Method validation study on determination of OC-pesticides and PCBs by GC-ECD and GC-MS(/MS) in animal feeding stuffs

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Summary

Due to new legislation setting maximum limits for non dioxin-like polychlorinated biphenyls (ndl-PCBs), the European Commission Mandate M/523 called for modification of current EN standards for determination of PCBs and organochlorine pesticides (OCPs). This concerns the standards EN-15741:2009 (GC-MS) and EN 15742:2009 (GC-ECD). WFSR (at that time RIKILT) Wageningen University & Research was requested by the European Committee for Standardization (CEN) to adapt current standards.

The modifications concern (a) reduction of the limit of quantification (LOQ) for each individual ndl-PCB congener from 5 ng/g to 0.5 ng/g and (b) extending the scope of the methods with the OCPs photoheptachlor, cis/trans nonachlor and keto-endrin. The modifications of EN 15742:2009 (GC-ECD) concern the inclusion of above mentioned additional OCPs. In addition, the determination of the ndl-PCBs is removed from this standard as GC-ECD is not capable of reaching the lowered LOQs for the ndl-PCBs. Therefore, the modified EN 15742:2009 (GC-ECD) limits to the determination of OCPs only. The modifications of EN 15741:2009 (GC-MS(/MS)) concern the inclusion of above mentioned additional OCPs, as well as the lowered LOQs for the ndl-PCBs.

The revised standard for GC-ECD (EN15741) could not be evaluated due to insufficient number of participating laboratories (n=3). The extension of the standard with keto-endrin, photo-heptachlor and cis/trans-nonachlor is therefore not possible. Nevertheless the current standard remains intact for the OCPs that were included in the original standard.

The standard for GC-MS(/MS) (EN15742) was successfully revised. The method is suitable for analysis of nearly all ndl-PCBs in all tested matrices. The method is also suitable for the majority of OCPs in the included feed matrices. The suitability of the standard could not be evaluated for the newly added organochlorine pesticides (endrin ketone, cis- and trans-nonachlor and photo heptachlor), mainly because only 5 out of 10 laboratories had sent in results and also because some laboratories were non-compliant.

1 Introduction

Due to new legislation setting maximum limits for non dioxin-like polychlorinated biphenyls (ndl-PCBs), current EN standards for determination of PCBs and organochlorine pesticides (OCPs) are modified. It concerns the standards EN-15741:2009 (GC-MS) [CEN, 2009a] and EN 15742:2009 (GC-ECD) [CEN, 2009b). RIKILT Wageningen University and Research was requested by the European Committee for Standardization (CEN) to modify the standards. The modifications concern (a) reduction of the limit of quantification (LOQ) for each individual ndl-PCB congener from 5 ng/g to 0.5 ng/g and (b) extending the scope of the methods with the OCPs photoheptachlor, cis/trans nonachlor and keto-endrin. The modifications of EN 15742:2009 (GC-ECD) concern the inclusion of above mentioned additional OCPs. In addition, the determination of the ndl-PCBs is removed from this standard as GC-ECD is not capable of reaching the lowered LOQs for the ndl-PCBs. Therefore, the modified EN 15742:2009 (GC-ECD) limits to the determination of OCPs only. The modifications of EN 15741:2009 (GC-MS(/MS)) concern the inclusion of above mentioned additional OCPs, as well as the lowered LOQs for the ndl-PCBs. The latter is achieved by either (i) a 10-fold higher sample intake combined with GC-MS (ii) the use of GC - triple quadrupole mass spectrometry (MS/MS). For the modified standards a collaborative trial was organised between March and May 2016 for the validation of the standards. The modified EN 15742:2009 (GC-ECD) was validated in a collaborative trial, focussing only on photoheptachlor, cis/trans nonachlor and keto-endrin, whereas for EN 15741:2009 (GC-MS(/MS)) all OCPs and ndl-PCBs were included in the validation. We here report on the results of these collaborative trials.

