ERM®-CC007a: Organochlorine pesticides in soil

Certification Report

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List of Abbreviations

BIPM	International Bureau of Weights and Measures
CCQM	Consultative Committee for the Amount of Substance – Metrology in Chemistry
CIPM	International Committee for Weights and Measures
CIPM MRA	Mutual Recognition Arrangement of CIPM members
DDD	Dichlordiphenyldichlorethane
DDE	Dichlordiphenyldichlorethene
DDT	Dichlordiphenyltrichlorethane
ECD	Electron capture detector
ERM®	European Reference Materials
FBE	Fluidised bed extraction
GC	Gas chromatography
HCH	Hexachlorocyclohexane
MS	Mass spectrometry
PFE	Pressurised fluid extraction
PTFE	Polytetrafluoroethylene
OCP	Organochlorine pesticides

1 Introduction

1.1 Organochlorine pesticides of interest and analytical procedures

Organochlorine pesticides (OCP) belong to the persistent organic pollutants to be monitored by field laboratories on contaminated sites. The quantification of OCP in soil using gas chromatography with electron capture detection (GC-ECD) has been standardised [1] and the alternative application of GC-MS for this purpose is widely used by routine laboratories.

ERM®-CC007a replaces the certified reference materials ERM®-CC007 which displayed a similar matrix/analyte combination and has recently been sold out. The certified properties of ERM®-CC007a are the extractable contents of selected hexachlorocyclohexane isomers as well as DDT isomers and metabolites.

1.2 Strategy of the certification project

A real world material, representative for an industrially polluted soil substrate taken from a former pesticide production site was preliminarily checked for its appropriateness regarding the contents of the target compounds. GC-MS and GC-ECD are the two analytical procedures equally often used in practice. Therefore, both chromatographic procedures along with usually applied extraction procedures were employed for the characterisation of the OCP contents. This certification exercise was conducted at BAM employing a number of independent operator/equipment combinations in-house rather than an external intercomparison since the calibration and measurement capability (CMC) of BAM with regard to OCP has internationally been recognised by way of key comparisons [2] of the Consultative Committee for Amount of Substance – Metrology in Chemistry (CCQM) of the International Bureau of Weights and Measures (BIPM). Every operator analysed two different units of the candidate material and two control solutions containing the congeners of question. Traceability was established using calibration standard solutions certified for the contents of these congeners and in case of GC-MS additionally the respective ^{13}C-labelled OCP were applied as internal standards (see clause 5.3.3).

2 Candidate material

The sandy soil was sampled from a former pesticide production site near Berlin, Germany. The specific location displayed an aged contamination originating from industrial pollution over decades. After drying to constant weight, the bulk material was classified by means of an automatic sieving station and a total amount of 29 kg of the fraction 125 - 250 µm was collected. Thereafter, this material was homogenised by means of a 120 L stainless steel barrel in a drum hoop mixer (J. Engelsmann AG, Ludwigshafen; Germany). The barrel was equipped with a mixing insert inside to improve the mixing intensity.

A total of 250 units were bottled in 100 mL amber screw-capped glass bottles containing (103.7 ± 0.5) g each and units were numbered in the order of leaving the bottling process. The screw caps equipped with PTFE foil inserts were tightly closed and sealed with shrinking foil. All units were stored at -20 °C directly after bottling. Table 1 comprises the matrix characterisation of the candidate material.

Table 1: Matrix characterisation of ERM®-CC007a

Parameter	Value	Method
Particle size range	125 - 250 µm	Sieving
Water content	(0.44 ± 0.02) %	*Karl Fischer* titration
Drying loss	(0.60 ± 0.07) %	Gravimetry after drying to constant weight at 105 °C (ISO 11465:1993)
Total organic carbon	(4.14 ± 0.06) mg g^{-1}	ISO 10694:1995
Total inorganic carbon	(0.55 ± 0.04) mg g^{-1}	ISO 10694:1995
CHN-Analysis (in %)	C: 0.53 ± 0.02; H: 0.106 ± 0.001; N: 0.046 ± 0.006	Combustion

3 Homogeneity study

The procedure employed for this study was pressurized fluid extraction (PFE) with acetone/n-hexane (1:1) followed by GC-ECD. Details can be found in ANNEX B.1. The minimum sample intake of 5 g for one determination was chosen form earlier experience with similar materials including ERM®-CC07 and is recommended as minimum sample intake in the certificate.

Fourteen bottles were selected equidistantly from the whole batch of 250 units in the order of bottling. The selected units were analysed four times each using the sample intake of 5 g. The extraction procedure and instrumental parameters are given in ANNEX B1. All 14 units were extracted once under repeatability conditions on three consecutive days. Thereafter, all extracts were analysed under repeatability conditions by quantifying all 42 extracts against one calibration after randomisation. (see ANNEX B for the individual measurement results). Table 2 reveals the synopsis of the 1-way analysis of variance (ANOVA).

Table 2: Results of the 1-way ANOVA on the candidate material (14 units were analysed in triple)

OCP	MS_{within} [a] ($\mu g^2\ kg^{-2}$)	$MS_{between}$ [b] ($\mu g^2\ kg^{-2}$)	F_{obs} [c]	F_{crit} [d]	$u_{bb,r}$ [e] ($\mu g\ kg^{-1}$)
α-HCH	70.0498	206.9935	2.95	2.09	0.03955971
β-HCH	4151.875	13209.54	3.18	2.09	0.04996002
γ-HCH	6.286023	5.497816	0.875	2.09	0.01693717
δ-HCH	4.291762	1.76943	0.412	2.09	0.01774768
p,p'-DDE	125.6527	446.2479	3.55	2.09	0.04091578
o,p'-DDD	19.92263	19.86017	0.997	2.09	0.01991951
o,p'-DDT	281,5599	268,017	0.952	2.09	0.01434746
p,p'-DDT	9242.412	7894.35	0.854	2.09	0.02285879

[a] *Mean of squared deviations within bottles (from 1-way ANOVA)*
[b] *Mean of squared deviations between bottles (from 1-way ANOVA)*
[c] *Observed F-value: $MS_{between}/MS_{within}$*
[d] *Critical F-value on the 95% level of confidence*
[e] *Standard uncertainty between the bottles: Estimate of inhomogeneity contribution to the total uncertainty of the respective property value according to [5].*

