	5.9	8.5	8.4	7.6	1.5
7	TENAX	92.86	99.32	666.26	96.09	4.57										
8	TENAX	8.41	11.86	6.80	9.02	2.58	373.7	535.8	590.0	499.8	112.6	2.2	2.2	1.1	1.8	0.6
9	TENAX	22.71	43.12	21.11	28.98	12.27	249.1	210.8	0.0	153.3	134.1	8.4	17.0		12.7	6.1

Benz(a)anthracene		**Available**					**Residu**					**% available**				
Lab no	extr. Liquid	Repl.1	Repl.2	Repl.3	average	stdev	Repl.1	Repl.2	Repl.3	average	stdev	Repl.1	Repl.2	Repl.3	average	stdev
		Sample 1														
1	Cyclodextrine	47.6	43.6	54.8	48.7	5.7	802	1034	802	880	133.7	5.6	4.0	6.4	5.3	1.2
2	Cyclodextrine	117.1	103.5	105.1	108.6	7.4	1388	1402	1400	1396	7.4	7.8	6.9	7.0	7.2	0.5
3	Cyclodextrine	160.5	183.9	158.7	167.7	14.1										
4	TENAX	180.1	227.0	172.8	193.3	29.4	725	768	768	754	25.2	19.9	22.8	18.4	20.4	2.3
5	TENAX	156.5	133.9	181.5	157.3	23.8	538	430	405	458	70.5	22.5	23.7	30.9	25.7	4.5
6	TENAX	168.2	185.7	192.0	182.0	12.3	576	518	490	528	43.9	22.6	26.4	28.1	25.7	2.8
7	TENAX	206.8	216.3	193.2	205.4	11.6										
8	TENAX	126.7	166.9	44.2	112.6	62.6	783	916	912	870	76.0	13.9	15.4	4.6	11.3	5.9
9	TENAX	63.6	118.3	134.6	105.5	37.2	348	374	398	373	24.9	15.4	24.0	25.3	21.6	5.4
		Sample 2														
1	Cyclodextrine	487	499	438	475	32.3	6699	7456	7378	7178	416.4	6.8	6.3	5.6	6.2	0.6
2	Cyclodextrine	1247	1362	1036	1215	165.5	6812	6697	7023	6844	165.5	15.5	16.9	12.9	15.1	2.1
3	Cyclodextrine	1475	1556	1589	1540	58.6										
4	TENAX	2006	1289	1861	1719	378.8	4245	5577	5479	5100	742.3	32.1	18.8	25.4	25.4	6.7
5	TENAX	963	1163	1037	1054	101.2	50	35	56	47	10.7	95.1	97.1	94.9	95.7	1.2
6	TENAX	1093	1070	1062	1075	16.1	4266	4886	4280	4477	354.1	20.4	18.0	19.9	19.4	1.3
7	TENAX	1210	1284	1290	1261	44.7										
8	TENAX	1388	1176	2095	1553	481.0	6244	5223	4798	5422	743.1	18.2	18.4	30.4	22.3	7.0

9	TENAX	2008	2184	52	2096	124.2	2102	2209	54	2155	75.4	48.9	49.7		49.3	0.6
		Sample 3														
1	Cyclodextrine	48.8	43.8	49.4	47.3	3.1	1310	874	1062	1082	218.9	3.6	4.8	4.4	4.3	0.6
2	Cyclodextrine	64.1	83.9	88.6	78.9	13.0	1421	1401	1396	1406	13.0	4.3	5.7	6.0	5.3	0.9
3	Cyclodextrine	163.9	132.2	169.5	155.2	20.1										
4	TENAX	209.2	187.5		198.3	15.4	620	468		544	107.3	20.9	22.0		21.5	0.8
5	TENAX	146.7	165.3	124.7	145.6	20.3	513	426	472	471	43.6	22.2	28.0	20.9	23.7	3.8
6	TENAX	134.4	174.0	147.4	151.9	20.2	674	540	580	598	68.7	16.6	24.4	20.3	20.4	3.9
7	TENAX	187.0	199.0	245.5	210.5	30.9										
8	TENAX	108.3	95.2	141.8	115.1	24.0	979	886	973	946	52.1	10.0	9.7	12.7	10.8	1.7
9	TENAX	174.8	136.9	60.9	124.2	58.0	244	227	357	276	71.0	41.8	37.6	14.6	31.3	14.7
		Sample 4														
1	Cyclodextrine	36.0	55.4	39.4	43.6	10.4	4230	3961	3728	3973	251.22	0.8	1.4	1.0	1.1	0.3
2	Cyclodextrine	199.1	164.9	113.7	159.2	43.0	3356	3390	3441	3396	42.96	5.6	4.6	3.2	4.5	1.2
3	Cyclodextrine	215.3	265.0	210.6	230.3	30.1										
4	TENAX	269.3	291.7	228.5	263.1	32.0	2566	2319	2581	2488	147.16	9.5	11.2	8.1	9.6	1.5
5	TENAX	100.2	250.1	170.3	173.5	75.0	2334	1913	1792	2013	284.50	4.1	11.6	8.7	8.1	3.8
6	TENAX	203.3	262.1	258.5	241.3	33.0	3033	2343	3030	2802	397.30	6.3	10.1	7.9	8.1	1.9
7	TENAX	282.8	320.6	303.9	302.4	18.9										
8	TENAX	96.1	131.5	82.3	103.3	25.4	2501	3035	4156	3231	844.51	3.7	4.2	1.9	3.3	1.2
9	TENAX	208.2	199.0	196.1	201.1	6.3	1317	1204		1260	80.12	13.7	14.2		13.9	0.4