2 Design of the study

2.1 Sample preparation

Twelve samples (Table 1) were prepared including compound feeds and feed materials. The compound feeds were prepared by Research Diet Service (Wijk bij Duurstede, The Netherlands). To that end, blank vegetable oils were prepared with relevant levels of the target analytes (except for sample A and B which is the blank feed sample). These oils were mixed together with other ingredients following the corresponding chicken and pig feed formulations. The ingredients were mixed thoroughly and the feeds were packed in individual containers. The fish oil was spiked with the target analytes at the desired levels. The fish meal was ordered from MTC, Hengelo, The Netherlands. The fish meal was spiked with additional analytes in oil and mixed thoroughly into the feed material. Concerning the analytes of interest, all were acquired from commercial suppliers, except for photo-heptachlor. Due to limited stocks available commercially, it was decided to prepare photo-heptachlor from heptachlor by UV radiation at wavelengths >290 nm for a prolonged period, according to Huhnerfuss *et al.*, 2005).

The homogeneous materials were distributed to the participating laboratories as specified in §3.4. Also, one vial containing ^{13}C mass labelled analogues of PCB-28, 52, 101, 138, 153 and 180 was prepared for each participant of the GC-MS(/MS) collaborative trial. These mass labelled analogues were provided by Cambridge Isotope Laboratories (www.isotope.com), free of charge.

The aimed levels of the materials are presented in Annex 1.

Table 1 *Samples of the method validation study.*

Material	Code
Chicken feed	A and B
Chicken feed	C and D
Pig feed	E and F
Vegetable oil	G and H
Fish oil	I and J
Fish meal	K and L

2.2 Sample identification

After homogenization, the feed materials were divided into sub-portions of 50 g, the oil samples into portions of 10 ml sub. The samples for the participants were randomly coded by a web application (Annex 2). Per material 10 randomly taken samples were used for homogeneity testing.

2.3 Homogeneity

The homogeneity of materials C, D, E, F, K and L was tested according to The International Harmonized Protocol for Proficiency Testing of Analytical Laboratories and ISO 13528, taking into account the insights discussed by Thompson (2000) regarding the Horwitz equation. Ten containers of each material were analysed in duplicate for PCB 138. The results are presented in Annex 3. The homogeneity of the other analytes was not tested, because the homogeneity test of PCB 138 test of the other analytes was adequate to prove the sufficient homogeneity of the materials. Materials A and B were not tested for homogeneity since this was the blank sample. Also, the oil-materials G, H, I and J were not tested for homogeneity since we assume, and experience shows, that these materials are easily homogenized by stirring/shaking for a prolonged time.

2.4 Sample distribution and participating laboratories

Each of the participating laboratories received a randomly assigned laboratory code, generated by a webapplication, designed for proficiency tests. The sample sets with the corresponding number, consisting of twelve coded samples (Annex 2) were sent to the participating laboratories on March 21 2016. The sample sets were packed in a polypropylene box at room temperature and were dispatched to the participants immediately by courier. The samples were accompanied by a letter (Annex 4 and 5) describing the requested analyses and an acknowledgement of receipt form. By e-mail the laboratories received instructions on how to use the web application for reporting results.

The laboratories were asked to store the samples in the refrigerator until analysis. A single analysis of each sample was requested using the prescribed method "*Animal feeding stuffs — Determination of OC-pesticides and PCB's by GC/MS"* (Annex 6) and "*Animal feeding stuffs – Determination of OC-pesticides and PCB's by GC/ECD*" (Annex 7). The deadline for submitting the results was May 16 2016, allowing 8 weeks for the analysis.

Participating laboratories
Seven laboratories registered for participation in the GC-ECD part of the method validation study. Four of them are situated in Europe, one in Africa, one in Asia and one in South-America. Since only three laboratories submitted results, these results were not statistically evaluated. The results section therefor limits to the GC-MS(/MS) results. Fifteen laboratories registered for participation in the GC-MS(/MS) part. Twelve of them are situated in Europe, two in Africa and one in Asia. Four of these labs registered for both GC-MS and GC-MS/MS analysis, so in principle 19 datasets could be obtained. However, not all laboratories submitted their results and ultimately, ten datasets were obtained.

2.5 Statistical evaluation

The main objective of this collaborative study is the validation of the new candidate CEN methods for the determination of OC-pesticides and PCB's in animal feeding stuffs.