The estimates of inhomogeneity contributions u_{bb} potentially hidden by the measurement uncertainty and to be included to the total uncertainty were estimated according to ISO Guide 35 as the maximum of the values obtained from Eq. (1) and (2).

$$u_{bb} = \sqrt{\frac{MS_{among} - MS_{within}}{n}} \quad (1) \qquad u_{bb} = \frac{S_{method}}{n}\sqrt[4]{\frac{2}{N(n-1)}} \quad (2)$$

where:

S_{method} = Method variability ($= \sqrt{MS_{within}}$)
n = Number of replicate determinations per sample
N = Number of bottles analysed

4 Stability study

4.1 Initial stability study

From earlier experience with ERM®-CC007 a temperature-driven deterioration of the OCP content was to be expected also for this material. Selected units of the candidate material were submitted to a so-called isochronous [3] accelerated ageing at temperatures between +4 and+ 60 °C over periods of 1-12 months as shown in Table 3. After the respective periods of time individual units were stored at (–20 ± 5) °C. All units were analysed for OCP using the procedure applied for the homogeneity study under repeatability conditions together with reference samples which had been kept at (–20 ± 5) °C since bottling. For the individual data see ANNEX C.

Table 3: Accelerated ageing of exposed units, exposition temperatures and periods

Ageing time [Months]	+ 4 °C	+ 20 °C	+ 40 °C	+ 60 °C	Remark
1	x	x	x	x	*initial study*
3	x	x	x	x	*initial study*
6	x	x	x		*initial study*
12	x	x	x		*post certification monitoring*
24	x	x			*post certification monitoring*
36	x	x			*post certification monitoring*

Data evaluation and expiry date estimation strictly follow the procedures as comprehensively described in [4]: From semi-logarithmic plots of measured single values over time, effective deterioration rates were determined and tested against an Arrhenius model describing the dependence on temperature of the deterioration rates. All of the OCP matched the model, some of them excellently. Activation energies, slopes and intercepts as determined from the model are collected in table 4. Figure 4.1 shows the dependence of the logarithm of the effective deterioration rate k_eff on the inverse temperature by way of example for α-HCH and p,p-DDT.

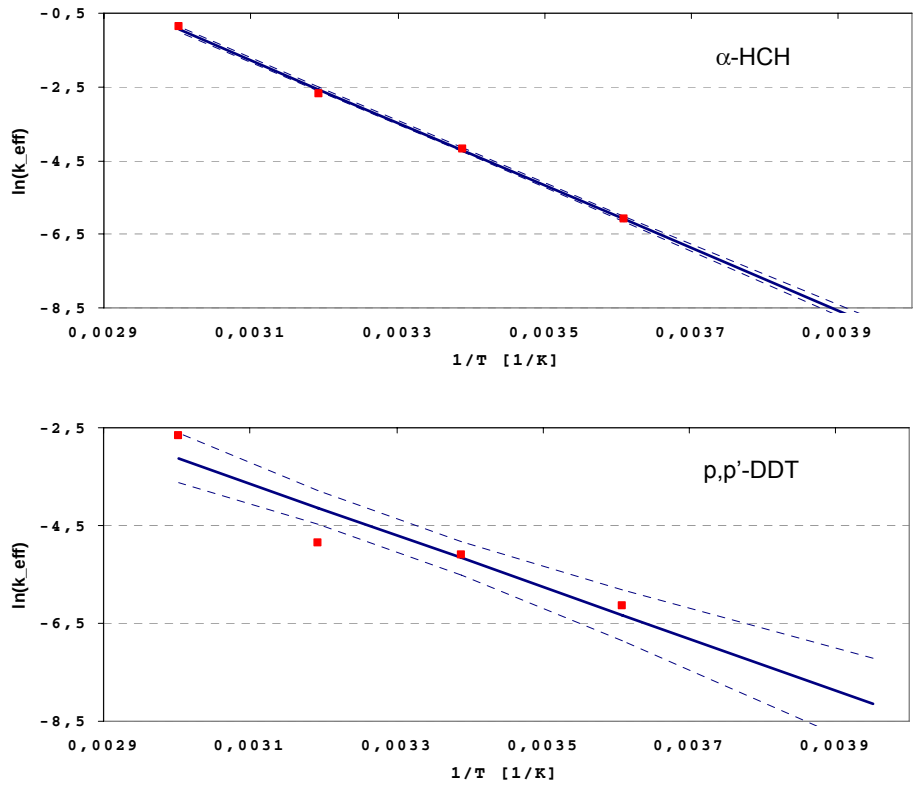

Fig. 4.1: Effective deterioration rate versus inverse temperature for α-HCH and p,p'-DDT.

Using the value at the upper confidence bound of the regressed line as a worst-case estimate for the rate of deterioration to be expected, the expiry term estimates as summarised in table 4 were calculated. The period given in table 4 started with the storage of the whole batch at (–20 ± 5) °C directly after production on 10 November 2006.

Although shelf life at a storage temperature of (–20 ± 5) °C is quite considerable for the OCP considered, any exposure to room or even higher temperatures may drastically reduce the time of validity of certified OCP contents of ERM®-CC007a. Therefore, a unique expiry date of **one year after dispatch from BAM** is established. Transportation/delivery time should be kept at the possible minimum and any exposure to heat or strong light should be avoided.

Table 4: Estimated period in months after which the certified or indicative values fall out of their certified uncertainties U

OCP	- 20 °C	+ 4 °C	+ 20 °C	ΔE_a (kJ mol^{-1})
α-HCH	486.8	28.5	4.5	4.5
β-HCH	318.7	40.7	9.9	9.9
γ-HCH	145.2	17.6	4.2	4.2
δ-HCH	1093.7	81.1	14.4	14.4
p,p'-DDE	167.2	41.4	15.6	15.6
o,p'-DDD	5498.5	301.2	41.2	41.2
o,p'-DDT	414.0	46.5	10.3	10.3
p,p'-DDT	244.9	37.4	10.2	10.2

4.2 Post-certification stability monitoring

The first rough estimation of stability will be updated by further measurements of units stored at +4 °C and +20 °C over the period of availability of the material. The post-certification measurements will be conducted according to the information given in table 3. Several units investigated during the initial stability study were stored again at +4 °C or +20 °C, respectively. That way, information on the long term stability of units of ERM®-CC007a having been opened at least once for withdrawal of material is expected in the course of the post certification monitoring. Earlier experience with ERM®-CC007 does not indicate any enhanced deterioration of once opened bottles if they are closed and stored thereafter according to the instructions given in the certificate (see also clause 6.2).