Benz(a)pyrene		**Available**					**Residu**					**% available**				
Lab no	extr. Liquid	Repl.1	Repl.2	Repl.3	average	stdev	Repl.1	Repl.2	Repl.3	average	stdev	Repl.1	Repl.2	Repl.3	average	stdev
		Sample 1														
1	Cyclodextrine	9.2	7.7	10.1	9.0	1.2	802.4	1034.0	802.4	879.6	133.7	1.1	0.7	1.2	1.0	0.3
2	Cyclodextrine	14.6	13.5	12.3	13.5	1.1	74.4	75.5	76.7	75.5	1.1	16.4	15.2	13.8	15.2	1.3
3	Cyclodextrine	38.5	43.8	37.4	39.9	3.4										
4	TENAX	97.7	126.3	94.4	106.1	17.5	676.7	708.6	707.3	697.5	18.1	12.6	15.1	11.8	13.2	1.7
5	TENAX	31.3	27.3	33.8	30.8	3.3	610.1	661.9	599.9	624.0	33.2	4.9	4.0	5.3	4.7	0.7
6	TENAX	42.7	53.5	38.2	44.8	7.9	694.4	633.8	624.2	650.8	38.1	5.8	7.8	5.8	6.4	1.2
7	TENAX	49.9	49.8	47.1	49.0	1.6										
8	TENAX	39.7	47.9	16.1	34.5	16.5	612.2	950.5	718.8	760.5	172.9	6.1	4.8	2.2	4.4	2.0
9	TENAX	22.2	35.1	39.2	32.2	8.9	339.9	384.7	440.8	388.4	50.5	6.1	8.4	8.2	7.6	1.2
		Sample 2														
1	Cyclodextrine	95.6	93.1	83.9	90.8	6.2	5775	6291	6188	6084.7	273.1	1.6	1.5	1.3	1.5	0.1
2	Cyclodextrine	127.8	142.5	107.4	125.9	17.6	4142	4128	4163	4144.1	17.6	3.0	3.3	2.5	2.9	0.4