Statistical evaluation of the results was carried out according to the Collaborative Study Guidelines of AOAC International for blind (unpaired) replicates and ISO 5725. According to ISO 5725, the results were assessed in the following manner: (i) visual inspection of the data for identification of irregular data (and removal of such data); (ii) identification of consistent irregular data of Mandel h and k plots and (iii) identification of outliers using numerical statistical tests. Irregular or outlying data were discarded, if applicable. After evaluation of the data, the HorRat (Horwitz ratio) is calculated. Horrat is described for evaluation of collaborative studies. The HorRat is the ratio of the reproducibility relative standard deviation, expressed as a percentage (RSD_R %) to the predicted reproducibility relative standard deviation, expressed as a percentage ($PRSD_R$ %,):

$$\text{HorRat} = \frac{\text{RSD}_R(\%)}{\text{PRSD}_R(\%)}$$

Where: $PRSD_R$ (%) = $2C^{-0.1505}$

C = estimated mean concentration expressed as a decimal fraction (10^{-6} for this test)

However, this equation is not applicable to the lower concentration range (<120 µg/kg) [Thompson, 2000]. Therefore a $PRSD_R$ (%) of 22% is used in this study (corresponding to the value when 120 µg/kg is entered in the Horwitz equation).

In the statistical evaluation of the results, the recommendations as described in the draft document CEN/TR 17421 (version available during evaluation of the results of the collaborative study, end of 2017) regarding the organization and evaluation of collaborative studies for multi-analyte methods of analysis [CEN, 2019] were taken into account. According to this draft version of CEN/TR 17421 the respective working group of CEN/TC 327 (WG 1, Organic Contaminants) must decide case by case whether the available data are acceptable in terms of HorRat values higher than 2 and number of accepted results. WG 1 decided in its meeting of 4 December 2017 that, if a minimum of 5 laboratories remains after exclusion of non-compliant laboratories and outliers, the method is fully validated for that specific analyte/matrix combination and that HorRat values up to 2,5 are deemed acceptable

3 Results and discussion

The results section limits to only the GC-MS(/MS) results (EN15741). Concerning the GC-ECD method (EN15742), the revised standard for GC-ECD could not be evaluated due to insufficient number of participating laboratories (n=3).

3.1 PCBs

Excellent results were obtained for the PCBs. In total 9 datasets were submitted (2 for GC-MS and 7 for GC-MS/MS). No data was excluded by visual inspection of the data, nor on the basis of statistical outlier tests. The results show excellent Horrat values of <2.0 in 29 out of 30 cases (5 matrices x 6 PCBs). Only in 1 case, Horrat was 3.0 (vegetable oil, PCB-180). This is a good achievement, also given the wide span of analyte concentrations in the samples spanning nearly 2 orders of magnitude. This clearly demonstrates the added value of the use of ^{13}C mass labelled internal standards which was obligatory to use for each individual congener.

PCB-28 - Results summary of the collaborative trial.

Material name	**Chicken feed**	**Pig feed**	**Vegetable oil**	**Fish oil**	**Fish oil**
Analyte	**PCB 28**	**PCB 28**	**PCB 28**	**PCB 28**	**PCB 28**
Year of inter-laboratory study	**2016**	**2016**	**2016**	**2016**	**2016**
Number of laboratories	9	9	9	9	9
Number of samples	2	2	2	2	2
Number of laboratories considered as non-compliant	0	0	0	0	0
Number of outlying laboratories	0	0	0	0	0
Number of accepted results	18	18	18	17	18
Mean value, x, µg/kg	0,8	0,9	1,4	20,4	8,3
Repeatability standard deviation sr, µg/kg	0,1	0,1	0,3	3,5	0,7
Repeatability relative standard deviation, RSDr, %	11,2	12,8	20,4	17,4	7,9
Repeatability limit r [r = 2,8 × sr], µg/kg	0,3	0,3	0,8	9,9	1,8
Reproducibility standard deviation sR, µg/kg	0,3	0,2	0,5	7,5	3,2
Reproducibility relative standard deviation, RSDR, %	35,0	26,1	38,4	38,6	38,2
Reproducibility limit R [R = 2,8 × sR], µg/kg	0,8	0,6	1,5	21,0	8,9
HorRat value	1,6	1,2	1,7	1,7	1,7

PCB-52 - Results summary of the collaborative trial.