5 Certification study

5.1 Selection of participants and methods

Since the calibration and measurement capability of BAM for OCP in natural matrices was demonstrated in a key comparisons conducted by the CCQM and is recognised under the CIPM MRA, it was decided to conduct the certification study based on in-house capabilities as it had been done in case of ERM®-CC007. Moreover, no external laboratories with proven expertise were available.

Eight different operator/equipment combinations from BAM (in the following called "laboratories") were invited to participate in the certification exercise. The methods employed covered both methods GC-MS and GC-ECD as well as modern and classical extraction procedures. The equivalence of Soxhlet, pressurised fluid extraction (PFE), fluid bed extraction (FBE), sonication, and shaking was investigated prior to the certification exercise with one operator/equipment combination under repeatability conditions on similar materials such as ERM®-CC007.

5.2 Design of the study

Two units of the candidate material were to be analysed by each laboratory in triple each. A rough information on the level of content of the OCP was obtained from preliminary investigations and the homogeneity study. Certified standard substances for each of the OCP (pure substances from Cerillant Science, Austin, TX, USA) were used as calibrants (see also clause 5.3.3). Each laboratory was to prepare its independent calibration from the purchased pure substances. Laboratories using GC-MS applied the respective ^{13}C-labelled OCP as internal standards (provided by LGC Standards, Wesel, Germany).
Extraction methods and solvents to be used by each laboratory are listed in Table 5. In order to allow an assessment of the interlaboratory variability each laboratory was asked to analyse two extracts

prepared and also measured by the respectively indicated other laboratory. Results for the contents of OCP were to be reported on basis of total mass intake, no dry mass determinations were asked for.

Table 5: Extraction and determination methods

Entry	Analytical method	Extraction method	Extraction solvent
1	GC-IDMS	Shaking	Cyclohexane; acetone (1:1)
2	GC-IDMS	Fluidised bed extraction	Toluene
3	GC-ECD	Sonication	Cyclohexane/acetone (1:1)
4	GC-ECD	Pressurised fluid extraction	Cyclohexane/acetone (1:1)
5	GC-ECD	Shaking	Cyclohexane; acetone (1:1)
6	GC-ECD	Fluidised bed extraction	Toluene
7	GC-IDMS	Sonication	Cyclohexane/acetone (1:1)
8	GC-IDMS	Pressurised fluid extraction	Cyclohexane/acetone (1:1)

5.3 Evaluation of results and certified values

The results of the certification study are listed comprehensively in table 6 and were evaluated in accordance with ISO Guide 35 [5] and the specific requirements of the ERM® agreement [6]. For all measurement data see ANNEX B.3. The computer software SoftCRM [7] was partially used for statistical tests and data treatment.

5.3.1 Technical evaluation

A thorough investigation of all measurement results led to the identification of several data from some operator/equipment combinations which are biased due to an obvious technical reason such as interference by other substances and other chromatographic reasons. These values were eliminated from the data sets prior to the statistical evaluation.

5.3.2 Statistical evaluation

Since the participants in the intercomparison used different extraction techniques and solvents, followed their own procedures, and applied both GC-ECD and GC-MS, a certain scatter of results was to be expected from experience. Thus, there was no good reason for assuming that the single values measured by the different laboratories would belong to a common mother distribution. This was confirmed by the statistical analysis within which the following statistical parameters were calculated:

- *the mean of laboratory means*
- *the standard deviation of the distribution of laboratory means, and the standard deviation of the mean of laboratory means*
- *the confidence interval of the mean of laboratory means at the 0.05 significance level*

and the following statistical tests were carried out (at significance levels of 0.05 and 0.01):

- *Cochran test for the identification of outliers with respect to laboratory variance*
- *Grubbs test for the identification of outliers with respect to the mean*
- *Dixon and Nalimov test for the verification of possible outlier indications*
- *Kolmogorov-Smirnov Test (Lilliefors version) for the normality test*
- *Test for skewness and kurtosis*

The results of the above calculations and tests for a data evaluation based upon the laboratory means are given in table 7 ct. The main features are as follows:

- *Scheffé- und Snedecor-F-Test: Data sets differ significantly.*
- *Bartlett-Test: Variances are inhomogeneous (at the significance level of 0.01).*
- *Cochran-Test: No outliers detected (significance level 0.05 and 0.01).*
- *Dixon-, Grubbs- und Nalimov-Test: Laboratory means do not contain outliers (significance level 0.01).*
- *Kolmogorov-Smirnov and skewness/kurtosis test: Based on the available data, the hypothesis of normality cannot be rejected.*

Table 6: Data sets received from the participating laboratories (mean ± standard deviation)

	No. 1	**No. 2**	**No. 3**	**No. 4**	**No. 5**	**No. 6**	**No. 7**	**No. 8**
	(µg kg^{-1})	(µg kg^{-1})	(µg kg^{-1})	(µg kg^{-1})	(µg kg^{-1})	(µg kg^{-1})	(µg kg^{-1})	(µg kg^{-1})
α-HCH	242.92±4.92	208.49±6.03	202.14±7.75	241.74±8.17	226.35±11.71	188.95±7.04	240.07±4.66	204.04±7.42
β-HCH	1696.33 ±72.24	1643.88 ±38.55	1219.81 ±130.64	1685.04 ±79.58	1805.56 ±93.37	1365.77 ±138.88	1594.32 ±113.90	1541.33 ±83.33
γ-HCH	20.10±1.14	19.75±0.87	15.93±1.60	19.16±1.65	25.62±2.26	21.21±2.14	24.94±3.18	24.79±4.36
δ-HCH	--	12.90±1.31	13.93±1.69	21.35±1.67	17.66±1.60	19.17±2.42	16.35±2.99	14.29±2.38
p,p'-DDE	350.87±12.10	465.12±22.35	322.59±12.58	431.60±30.25	360.31±22.39	--	312.32±11.06	418.07±20.46
o,p'-DDD	22.81±0.30	23.64±2.18	10.40±0.58	12.04±0.80	14.52±0.71	14.91±0.98	20.28±2.09	24.21±5.80
o,p'-DDT	379.18±7.79	381.76±16.88	265.01±13.08	377.46±15.79	435.42±20.22	275.29±12.06	320.92±9.22	323.41±16.87
p,p'-DDT	883.95±12.25	854.77±25.25	982.59±36.88	815.48±28.30	--	766.22 ±109.11	1215.74 ±24.70	1176.14 ±58.49