3	Cyclodextrine	171.3	179.8	182.2	177.8	5.7										
4	TENAX	270.2	198.9	262.5	243.8	39.1	3442	4461	4531	4144.9	609.3	7.3	4.3	5.5	5.7	1.5
5	TENAX	208.5	215.8	186.7	203.7	15.1	3460	3622	3928	3670.0	237.7	5.7	5.6	4.5	5.3	0.6
6	TENAX	198.0	194.0	197.7	196.5	2.2	4018	4664	4126	4269.5	345.6	4.7	4.0	4.6	4.4	0.4
7	TENAX	227.4	246.3	246.5	240.1	11.0										
8	TENAX	352.8	304.7	583.1	413.6	148.8	4233	3474	3232	3646.1	522.5	7.7	8.1	15.3	10.3	4.3
9	TENAX	131.3	123.2	153.9	136.1	15.9	2105	2111	1873	2029.6	135.7	5.9	5.5	7.6	6.3	1.1
		Sample 3														
1	Cyclodextrine	8.50	6.87	6.93	7.43	0.92	1100	690	826	871.9	208.7	0.8	1.0	0.8	0.9	0.1
2	Cyclodextrine	6.43	6.03	9.42	7.30	1.85	693	693	690	691.7	1.9	0.9	0.9	1.3	1.0	0.3
3	Cyclodextrine	34.10	27.06	34.15	31.77	4.08										
4	TENAX	153.87	142.50		148.19	8.04	420	595	527	507.3	123.6	7.5	4.4		5.9	2.2
5	TENAX	23.64	24.97	19.23	22.61	3.00	661	555	630	615.1	54.6	3.5	4.3	3.0	3.6	0.7
6	TENAX	31.21	30.82	27.49	29.84	2.05	723	588	618	642.8	70.8	4.1	5.0	4.3	4.5	0.5
7	TENAX	40.64	43.32	45.91	43.29	2.63										
8	TENAX	21.62	17.97	27.79	22.46	4.96	633	548	655	611.9	56.2	3.3	3.2	4.1	3.5	0.5
9	TENAX	39.54	28.33	14.71	27.53	12.43	232	200	311	247.8	57.4	14.6	12.4	4.5	10.5	5.3
		Sample 4														
1	Cyclodextrine	11.49	15.78	12.03	13.10	2.34	4465	4194	4194	4284	156	0.3	0.4	0.3	0.3	0.1
2	Cyclodextrine	31.48	22.34	17.90	23.90	6.92	550	559	563	557	7	5.4	3.8	3.1	4.1	1.2
3	Cyclodextrine	59.66	75.16	57.92	64.24	9.49										
4	TENAX	69.51	83.39	64.16	72.35	9.93	2587	2357	2613	2519	141	2.6	3.4	2.4	2.8	0.5
5	TENAX	21.19	53.24	35.94	36.79	16.04	3815	4035	3824	3891	125	0.6	1.3	0.9	0.9	0.4
6	TENAX	47.34	29.59	38.01	38.31	8.88	3675	2982	3654	3437.0	394	1.3	1.0	1.0	1.1	0.2
7	TENAX	57.55	53.81	80.56	63.97	14.48										
8	TENAX	20.86	27.32	17.46	21.88	5.01	1894	2662	3317	2624.1	713	1.1	1.0	0.5	0.9	0.3
9	TENAX	54.31	53.03	58.33	55.22	2.76	1455	1378		1416	54	3.6	3.7		3.7	0.1

Benzo(k)fluoranthene		**Available**					**Residu**					**% available**				
Lab no	extr. Liquid	Repl.1	Repl.2	Repl.3	average	stdev	Repl.1	Repl.2	Repl.3	average	stdev	Repl.1	Repl.2	Repl.3	average	stdev
		Sample 1														
1	Cyclodextrine	14.8	15.3	16.8	15.6	1.1	590	729	566	628	87.6	2.4	2.1	2.9	2.5	0.4
2	Cyclodextrine	25.3	23.1	23.4	23.9	1.2	559	561	561	560	1.2	4.3	4.0	4.0	4.1	0.2
3	Cyclodextrine	54.3	58.1	50.9	54.5	3.6										
4	TENAX	95.2	122.1	91.6	103.0	16.7	452	478	485	472	17.3	17.4	20.3	15.9	17.9	2.3
5	TENAX	35.5	30.0	39.3	34.9	4.7	353	390	368	371	18.4	9.1	7.1	9.6	8.6	1.3
6	TENAX	51.2	63.4	49.9	54.8	7.5	396	367	353	372	22.2	11.4	14.7	12.4	12.9	1.7