Material name	**Chicken feed**	**Pig feed**	**Vegetable oil**	**Fish oil**	**Fish meal**
Analyte	**PCB 52**	**PCB 52**	**PCB 52**	**PCB 52**	**PCB 52**
Year of inter-laboratory study	**2016**	**2016**	**2016**	**2016**	**2016**
Number of laboratories	9	9	9	9	9
Number of samples	2	2	2	2	2
Number of laboratories considered as non-compliant	0	0	0	0	0
Number of outlying laboratories	0	0	0	0	0
Number of accepted results	18	18	18	18	18
Mean value, x, µg/kg	0,8	0,8	1,3	19,5	7,2
Repeatability standard deviation sr, µg/kg	0,0	0,1	0,3	3,3	0,7
Repeatability relative standard deviation, RSDr, %	6,4	15,6	23,5	16,7	9,3
Repeatability limit r [r = 2,8 × sr], µg/kg	0,1	0,3	0,9	9,1	1,9
Reproducibility standard deviation sR, µg/kg	0,3	0,2	0,5	8,6	2,6
Reproducibility relative standard deviation, RSDR, %	34,3	33,1	38,7	44,4	36,3
Reproducibility limit R [R = 2,8 × sR], µg/kg	0,7	0,7	1,4	24,2	7,3
HorRat value	1,6	1,5	1,8	2,0	1,6

PCB-101 - Results summary of the collaborative trial.

Material name	**Chicken feed**	**Pig feed**	**Vegetable oil**	**Fish oil**	**Fish meal**
Analyte	**PCB 101**	**PCB 101**	**PCB 101**	**PCB 101**	**PCB 101**
Year of inter-laboratory study	**2016**	**2016**	**2016**	**2016**	**2016**
Number of laboratories	9	9	9	9	9
Number of samples	2	2	2	2	2
Number of laboratories considered as non-compliant	0	0	0	0	0
Number of outlying laboratories	0	0	0	0	0
Number of accepted results	18	18	18	18	18
Mean value, x, µg/kg	0,7	0,7	1,2	18,2	6,6
Repeatability standard deviation sr, µg/kg	0,1	0,1	0,3	2,8	0,6
Repeatability relative standard deviation, RSDr, %	10,3	13,1	25,4	15,6	8,9
Repeatability limit r [r = 2,8 × sr], µg/kg	0,2	0,2	0,9	7,9	1,6
Reproducibility standard deviation sR, µg/kg	0,2	0,2	0,5	6,0	2,0
Reproducibility relative standard deviation, RSDR, %	32,7	32,9	39,5	33,0	30,1
Reproducibility limit R [R = 2,8 × sR], µg/kg	0,6	0,6	1,3	16,8	5,6
HorRat value	1,5	1,5	1,8	1,5	1,4

PCB-138 - Results summary of the collaborative trial.

Material name	**Chicken feed**	**Pig feed**	**Vegetable oil**	**Fish oil**	**Fish meal**
Analyte	**PCB 138**	**PCB 138**	**PCB 138**	**PCB 138**	**PCB 138**
Year of inter-laboratory study	**2016**	**2016**	**2016**	**2016**	**2016**
Number of laboratories	9	9	9	9	9
Number of samples	2	2	2	2	2
Number of laboratories considered as non-compliant	0	0	0	0	0
Number of outlying laboratories	0	0	0	0	0
Number of accepted results	18	18	18	18	18
Mean value, x, µg/kg	1,4	1,5	2,1	37,1	14,4
Repeatability standard deviation sr, µg/kg	0,0	0,2	0,4	6,0	1,6
Repeatability relative standard deviation, RSDr, %	2,4	15,6	18,2	16,2	11,1
Repeatability limit r [r = 2,8 × sr], µg/kg	0,1	0,6	1,1	16,8	4,5
Reproducibility standard deviation sR, µg/kg	0,4	0,6	0,7	13,0	4,3
Reproducibility relative standard deviation, RSDR, %	32,7	37,6	33,3	34,9	29,5
Reproducibility limit R [R = 2,8 × sR], µg/kg	1,3	1,6	1,9	36,3	11,9
HorRat value	1,5	1,7	1,5	1,6	1,3

PCB-153 - Results summary of the collaborative trial.

Material name	**Chicken feed**	**Pig feed**	**Vegetable oil**	**Fish oil**	**Fish meal**
Analyte	**PCB 153**	**PCB 153**	**PCB 153**	**PCB 153**	**PCB 153**
Year of inter-laboratory study	**2016**	**2016**	**2016**	**2016**	**2016**
Number of laboratories	9	8	9	9	9
Number of samples	2	2	2	2	2
Number of laboratories considered as non-compliant	0	0	0	0	0
Number of outlying laboratories	0	0	0	0	0
Number of accepted results	18	16	18	18	18
Mean value, x, µg/kg	1,4	1,5	2,1	38,9	14,3
Repeatability standard deviation sr, µg/kg	0,1	0,2	0,3	6,3	1,4
Repeatability relative standard deviation, RSDr, %	8,9	14,2	13,4	16,3	9,9
Repeatability limit r [r = 2,8 × sr], µg/kg	0,3	0,6	0,8	17,7	4,0
Reproducibility standard deviation sR, µg/kg	0,5	0,6	0,6	15,0	4,3
Reproducibility relative standard deviation, RSDR, %	36,4	38,5	28,0	38,5	30,1
Reproducibility limit R [R = 2,8 × sR], µg/kg	1,4	1,6	1,6	42,0	12,1
HorRat value	1,6	1,7	1,3	1,7	1,4