The means of laboratory means were taken as the best estimates w_{char} for the values to be certified, and the standard deviations of the mean of laboratory means were taken as the uncertainty contributions u_{char} from characterisation by this intercomparison exercise.

Table 7: Values and statistical parameters for the intercomparison

Compound	W_{char}[a] (µg/kg)	**SD**[b] (µg/kg)	u_{char}[c] (µg/kg)	**CI**[d] (µg/kg)	**Pooling**	**Data sets**
α-HCH	219.338	21.073	7.450	17.617	no	8
β-HCH	1569	191.2	67.599	159.9	no	8
γ-HCH	21.438	3.406	1.204	2.847	no	8
δ-HCH	16.522	3.067	1.159	2.836	no	7
p,p'-DDE	380.126	58.409	22.077	54.019	no	7
o,p'-DDD	17.85	5.521	1.952	4.616	no	8
o,p'-DDT	344.805	58.618	20.725	49.006	no	8
p,p'-DDT	956.414	176.934	66.875	163.637	no	7

[a] *mean of laboratory means*
[b] *standard deviation of the population of laboratory means*
[c] *standard deviation of the mean of means*
[d] *confidence interval*

Table 7 continued

Compound	**Scheffè**[f]	**Bartlett**[g] 0.01	**Cochran**[h] 0.01(0.05)	**Grubbs**[h] 0.01(0.05)	**Dixon**[h] 0.01(0.05)	**Nalimov**[h] 0.01(0.05)	**Gauss**[i] 0.05
α-HCH	no	hom	-(-)	-(-)	-(-)	-(-)	yes
β-HCH	no	hom	-(-)	-(-)	-(-)	-(3)	yes
γ-HCH	no	hom	-(8)	-(-)	-(-)	-(-)	yes
δ-HCH	no	hom	-(-)	-(-)	-(-)	-(-)	yes
p,p'-DDE	no	hom	-(-)	-(-)	-(-)	-(-)	yes
o,p'-DDD	no	inhom	8(8,2)	-(-)	-(-)	-(-)	yes
o,p'-DDT	no	hom	-(-)	-(-)	-(-)	-(-)	yes
p,p'-DDT	no	inhom	7(7, 5)	-(-)	-(-)	-(-)	yes

[f] *Scheffé test for two-by-two data set compatibility*
[g] *Bartlett test for homogeneity of laboratory variances (significance level 0.01)*
[h] *Outlier tests: outlying laboratory values are indicated, significance level 0.01 (0.05 in brackets)*
[l] *Normal at a signifcance level of 0.05*

5.3.3 Traceability

In order to ensure traceability of the extractable contents of the investigated OCP, commercially available pure substances were used as calibration standards. The information on the purity of the calibration standard substances supplied by the provider was checked The standard substances were obtained from Cerillant Science, Austin, TX, USA. The reported purity was checked as for ERM®-CC007 by means of two different columns using GC-FID (100% method). The updated purity information was used to adjust both the certified value and its uncertainty according to usually applied statistical procedures. The resulting contributions of the calibrant purities to the uncertainty u_{pur} are given in table 8. It should however be noted that the contribution of the purity of the calibrants u_{pur} to the combined uncertainty u_{com} of the certified values is negligibly small.

5.3.4 Certified values and combined uncertainties

As mentioned in clause 5.3.2, the means of laboratory means were taken as the best estimates w_{char} for the values to be certified. The standard deviations of the mean of laboratory means were taken as the uncertainty contributions u_{char} from characterisation.
Besides this uncertainty of characterisation u_{char}, the contribution from a possibly undetected inhomogeneity u_{bb}, and the uncertainty of the purity of the calibration standard u_{pur} contribute to the combined uncertainty according to

$$u_{com,\,r}^2 = u_{char,\,r}^2 + u_{bb,\,r}^2 + u_{pur,\,r}^2$$

where the index r refers to the corresponding relative uncertainties. The uncertainties of purity of the calibration standards were taken from the certificates of Cerillant Science. The contributions of u_{char} are given in clause 5.3.2, and those of u_{bb} are evaluated in clause 3.

The final values are given in table 8 where the expansion factor for the expanded uncertainties is $k = 2$. The value and the expanded uncertainty are rounded according to the recommendations of the GUM [8] and are given with respect to raw sample mass in table 9. It should be noted that u_{pur} does not influence u_{com} after rounding. The water content of ERM®-CC007a was seen as remaining stable if the material is handled according to the instructions given in the certificate (see also clause 6).

Table 8: Certified mass fractions of OCP in ERM®-CC007a (before rounding)

Compound	w_{char} (µg kg^{-1})	u_{char} (µg kg^{-1})	u_{bb} (µg kg^{-1})	$u_{bb,r}$	u_{pur} (µg kg^{-1})	u_{com} (µg kg^{-1})
α-HCH	219.338	7.4504306	8.67694728	0.03955971	0.658014	22.9112473
β-HCH	1569	67.5994083	78.387273	0.04996002	15.69	209.384055
γ-HCH	21.438	1.20420285	0.36309907	0.01693717	0.21438	2.551787
δ-HCH*	16.522	1.15921704	0.29322717	0.01774768	0.16522	2.41417809
p,p'-DDE	380.126	22.0765269	15.5531519	0.04091578	1.140378	54.0582662
o,p'-DDD*	17.85	1.95196827	0.35556325	0.01991951	0.1785	3.98420261
o,p'-DDT	344.805	20.7245926	4.94707749	0.01434746	0.68961	42.6360354
p,p'-DDT	956.414	66.8747661	21.8624631	0.02285879	2.869242	140.8323

* indicative values

Table 9: Certified mass fractions of OCP in ERM®-CC007a (after rounding)

Compound	Mass fraction in (µg kg^{-1})
α-HCH	219 ± 23
β-HCH	1570 ± 210
γ-HCH	21.4 ± 2.6
p,p'-DDE	380 ± 60
o,p'-DDT	340 ± 50
p,p'-DDT	960 ± 140

6 Information on the proper use of ERM®-CC007a

6.1 Shelf life

From the initial stability study a preliminary shelf life of two years at storage temperatures not higher than +4 °C is estimated. Since the dispatch to the end user may occur at any time during this period the certified properties will be valid for 12 months beginning with the dispatch of the material from BAM. The validity of this information will be maintained by post-certification stability monitoring.