7	TENAX	64.4	67.8	62.4	64.9	2.7									
8	TENAX	51.4	52.0	16.9	40.1	20.1	386	410	435	410	24.2	11.8	11.3	3.7	8.9	4.5
9	TENAX	24.5	39.5	45.4	36.5	10.8	221	245	269	245	24.0	10.0	13.9	14.4	12.8	2.4
		Sample 2														
1	Cyclodextrine	214	264	208	228	31	4389	4660	4760	4603	192.0	4.6	5.4	4.2	4.7	0.6
2	Cyclodextrine	366	411	310	362	50	3298	3253	3354	3302	50.3	10.0	11.2	8.5	9.9	1.4
3	Cyclodextrine	491	526	542	519	26										
4	TENAX	547	349	494	463	102	2385	3092	3100	2859	410.6	18.6	10.1	13.8	14.2	4.3
5	TENAX	347	401	356	368	29	2216	2313	2500	2343	144.4	13.5	14.8	12.5	13.6	1.2
6	TENAX	334	327	335	332	5	2392	2626	2326	2448	157.8	12.3	11.1	12.6	12.0	0.8
7	TENAX	405	402	474	427	40										
8	TENAX	522	445	800	589	187	2598	2181	2011	2263	302.0	16.7	17.0	28.5	20.7	6.7
9	TENAX	234	221	271	242	26	1372	1466	1249	1362	108.5	14.6	13.1	17.8	15.1	2.4
		Sample 3														
1	Cyclodextrine	17.7	16.8	17.5	17.3	0.5	983	667	779	810	160.1	1.8	2.5	2.2	2.1	0.3
2	Cyclodextrine	21.1	25.4	27.4	24.6	3.2	551	547	545	547	3.2	3.7	4.4		4.1	0.5
3	Cyclodextrine	65.8	53.6	65.5	61.6	6.9										
4	TENAX	175.3	160.5		167.9	10.5	327	466		397	98.0	16.7	10.3		13.5	4.5
5	TENAX	42.4	45.4	34.6	40.8	5.5	453	381	432	422	37.1	8.5	10.6	7.4	8.9	1.6
6	TENAX	48.5	51.0	41.9	47.2	4.7	496	390	412	433	56.3	8.9	11.6	9.2	9.9	1.5
7	TENAX	79.2	69.1	79.7	76.0	6.0										
8	TENAX	39.8	31.9	46.8	39.5	7.4	438	393	416	415	22.4	8.3	7.5	10.1	8.7	1.3
9	TENAX	75.6	51.2	28.7	51.8	23.4	179	172	262	204	50.0	29.7	22.9	9.9	20.8	10.1
		Sample 4														
1	Cyclodextrine	23.1	34.2	23.0	26.8	6.4	2585	2330	2330	2415	147.22	0.9	1.4	1.0	1.1	0.3
2	Cyclodextrine	74.2	61.0	42.4	59.2	16.0	520	533	552	535	15.98	12.5	10.3	7.1	10.0	2.7
3	Cyclodextrine	102.3	127.5	95.9	108.6	16.7										
4	TENAX	118.0	129.9	96.5	114.8	16.9	1383	1253	1382	1339	74.90	7.9	9.4	6.5	7.9	1.4
5	TENAX	39.3	98.2	67.6	68.4	29.5	1357	1427	1375	1386	36.35	2.8	6.4	4.7	4.6	1.8
6	TENAX	75.7	82.3	83.2	80.4	4.1	1560	1181	1527	1423	209.77	4.6	6.5	5.2	5.4	1.0
7	TENAX	109.9	102.1	115.2	109.1	6.6										
8	TENAX	38.2	64.2	33.8	45.4	16.4	941	1112	1617	1223	351.62	3.9	5.5	2.0	3.8	1.7
9	TENAX	95.6	90.1	95.9	93.9	3.3	758	700		729	41.04	11.2	11.4		11.3	0.1