PCB-180 - Results summary of the collaborative trial.

Material name	Chicken feed	Pig feed	Vegetable oil	Fish oil	Fish meal
Analyte	**PCB 180**	**PCB 180**	**PCB 180**	**PCB 180**	**PCB 180**
Year of inter-laboratory study	**2016**	**2016**	**2016**	**2016**	**2016**
Number of laboratories	9	9	9	8	9
Number of samples	2	2	2	2	2
Number of laboratories considered as non-compliant	0	0	0	0	0
Number of outlying laboratories	0	0	0	0	0
Number of accepted results	18	18	18	16	18
Mean value, x, µg/kg	0,7	1,5	1,5	17,6	6,6
Repeatability standard deviation sr, µg/kg	0,0	0,2	0,5	2,9	0,8
Repeatability relative standard deviation, RSDr, %	7,5	13,3	32,8	16,7	11,7
Repeatability limit r [r = 2,8 × sr], µg/kg	0,1	0,6	1,3	8,2	2,2
Reproducibility standard deviation sR, µg/kg	0,2	0,5	1,0	4,1	1,9
Reproducibility relative standard deviation, RSDR, %	28,5	35,8	66,2	23,4	28,3
Reproducibility limit R [R = 2,8 × sR], µg/kg	0,5	1,5	2,7	11,5	5,2
HorRat value	1,3	1,6	3,0	1,1	1,3

3.2 OCPs

Visual inspection of the data of the OCPs showed a different picture. As the samples were spiked during the sample preparation phase (Paragraph 2.1), we can compare the submitted results with the spiking levels (Annex 1), particularly for the feed and vegetable oil materials where the samples were spiked on blank material. For fish oil and meal, we need to take into account that residues of contaminants may already have been present in the material prior to spiking. When comparing the laboratory results with the spiked levels, we investigated if laboratory results lie within a 30-300% range of the spiked values (an upper limit of 400% was used for fish matrices as they may have contained background levels of the analytes). We have assessed all matrix-analyte combinations, submitted by all laboratories, and summarised these per dataset (e.g. PT 703) in Table 2. If a value is <30% of the spiked value, severe losses have occurred during analysis, whereas extremely high levels (>300% resp. 400%) should be questioned as well for the reason that the analytes cannot be generated during the analysis. Overestimating results may indicate calculation errors or losses of the internal standards, resulting in overestimations of the results or poor quality of calibrants. The laboratories were contacted to ask if irregularities occurred during their analysis, but they reported back that no irregularities occurred. It was decided to discard these extreme values from the dataset.

The results in Table 2 show that for PT703, 704, 749 and 750 over 95% and for PT748 over 90% of their submitted OCPs results lie in the indicated ranges, showing that very limited severe under- or overestimation occurred. PT706, 742 and 744 suffered from severe overestimation (36-39% of the matrix-analyte combinations). PT705 and 755 suffered primarily from severe underestimation (25 and 27% resp.) but also reported a number of results that were severely overestimated (7 and 14% resp.). Looking closer at the data (Annex 10), it is clear that overestimation occurred for aldrin (PT705, 706, 742 and 744), and to a lesser degree deviations occurred for the other drin compounds as well. The laboratories have not provided reasons for these deviating results. As a result of this, for aldrin a bimodal dataset is obtained, but after discarding the extreme data, the bimodality is resolved and the retained datasets are close to the spiked levels. For oxychlordane only 1-2 laboratories provided results close to the spiked level for all matrices, therefore insufficient data remains for statistical analysis and oxychlordane cannot be included in the standard. Two datasets (PT705 and PT755), both for GC-MS and GC-MS/MS, were deviating to a large degree, being often much higher than the other laboratories (see Annex 10) and much higher than the spiked levels (see Annex 1). These datasets originate from the same laboratory, that submitted two datasets on two different methods (GC-MS and GC-MS/MS). They reported up to 15-fold overestimation of the spiked levels (e.g. oxychlordane in vegetable oil). In addition, lab PT705 suffered from consistently outlying Mandel k values, with 44 of the 77 submitted results being above the 5% chance level (Annex 8), indicating a very poor repeatability. This can also be seen from the submitted results (Annex 10), e.g. for heptachlor, p,p'-DDE, o,p'-DDT, most of the chlordane's and dieldrin and endrin observations. The laboratory was asked for