6.2 Transport, storage and use

The stability of the content of OCP allows the dispatch of the material at ambient temperature. On receiving, it is to be stored at (–20 ± 5) °C. Before withdrawing a sub-sample the bottle has to have reached ambient temperature. Thereafter, the bottle must be closed tightly and stored at (–20 ± 5) °C. The water content remains stable when the material is treated as described.

6.3 Safety instructions

The sediment was not sterilised, however, it is supposed to not exhibit any biological activity due to having been dried to constant weight. No hazardous effect is to be expected when the material is used under conditions usually adopted for the analysis of environmental matrices moderately contaminated with organochlorine pesticides.

It is strongly recommended to handle and dispose the reference material in accordance with the guidelines for hazardous materials legally in force at the site of end use and disposal.

6.4 Legal notice

Neither the BAM Federal Institute for Materials Research and Testing nor any person acting on their behalf make any warranty or representation, express or implied, that the use of any information, material, apparatus, method or process disclosed in this document may not infringe privately owned rights, or assume any liability with respect to the use of, or damages resulting from the use of any information, material, apparatus, method or process disclosed in this document.

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[5] ISO Guide 35:2006. Reference materials – General and statistical principles for certification. ISO, Geneva, Switzerland

[6] For detailed information on the ERM® agreement see: www. erm-crm.org/ermcrm

[7] SoftCRM V.1.22 (developed and funded under Contract SMT4 - CT98 – 6533 of the STANDARDS, MEASUREMENTS & TESTING PROGRAMME)

[8] Guide To The Expression Of Uncertainty In Measurement. ISO, Geneva (1993). (ISBN 92-67-10188-9, Reprinted 1995)

ANNEX A: Measurement methods

Entries	Extraction
1, 5	Shaking; 24 h; 300 rounds min^{-1} with acetone followed by cyclohexane (10 ml each)
2, 6	FBE (fexIKA®); toluene; 20 cycles; heating 160°C for 5 min; cooling: 80°C
3, 7	Sonication, cyclohexane/acetone (1:1), 10 ml; 40°C, 1 h; 2 cycles
4, 8	PFE (ASE®), cyclohexane/acetone (1:1); 140 bar; 100°C; 1 cycle; 10 min static; 60% purge

Entries	Chromatography
1, 2	GC-MS, column: DB1MS, 30 m x0.25 mm x 025 µm; splitless; injection volume: 3 µl; ISTD*: ^{13}C-OCP**
3, 4	GC-ECD, column: DB1701, 30x0.25x025; KAS on column; injection volume: 3 µl; ISTD*: PCB52
7, 8	GC-MS, column: SGE-HT8, 30 m x 0.25 mm x 0.25 µm; KAS on column; injection volume: 1 µl; ISTD*: ^{13}C-OCP**
5, 6	GC-ECD; column: DB-XLB, 60 m x 0.25 mm x 0.25 µm; splitless; injection volume: 1 µl; ISTD*: PCB52

* Internal standard were added to the soil sample directly before extraction.

** $[^{13}C]_6$-α-HCH, $[^{13}C]_6$-β-HCH, $[^{13}C]_6$-γ-HCH, $[^{13}C]_6$-δ-HCH, $[^{13}C]_{12}$-p,p'-DDE, $[^{13}C]_{12}$-o,p'-DDD, $[^{13}C]_{12}$-o,p'-DDT, $[^{13}C]_{12}$-p,p'-DDT

ANNEX B: Measurement results

B.1 Homogeneity study

The measurements for the homogeneity and stability study were both performed under repeatability conditions with a randomised order of extracts and using GC-ECD after PFE. It should be noted that in case or testing the homogeneity the accuracy of the determined values is of minor importance as long as the precision of the measurements is sufficient. Therefore, the calibration was chosen in a way to allow a rapid and robust performance of the extended measurement series. As a result, the determined OCP contents differ from those values obtained during the certification measurements.
In order to control any possible drift of the chromatographic system (e. g. of the detector) one selected calibration solution was injected after every seventh run in the homogeneity and stability studies (data not given). No significant variability compared with the variability of analytical results obtained from replicate extractions of the candidate material was observed.

α-HCH				β-HCH			
Bottle	Replicate			Bottle	Replicate		
	1 (µg kg^{-1})	2 (µg kg^{-1})	3 (µg kg^{-1})		1 (µg kg^{-1})	2 (µg kg^{-1})	3 (µg kg^{-1})
1	162.43	158.88	160.11	1	1152.88	999.58	997.04
2	159.69	173.73	161.53	2	1053.57	1116.12	1045.80
3	160.36	161.02	159.97	3	1016.37	1006.37	987.29
4	160.13	166.03	154.36	4	1043.08	1038.63	998.71
5	173.67	179.63	163.40	5	1093.55	1222.12	1027.43
6	197.79	185.78	175.32	6	1267.42	1221.42	1111.59
7	193.09	176.43	159.63	7	1067.86	1105.95	1088.32
8	172.83	194.47	174.46	8	1240.72	1356.65	1114.27
9	169.21	177.90	167.44	9	1049.95	1138.72	1078.33
10	168.78	175.88	174.01	10	1079.13	1167.46	1095.92
11	164.95	172.02	177.68	11	1008.25	1102.01	1144.62
12	179.33	184.07	163.90	12	1144.02	1213.62	1083.82
13	165.29	165.17	151.97	13	1070.20	1035.68	975.92
14	174.78	175.28	180.68	14	1129.71	1179.87	1122.79