Annex 2 Results PCBs of individual laboratories

Orange: Outliers, not used in evaluation
Yellow: stragglers

PCB 52		Available					Residue					%Available				
Lab no	extr. Liquid	Repl.1	Repl.2	Repl.3	average	stdev	Repl.1	Repl.2	Repl.3	average	stdev	Repl.1	Repl.2	Repl.3	average	stdev
	Sample 1															
1	cyclodextrin	11.8	12.1	15.1	13.0	1.8	35.4	40.0	30.7	35.3	4.6	33.1	25.0	23.3	27.1	5.2
2	cyclodextrin	11.5	14.8	17.8	14.7	3.2	33.1	29.8	26.8	29.9	3.2	40.0	25.8	33.1	33.0	7.1
3	cyclodextrin	28.3	25.5	38.1	30.6	6.6										
4	TENAX	26.8	34.4	26.2	29.1	4.5	26.9	26.9	24.9	26.3	1.2	51.2	49.9	56.0	52.4	3.2
5	TENAX	11.9	9.7	14.1	11.9	2.2	13.2	14.4	10.8	12.8	1.8	56.7	47.4	40.2	48.1	8.3
6	TENAX	n.d.	n.d.	n.d.			22.6	29.2	32.2	28.0	4.9					
7	TENAX	21.2	38.5	26.8	28.8	8.8										
8 [1]	TENAX	11.9	17.8	13.8	14.5	3.0	25.8	24.5	34.6	28.3	5.5	28.6	31.6	42.0	34.1	7.1
	Sample 2															
1	cyclodextrin	11.4	17.8	14.0	14.4	3.2	43.9	46.6	50.0	46.8	3.1	21.8	20.6	27.7	23.4	3.8
2	cyclodextrin	21.1	6.4	8.5	12.0	8.0	32.5	47.2	45.1	41.6	8.0	15.9	39.4	12.0	22.4	14.9
3	cyclodextrin	35.5	42.4	21.4	33.1	10.7										
4	TENAX	23.5	16.6	22.2	20.8	3.7	28.1	42.2	43.3	37.9	8.5	33.8	45.6	28.2	35.9	8.9
5	TENAX	9.9	10.6	8.5	9.7	1.1	19.4	19.9	20.8	20.0	0.7	28.9	33.8	34.8	32.5	3.1
6	TENAX	67.9	69.7	72.2	69.9	2.1	100.1	118.0	109.1	109.1	8.9	39.8	40.4	37.2	39.1	1.7
7	TENAX	27.6	n.d.	n.d.	27.6											
8 [1]	TENAX	18.1	12.5	15.5	15.4	2.8	29.5	37.8	44.5	37.3	7.5	25.8	38.0	24.9	29.6	7.3
	Sample3															
1	cyclodextrin	24.6	34.2	25.6	28.2	5.3	133.4	115.0	129.8	126.1	9.7	16.5	15.6	22.9	18.3	4.0
2	cyclodextrin	38.4	36.5	35.4	36.8	1.5	114.6	116.5	117.6	116.2	1.5	23.1	25.1	23.9	24.1	1.0
3	cyclodextrin	171.0	74.7	38.6	94.8	68.4										
4	TENAX	63.8	69.2		66.5	3.8	61.3	47.2	68.3	58.9	10.7	0.0	51.0		25.5	36.1
5	TENAX	4.8	25.5	20.3	16.8	10.8	34.2	36.0	41.2	37.1	3.6	33.0	12.2	41.4	28.9	15.0
6	TENAX	86.0	72.9	76.6	78.5	6.7						100.0	100.0	100.0	100.0	0.0

7	TENAX	65.9	0.7	n.d.	33.3	46.1									
8 (1)	TENAX	31.7	34.1	39.1	35.0	3.7	90.5	88.3	103.3	94.0	8.1	27.4	26.0	27.9	27.1	1.0
	Sample 4															
1	cyclodextrin	n.d.	n.d.	n.d.												
2	cyclodextrin	0.39	0.00	0.00	0.13	0.04	2.63	2.63	2.24	2.50	0.22	14.76	12.91	0.00	9.22	8.04
3	cyclodextrin	4.58	1.28	1.24	2.36	1.92										
4	TENAX	0.14	0.18	0.15	0.16	0.02	2.01	1.00	2.02	1.68	0.58	6.93	6.65	14.91	9.50	4.69
5	TENAX	0.38	0.79	0.48	0.55	0.22	0.80	2.48	1.37	1.55	0.86	25.89	32.28	24.21	27.46	4.26
6	TENAX	23.68	26.22	25.17	25.02	1.28	105.95	91.03	96.93	97.97	7.51	20.61	18.27	22.36	20.41	2.05
7	TENAX	0.98	<0.050	1.30	1.14	0.22										
8 (1)	TENAX	0.24			0.24		8.80	9.28	0.43	6.17	4.97	0.00	2.65	0.00	0.88	1.53