γ-HCH				δ-HCH			
Bottle	Replicate			Bottle	Replicate		
	1 (µg kg^{-1})	2 (µg kg^{-1})	3 (µg kg^{-1})		1 (µg kg^{-1})	2 (µg kg^{-1})	3 (µg kg^{-1})
1	21.70	21.53	20.96	1	18.21	17.67	16.30
2	20.27	21.32	19.13	2	17.36	18.11	14.65
3	20.65	19.01	22.76	3	20.48	14.80	18.26
4	19.51	18.43	21.12	4	16.05	16.13	18.94
5	21.50	26.23	19.69	5	16.42	21.86	14.02
6	25.83	22.68	23.47	6	19.06	18.12	16.21
7	28.44	20.34	19.61	7	22.42	16.52	15.38
8	20.19	22.11	20.16	8	17.13	16.01	14.41
9	20.28	21.41	21.66	9	16.35	15.64	17.81
10	18.98	21.05	21.63	10	16.10	16.66	16.08
11	19.87	22.73	21.44	11	17.56	18.86	15.78
12	21.22	22.32	19.30	12	16.15	17.30	15.47
13	21.07	21.30	18.17	13	18.67	15.96	14.37
14	30.50	20.70	20.68	14	16.60	16.06	14.47

o,p'-DDE				p,p'-DDE			
Bottle	Replicate			Bottle	Replicate		
	1 (μg kg^{-1})	2 (μg kg^{-1})	3 (μg kg^{-1})		1 (μg kg^{-1})	2 (μg kg^{-1})	3 (μg kg^{-1})
1	18.09	19.04	17.56	1	249.47	236.21	229.14
2	17.64	20.92	18.39	2	239.85	257.07	246.03
3	17.28	17.71	20.60	3	237.04	234.02	231.32
4	17.40	18.72	19.44	4	244.01	243.62	226.60
5	18.16	22.14	18.33	5	249.84	264.75	242.78
6	19.37	20.65	19.47	6	279.37	280.31	247.95
7	22.14	19.32	18.12	7	251.34	258.11	231.32
8	19.26	20.17	18.98	8	260.31	282.52	260.51
9	17.56	20.19	23.27	9	243.45	260.83	255.02
10	18.02	18.97	20.82	10	256.70	265.81	263.47
11	17.41	21.81	20.24	11	237.89	254.16	265.96
12	18.77	20.41	19.35	12	269.07	278.70	254.64
13	22.08	18.35	17.39	13	249.55	247.12	227.66
14	21.12	20.41	18.15	14	257.71	272.61	267.66

o,p'-DDT				p,p'-DDT			
Bottle	Replicate			Bottle	Replicate		
	1 (μg kg^{-1})	2 (μg kg^{-1})	3 (μg kg^{-1})		1 (μg kg^{-1})	2 (μg kg^{-1})	3 (μg kg^{-1})
1	202.29	170.94	149.15	1	760.29	612.62	517.20
2	180.89	170.84	159.21	2	698.66		547.78
3	189.62	162.83	152.06	3	704.73	580.60	493.77
4	178.85	158.77	131.11	4	697.52	579.85	420.47
5	183.64	182.80	159.23	5	725.78	660.97	533.34
6	196.72	184.08	159.87	6	746.35	663.59	557.49
7	168.37	168.72	147.53	7	599.61	594.94	500.98
8	186.86	192.61	175.27	8	733.82	742.60	623.36
9	180.22	161.84	161.94	9	697.46	554.54	524.75
10	177.86	180.11	182.46	10	685.86	653.93	620.60
11	165.55	164.66	162.16	11	646.25	557.68	517.06
12	186.07	190.44	135.82	12	702.86	658.75	409.93
13	160.21	155.83	135.07	13	545.91	510.18	419.53
14	176.42	153.30	175.63	14	635.77	505.89	586.78

o,p'-DDD			
Bottle	Replicate		
	1 (µg kg^{-1})	2 (µg kg^{-1})	3 (µg kg^{-1})
1	25.77	27.89	30.54
2	26.02	33.09	32.43
3	26.17	28.83	35.19
4	25.58	28.69	35.28
5	26.33	33.71	32.21
6	29.60	33.65	31.74
7	37.35	32.96	32.12
8	27.43	33.58	32.70
9	25.95	35.01	39.05
10	29.64	31.82	35.56
11	27.94	36.93	39.76
12	29.35	36.08	43.10
13	37.92	32.95	35.95
14	31.44	41.81	34.63

B.2 Stability study

The measurements for the homogeneity and stability study were both performed under repeatability conditions with a randomised order of extracts and using GC-ECD after PFE. For drift control see B.1.