PCB 101		**Available**					**Residue**					**%Available**				
Lab no	extr. Liquid	Repl.1	Repl.2	Repl.3	average	stdev	Repl.1	Repl.2	Repl.3	average	stdev	Repl.1	Repl.2	Repl.3	average	stdev
	Sample 1															
1	cyclodextrin	11.7	11.8	14.9	12.8	1.8	44.8	51.7	40.1	45.6	5.8	20.7	18.6	27.1	22.1	4.4
2	cyclodextrin	13.6	18.1	23.2	18.3	4.8	40.8	36.3	31.2	36.1	4.8	25.0	33.3	42.6	33.6	8.8
3	cyclodextrin	20.6	18.4	25.1	21.3	3.4										
4	TENAX	21.2	27.2	19.6	22.7	4.0	38.9	35.9	33.9	36.3	2.5	35.2	43.1	36.7	38.3	4.2
5	TENAX	13.0	10.4	14.7	12.7	2.2	16.5	18.0	14.8	16.4	1.6	44.1	36.6	49.8	43.5	6.6
6	TENAX	n.d.	n.d.	n.d.			n.d.	n.d.	n.d.							
7	TENAX	4.4	8.0	5.6	6.0	1.8										
8 (1)	TENAX	10.3	16.0	13.1	13.1	2.8	25.9	22.3	27.8	25.3	2.8	28.4	41.7	32.1	34.1	6.9
	Sample 2															
1	cyclodextrin	3.88	5.94	4.85	4.89	1.03	27.72	27.96	30.94	28.87	1.79	12.29	17.51	13.56	14.45	2.72
2	cyclodextrin	10.59	3.31	4.83	6.25	3.84	17.71	24.99	23.47	22.05	3.84	37.43	11.71	17.06	22.07	13.57
3	cyclodextrin	10.07	5.22	6.05	7.11	2.59										
4	TENAX	7.05	5.04	6.21	6.10	1.01	25.11	19.09	20.16	21.45	3.21	21.93	20.91	23.55	22.13	1.33
5	TENAX	3.18	4.24	3.64	3.69	0.53	11.77	11.47	11.63	11.62	0.15	21.27	27.01	23.86	24.05	2.87
6	TENAX	n.d.	n.d.	n.d.			n.d.	n.d.	n.d.							
7	TENAX	5.10	n.d.	4.17	4.63	0.66										
8 (1)	TENAX	6.95	3.72	4.50	5.06	1.69	13.68	14.46	14.90	14.35	0.62	33.68	20.46	23.20	25.78	6.98
	Sample3															
1	cyclodextrin	18.9	24.0	19.3	20.7	2.8	152.1	142.6	158.1	150.9	7.8	11.1	14.4	10.9	12.1	2.0
2	cyclodextrin	32.9	30.6	29.8	31.1	1.6	106.1	108.4	109.2	107.9	1.6	23.7	22.0	21.4	22.4	1.2
3	cyclodextrin	61.7	27.7	17.5	35.7	23.1										
4	TENAX	46.0	51.4		48.7	3.8	66.3	63.3	75.3	68.3	6.3	41.0	44.8		42.9	2.7

5	TENAX	16.4	26.6	20.4	21.1	5.2	45.4	44.2	50.0	46.5	3.1	26.5	37.5	28.9	31.0	5.8
6	TENAX	n.d.	n.d.	n.d.			n.d.	n.d.	n.d.							
7	TENAX	7.2	n.d.	n.d.	7.2											
8 (1)	TENAX	19.9	21.7	25.3	22.3	2.8	81.6	71.9	70.7	74.7	5.9	19.6	23.2	26.4	23.1	3.4
	Sample 4															
1	cyclodextrin	n.d.	n.d.	n.d.			n.d.	n.d.	n.d.							
2	cyclodextrin	0.00	0.00	0.46	0.15	0.26	2.90	2.90	2.44	2.75	0.26	0.00	0.00	15.78	5.26	9.11
3	cyclodextrin	3.97	1.46	1.05	2.16	1.58										
4	TENAX	0.41	0.47	0.41	0.43	0.04	1.00	0.00	2.02	1.01	1.01	29.03		16.75	22.89	8.68
5	TENAX	0.14	0.36	0.32	0.27	0.12	1.03	1.67	0.77	1.16	0.46	11.67	17.57	29.49	19.58	9.07
6	TENAX	n.d.	n.d.	n.d.			n.d.	n.d.	n.d.							
7	TENAX	0.63	n.d.	1.07	0.57	0.54										
8 (1)	TENAX	0.37	0.33	0.00	0.24	0.20	1.19	4.09	0.87	2.05	1.78	23.85	7.56	0.00	10.47	12.19

PCB 138		**Available**					**Residue**					**%Available**				
Lab no	extr. Liquid	Repl.1	Repl.2	Repl.3	average	stdev	Repl.1	Repl.2	Repl.3	average	stdev	Repl.1	Repl.2	Repl.3	average	stdev
	Sample 1															
1	cyclodextrin	5.51	5.30	6.19	5.67	0.47	20.53	30.55	22.66	25.33	5.28	21.15	14.78	21.46	19.13	3.77
2	cyclodextrin	4.04	4.72	5.97	4.91	0.98	18.06	17.38	16.13	17.19	0.98	18.27	21.36	27.02	22.22	4.44
3	cyclodextrin	6.42	6.00	7.53	6.65	0.79										
4	TENAX	3.40	4.39	3.32	3.71	0.60	23.95	24.95	22.94	23.95	1.00	12.44	14.98	12.65	13.36	1.41
5	TENAX	8.16	6.87	9.60	8.21	1.37	18.54	18.17	17.31	18.01	0.63	30.56	27.43	35.68	31.22	4.17
6	TENAX	n.d.	n.d.	n.d.			13.46	13.08	10.94	12.49	1.36					
7	TENAX	6.05	11.13	8.53	8.57	2.54										
8 (1)	Tenax	9.97	14.82	13.11	12.63	2.46	34.86	37.07	37.03	36.32	1.27	22.23	28.56	26.14	25.65	3.19
	Sample 2															
1	cyclodextrin	1.86	2.76	2.31	2.31	0.45	14.32	13.51	15.23	14.36	0.86	11.50	16.95	13.17	13.87	2.79
2	cyclodextrin	2.79	0.81	1.25	1.61	1.04	11.01	12.99	12.55	12.19	1.04	20.18	5.84	9.04	11.69	7.53
3	cyclodextrin	3.91	4.49	2.24	3.55	1.17										
4	TENAX	0.91	0.58	0.71	0.73	0.17	11.05	14.11	43.19	22.79	17.74	7.63	3.92		5.78	2.62
5	TENAX	1.63	1.99	1.48	1.70	0.26	9.11	6.65	7.65	7.80	1.24	15.15	23.00	16.21	18.12	4.26
6	TENAX	n.d.	n.d.	n.d.												
7	TENAX	5.11	4.67	4.41	4.73	0.35										
8 (1)	TENAX	5.45	2.64	3.22	3.77	1.48	14.96	13.96	13.35	14.09	0.81	26.68	15.90	19.44	20.67	5.50
	Sample3															
1	cyclodextrin	7.96	9.22	7.94	8.37	0.74	53.82	52.90	51.92	52.88	0.95	12.88	14.84	13.26	13.66	1.04
2	cyclodextrin	7.45	7.51	7.32	7.43	0.10	49.75	49.69	49.88	49.77	0.10	13.02	13.13	12.79	12.98	0.17

3	cyclodextrin	16.62	7.84	5.36	9.94	5.92									
4	TENAX	4.76	4.65		4.71	0.08	43.19	61.28	51.23	51.90	9.06	9.93	7.06		8.50	2.03
5	TENAX	11.10	19.09	13.16	14.45	4.15	49.84	53.11	58.26	53.74	4.24	18.21	26.44	18.43	21.03	4.69
6	TENAX	n.d.	n.d.	n.d.			48.58	54.61	53.28	52.16	3.17					
7	TENAX	5.37	7.75	0.37	4.49	3.76										
8 (1)	TENAX	19.24	20.06	23.89	21.06	2.48	130.90	87.58	103.83	107.44	21.88	12.82	18.64	18.70	16.72	3.38
	Sample 4															
1	cyclodextrin	n.d.	n.d.	n.d.												
2	cyclodextrin	0.36	0.35	0.39	0.37	0.02	2.39	2.40	2.36	2.38	0.02	13.24	12.75	14.08	13.36	0.67
3	cyclodextrin	5.25	2.95	1.46	3.22	1.91										
4	TENAX	0.87	0.94	0.80	0.87	0.07	4.02	2.00	3.02	3.02	1.01	17.79	31.83	20.98	23.53	7.36
5	TENAX	0.28	0.77	0.55	0.53	0.25	1.32	0.33	2.85	1.50	1.27	17.40	70.24	16.24	34.62	30.85
6	TENAX	n.d.	n.d.	n.d.												
7	TENAX	2.40	n.d.	5.23	2.54	2.62										
8 (1)	Tenax	0.87	0.76	0.44	0.69	0.22	4.53	4.36	2.60	3.83	1.07	16.16	14.86	14.54	15.19	0.86