Bottle/ replicate	ageing period	T	α-HCH	γ-HCH	δ-HCH	δ-HCH	p,p'-DDE	o,p'-DDD	o,p'-DDT	p,p'-DDT
	(months)	(°C)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)
193-1	reference		217.70	12.97	1784.52	13.43	337.16	10.85	23.83	1410.84
193-2	reference		207.34	13.06	1644.47	12.32	323.42	14.34	20.07	1174.78
193-3	reference		242.56	19.82	1930.01	15.24	382.39	16.99	25.54	1366.16
193-4	reference		225.61	13.42	1811.14	11.61	366.39	15.88	24.52	1360.01
194-1	reference		214.76	12.22	1722.67	12.16	339.99	13.12	23.33	1345.09
194-2	reference		201.04	14.44	1598.87	18.71	323.56	13.82	22.52	1243.48
194-3	reference		216.83	13.08	1877.71	13.03	341.09	16.36	21.99	1193.06
194-4	reference		230.38	13.64	1849.30	12.68	372.69	17.92	23.71	1257.69
201-1	1	4	224.09	18.33	2105.34	17.04	349.92	9.58	26.39	1556.80
201-2	1	4	218.32	13.14	1881.82	20.81	355.84	15.83	22.81	1255.61
201-3	1	4	218.90	13.27	1821.84	13.11	349.63	14.47	23.82	1305.72
201-4	1	4	212.81	15.38	1657.06	14.10	336.36	13.71	22.13	1202.39
202-1	3	4	259.51	15.55	1904.53	41.15	364.11	14.69	29.68	1682.94
202-2	3	4	229.17	16.14	1973.54	16.27	360.47	12.66	25.52	1470.24
202-3	3	4	225.59	16.94	1790.66	13.03	353.43	17.25	22.96	1221.33
202-4	3	4	214.08	18.12	1687.04	11.88	349.26	15.18	22.13	1228.80
203-1	6	4	228.81	29.14	1915.01	20.53	357.57	11.37	25.23	1506.56
203-2	6	4	215.94	13.51	1807.10	13.81	341.53	10.98	23.87	1399.71
203-3	6	4	215.62	12.87	1771.99	11.44	360.42	17.49	22.62	1215.17
203-4	6	4	231.79	16.27	1733.45	13.48	355.51	18.02	25.74	1361.08
204-1	12	4	214.05	13.36	1682.84	13.65	326.66	10.52	23.70	1410.52
204-2	12	4	219.34	14.11	1709.72	13.40	352.21	14.51	24.06	1253.33
204-3	12	4	221.44	14.41	1733.98	12.45	371.17	18.22	24.14	1238.69
204-4	12	4	212.89	16.86	1716.79	15.77	341.94	16.23	21.71	1163.45
209-1	1	22	219.83	15.44	1718.90	11.88	348.07	9.09	26.38	1520.87
209-2	1	22	217.69	12.81	1735.65	11.67	352.33	14.54	23.54	1340.23
209-3	1	22	215.68	12.71	1780.49	12.42	359.59	17.08	22.66	1239.31
209-4	1	22	213.78	11.98	1736.38	11.65	350.73	15.19	23.21	1232.84
210-1	3	22	203.20	14.23	1721.50	14.24	328.82	9.59	24.77	1488.09
210-2	3	22	210.88	12.83	1776.13	12.11	351.84	14.65	23.46	1281.84
210-3	3	22	219.14	14.09	1758.30	10.88	353.52	21.47	21.67	1118.39
210-4	3	22	221.19	15.71	1869.69	13.09	369.16	14.97	24.83	1339.29
211-1	6	22	215.97	15.54	1858.65	14.50	373.70	10.91	27.36	1624.31
211-2	6	22	216.25	16.26	1846.81	13.79	366.87	12.99	25.63	1451.62
211-3	6	22	198.70	11.09	1694.58	10.13	343.37	15.09	21.66	1184.17
211-4	6	22	220.82	20.67	1844.30	16.50	369.81	15.92	27.32	1380.08
212-2	12	22	184.80	12.04	1711.35	12.23	324.55	12.35	21.12	1238.97
212-3	12	22	186.34	12.81	1658.36	13.66	337.10	13.97	22.42	1655.61
212-4	12	22	182.60	12.13	1647.75	11.10	335.48	14.96	21.62	1133.00
218-1	1	40	294.21	25.95	2031.09	23.61	403.94	20.82	38.37	2206.03
218-2	1	40	202.48	14.68	1688.17	18.70	347.62	12.96	23.91	1294.15
218-2	1	40	194.56	12.40	1741.19	11.61	352.67	13.43	24.28	1314.26
218-4	1	40	185.62	11.62	1634.51	10.45	331.31	13.32	21.90	1165.75
219-1	3	40	131.84	12.47	1436.53	11.11	267.02	7.67	19.34	1165.42

219-2	3	40	157.54	12.04	1654.17	17.22	334.13	12.47	21.33	1202.09
219-3	3	40	160.09	11.33	1675.71	9.79	355.26	14.33	22.64	1231.41
Bottle/ replicate	ageing period (months)	T (°C)	α-HCH (mg/kg)	γ-HCH (mg/kg)	δ-HCH (mg/kg)	δ-HCH (mg/kg)	p,p'-DDE (mg/kg)	o,p'-DDD (mg/kg)	o,p'-DDT (mg/kg)	p,p'-DDT (mg/kg)
219-4	3	40	190.98	13.53	1744.03	12.28	359.50	19.41	24.78	1284.39
220-1	6	40	128.93	15.24	1519.38	13.91	326.88	10.29	22.28	1239.78
220-2	6	40	123.61	12.88	1529.34	12.42	336.39	13.41	22.35	1147.03
220-3	6	40	114.11	10.00	1431.76	8.89	311.44	14.33	18.89	983.50
220-4	6	40	116.37	10.01	1513.49	11.22	327.35	13.67	19.92	1033.89
221-1	12	40	92.81	9.46	1611.20	9.45	356.27	10.10	21.46	1218.13
221-2	12	40	86.13	8.13	1494.26	8.59	332.78	12.43	18.93	1009.10
221-3	12	40	90.74	9.17	1626.15	8.47	362.22	16.17	19.52	994.56
221-4	12	40	94.13	9.89	1575.54	7.92	361.82	14.46	20.54	1041.79
222-1	1	60	113.30	12.33	1591.47	11.96	376.03	14.69	26.15	1598.05
222-2	1	60	104.01	15.05	1678.52	12.53	397.88	12.11	20.90	1235.27
222-3	1	60	94.57	9.29	1662.63	7.82	401.08	15.17	19.70	1007.71
222-4	1	60	92.42	8.30	1614.56	7.93	378.22	14.71	18.22	954.15
223-1	3	60	48.88	4.76	1004.36	6.14	294.62	6.35	11.24	756.06
223-2	3	60	45.56	2.95	1013.43	5.37	305.72	9.32	10.09	596.84
223-4	3	60	47.23	3.11	1067.44	5.97	324.72	10.58	10.80	624.50

B.3 Certification study

α-HCH	Replicate						
Entry	1	2	3	4	5	6	7
	(µg kg^{-1})	(µg kg^{-1})	(µg kg^{-1})	(µg kg^{-1})	(µg kg^{-1})	(µg kg^{-1})	(µg kg^{-1})
1	252.10	242.68	241.27	243.46	237.81	240.19	
2	211.42	217.87	206.87	212.02	207.69	198.97	204.59
3	196.89	201.56	190.02	208.19	205.06	211.10	
4	239.40	233.58	233.85	254.36	248.04	241.23	
5	241.85	233.80	228.92	233.54	219.77	219.79	206.81
6	196.81	188.42	197.52	182.54	190.34	178.04	188.95
7	245.55	234.72	237.65	245.84	239.91	236.76	
8	196.71	211.90	199.48	198.52	214.41	203.21	