PCB 180		**Available**					**Residue**					**%Available**				
Lab no	extr. Liquid	Repl.1	Repl.2	Repl.3	average	stdev	Repl.1	Repl.2	Repl.3	average	stdev	Repl.1	Repl.2	Repl.3	average	stdev
2	cyclodextrin	0.44	0.61	0.87	0.64	0.21	15.96	15.79	15.53	15.76	0.21	2.68	3.70	5.28	3.88	1.31
3	cyclodextrin	1.36	1.10	1.03	1.16	0.18										
4	TENAX	2.07	2.61	2.12	2.26	0.30	19.96	14.97	15.96	16.96	2.64	9.38	14.83	11.70	11.97	2.74
5	TENAX	2.37	2.60	1.71	2.23	0.46	9.56	9.26	8.93	9.25	0.32	19.87	11.01	4.83	11.90	7.56
6	TENAX	n.d.	n.d.	n.d.			n.d.	n.d.	n.d.							
7	TENAX	2.57	4.26	3.36	3.40	0.84										
8 (1)	TENAX	2.20	3.03	1.75	2.33	0.65	25.82	24.49	34.58	28.30	5.48	7.84	11.01	4.83	7.89	3.09
	Sample 2															
1	cyclodextrin	0.51	0.71	0.54	0.59	0.11	12.71	11.88	14.99	13.19	1.61	3.86	5.63	3.48	4.32	1.15
2	cyclodextrin	0.49	0.07	0.18	0.25	0.22	12.41	12.83	12.72	12.65	0.22	3.79	0.57	1.37	1.91	1.68
3	cyclodextrin	1.29	1.07	0.30	0.88	0.52										
4	TENAX	1.32	0.96	1.17	1.15	0.18	10.05	11.09	41.19	20.77	17.69	11.58	7.94		9.76	2.58
5	TENAX	6.05	8.06	4.25	6.12	1.91	3.10	6.10	3.64	4.28	1.60	66.12	7.33	8.21	27.22	33.69
6	TENAX	n.d.	n.d.	n.d.			n.d.	n.d.	n.d.							
7	TENAX	2.16	2.48	2.18	2.28	0.18										
8 (1)	TENAX	1.64	1.10	1.39	1.37	0.27	12.55	13.91	15.49	13.98	1.47	11.56	7.33	8.21	9.03	2.23
	Sample3															
1	cyclodextrin	1.25	1.53	1.10	1.29	0.22	51.48	50.60	54.28	52.12	1.92	2.37	2.94	1.98	2.43	0.48
2	cyclodextrin	1.22	1.25	1.18	1.22	0.04	42.18	42.15	42.22	42.18	0.04	2.81	2.88	2.71	2.80	0.09

3	cyclodextrin	3.54	1.49	0.89	1.98	1.39										
4	TENAX	5.21	4.39		4.80	0.57	38.17	49.22	36.16	41.19	7.03	12.00	8.20		10.10	2.69
5	TENAX	1.25	2.45	1.95	1.88	0.60	24.00	19.40	23.99	22.46	2.65	4.95	7.64	7.22	6.60	1.45
6	TENAX	n.d.	n.d.	n.d.			23.81	27.90	28.63	26.78	2.59					
7	TENAX	2.29	4.81	n.d.	2.36	2.40										
8 [1]	TENAX	3.70	3.76	4.65	4.04	0.53	56.17	45.53	59.70	53.80	7.37	6.18	7.64	7.22	7.01	0.75
	Sample 4															
1	cyclodextrin	n.d.	n.d.	n.d.			n.d.	n.d.	n.d.							
2	cyclodextrin	0.12	0.16	0.06	0.11	0.05	3.53	3.49	3.59	3.54	0.05	3.39	4.25	1.70	3.11	1.30
4	TENAX	0.94	0.97	0.82	0.91	0.08										
5	TENAX	0.44	0.54	0.42	0.46	0.06	7.03	4.01	5.04	5.36	1.54	11.75	19.54	13.98	15.09	4.01
6	TENAX	n.d.	n.d.	n.d.			33.80	43.63	44.67	40.70	6.00	1.29	8.87	6.01	5.39	3.83
7	cyclodextrin	2.94	0.65	0.25	1.28	1.45	n.d.	n.d.	n.d.							
7	TENAX	1.06	2.16	4.98	2.73	2.02										
8 [1]	TENAX	0.47	0.53	0.29	0.43	0.12	7.44	5.45	4.59	5.83	1.47	5.97	8.87	6.01	6.95	1.66

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