β-HCH	Replicate						
Entry	1	2	3	4	5	6	7
	(µg kg^{-1})	(µg kg^{-1})	(µg kg^{-1})	(µg kg^{-1})	(µg kg^{-1})	(µg kg^{-1})	(µg kg^{-1})
1	1685.74	1841.57	1668.66	1671.12	1663.86	1647.05	
2	1632.03	1683.40	1608.95	1711.92	1617.82	1620.15	1632.89
3	1135.86	1460.63	1100.03	1198.01	1160.42	1263.90	
4	1776.25	1621.24	1587.18	1764.89	1720.85	1639.86	
5	1925.29	1873.81	1814.14	1879.11	1727.46	1750.25	1668.89
6	1329.53	1300.63	1429.35	1263.16	1643.02	1228.93	1365.77
7	1511.03	1533.30	1486.53	1647.11	1793.83	1594.11	
8	1424.61	1608.49	1551.77	1528.02	1653.93	1481.15	

γ-HCH	Replicate						
Entry	1	2	3	4	5	6	7
	(µg kg^{-1})	(µg kg^{-1})	(µg kg^{-1})	(µg kg^{-1})	(µg kg^{-1})	(µg kg^{-1})	(µg kg^{-1})
1	20.27	21.41	19.93	20.58	17.99	20.39	
2	20.00	20.22	20.48	18.44	19.63	20.75	18.73
3	17.44	15.51	13.38	15.03	16.82	17.43	
4	17.58	18.19	17.93	22.01	19.78	19.45	
5	29.90	25.11	26.15		24.47	23.59	24.51
6	19.03	22.08	23.25	23.17	22.16	17.59	21.21
7	25.29	22.07	24.37	26.50	30.08	21.34	
8	21.18	21.17	32.70	22.74	24.68	26.27	

δ-HCH	Replicate						
Entry	1	2	3	4	5	6	7
	(µg kg^{-1})	(µg kg^{-1})	(µg kg^{-1})	(µg kg^{-1})	(µg kg^{-1})	(µg kg^{-1})	(µg kg^{-1})
1							
2	14.89	12.09	12.45	13.03	10.98	12.68	14.18
3	13.84	16.67	12.48	11.89	14.06	14.67	
4	23.55	22.62	19.57	21.74	21.30	19.31	
5	17.96	16.97	16.79	20.62	15.75	16.84	18.66
6	19.30	18.35	22.06	15.10	22.08	18.14	19.17
7	13.11	14.73	15.37	18.43	21.34	15.12	
8	12.07	11.27	13.31	15.97	16.64	16.50	

p,p'-DDE				Replicate			
Entry	1	2	3	4	5	6	7
	(μg kg^{-1})	(μg kg^{-1})	(μg kg^{-1})	(μg kg^{-1})	(μg kg^{-1})	(μg kg^{-1})	(μg kg^{-1})
1	365.89	364.60	352.72	341.90	340.10	340.01	
2	428.58	492.44	470.24	483.69	448.51	454.62	477.76
3	323.28	320.15	304.23	343.01	318.49	326.39	
4	479.26	422.72	398.29	452.93	430.44	405.94	
5	390.79	380.03	362.94	364.83	349.30	352.47	321.84
6							
7	301.72	308.33	302.80	327.98	324.00	309.08	
8	410.63	425.80	411.07	387.80	449.38	423.76	

o,p'-DDE				Replicate			
Entry	1	2	3	4	5	6	7
	(μg kg^{-1})	(μg kg^{-1})	(μg kg^{-1})	(μg kg^{-1})	(μg kg^{-1})	(μg kg^{-1})	(μg kg^{-1})
1	22.61	22.78	22.54	22.91	22.62	23.36	
2	22.18	25.33	22.95	22.89	21.42	27.77	22.97
3	9.71	11.25	9.95	10.91	10.27	10.33	
4	12.68	11.37	10.80	12.87	12.33	12.17	
5	15.15	14.73	15.02	14.97	14.66	13.89	13.21
6	16.86	15.34	14.66	13.89	14.43	14.27	14.91
7	19.22	17.78	19.09	19.96	22.54	23.07	
8	19.15	21.33	33.05	18.98	29.49	23.25	

o,p'-DDT				Replicate			
Entry	1	2	3	4	5	6	7
	(μg kg^{-1})	(μg kg^{-1})	(μg kg^{-1})	(μg kg^{-1})	(μg kg^{-1})	(μg kg^{-1})	(μg kg^{-1})
1	385.32	388.12	383.20	378.18	368.61	371.65	
2	366.71	394.76	373.70	398.71	360.18	375.20	403.04
3	256.31	264.50	248.00	275.20	261.66	284.39	
4	375.14	366.34	353.12	393.85	391.87	384.44	
5	438.35	441.42	443.03	454.52	433.11	445.43	392.05
6	264.01	258.20	286.87	278.61	292.45	271.58	275.29
7	310.73	311.61	316.57	331.67	330.18	324.75	
8	301.29	347.24	317.69	311.21	336.69	326.31	

p,p'-DDT				Replicate			
Entry	1	2	3	4	5	6	7
	(μg kg^{-1})	(μg kg^{-1})	(μg kg^{-1})	(μg kg^{-1})	(μg kg^{-1})	(μg kg^{-1})	(μg kg^{-1})
1	894.83	886.56	889.43	872.95	894.86	865.10	
2	851.63	844.15	832.42	902.32	837.40	840.09	875.40
3	962.98	973.46	931.14	1012.89	979.94	1035.12	
4	818.08	834.95	779.66	781.84	832.75	845.56	
5	--						
6	643.70	613.37	833.73	822.37	925.25	758.91	766.22
7	1181.44	1229.14	1193.18	1249.45	1221.59	1219.67	
8	1149.74	1273.45	1097.12	1167.51	1200.02	1169.00	