clarifications, but they indicated that they found no ground to doubt their results. In additional cases, Mandel k plots indicate poor precision for e.g. o,p'-DDT for PT703, 742 and 749 and HCB for PT703.

Table 2 *Proportion of the reported data that lies within a specified range of the contaminant levels that were spiked to the samples, for all matrix-analyte combinations summarised.*

	Matrix →	**Chicken feed, pig feed, vegetable oil**		**Fish oil, fish meal**	
Dataset	**within ranges**	**<30% of spiked value**	**>300% of spiked value**	**<30% of spiked value**	**>400% of spiked value**
PT703	96%	0%	0%	0%	4%
PT704	96%	3%	0%	0%	1%
PT705	68%	15%	4%	10%	3%
PT706	64%	0%	16%	0%	20%
PT742	60%	1%	19%	0%	20%
PT744	62%	2%	14%	0%	23%
PT748	92%	5%	1%	1%	2%
PT749	96%	0%	3%	0%	1%
PT750	98%	0%	0%	0%	2%
PT755	59%	19%	8%	8%	6%

The drin analytes (excl. endrin keton), the standard proved to be suitable for all 15 matrix-analyte combinations with Horrat values <2.5 (based on generally 10-16 accepted results). For endrin keton, a newly added analyte in this revised standard, too limited data was submitted, and only for feeds and vegetable oil a Horrat could be calculated which were all >2.5. Therefore, it is not possible to evaluate if the standard is suitable for this analyte. The grand mean values in particularly the feeds and vegetable oils are close to the theoretical (spiked) values. For the fish meal and fish oil some overestimation occurs compared to the spiked levels (Annex 1), most likely due to the presence of these contaminants in the material prior to spiking.

Concerning cis- and trans-chlordane, several Horrat values were <2.5, mostly based on 12-16 accepted results, except for the feeds and fish oil (cis-chlordane). The quantification of oxychlordane in chicken feed resulted in data that heavily overestimated the spiked value (Annex 1) and were rejected for that reason. Only 4 results remained, which is insufficient for validation. For chicken feed no Horrat value could be calculated. The grand mean values in particularly the chicken feed and oils were close to the theoretical (spiked) values (Annex 1).

The collaborative trial results show that 16 out of 20 analyte-matrix combinations for the DDTs resulted in acceptable HorRat values ≤2.5. The quantification of p,p'-DDT in pig feed resulted in a HorRat-value of 2.8 and 4.4 for the vegetable oil. Nevertheless, the mean values agreed reasonably well with the spiked levels. For o,p'-DDT and p,p'-DDE, all Horrat values met the criterium. The quantification of p,p'-TDE in the pig feed and fish meal resulted in HorRat-values >2.5. DDTs are susceptible to degradation in the analysis, particularly in GC-liners (van Leeuwen and de Boer, 2008). On the other hand, the grand mean values in particularly the feeds and vegetable oils are close to or above the theoretical (spiked) values, which makes it difficult to pinpoint the exact reason for the elevated HorRat values. For the fish meal and fish oil some overestimation occurs compared to the spiked levels (Annex 1), most likely due to the presence of these contaminants in the material prior to spiking.

The standard shows to be capable of the analysis of α- and b-endosulfan and endosulfan sulphate in most matrices (13 out of 15 analyte-matrix combinations) with Horrat values ≤2.5, based on 8-16 accepted results). The quantification of endosulfan sulfate in pig feed and vegetable oil resulted in HorRat-values >2.5. The grand mean values for all samples except fish meal agreed well with the spiked level. For the fish oil some overestimation occurs compared to the spiked levels (Annex 1), most likely due to the presence of these contaminants in the material prior to spiking.

The analysis of heptachlor and heptachlor epoxide by the standard showed good HorRat values in all 10 cases (based on 12-18 accepted results). Photo-heptachlor was added as a new OCP to the revised version of this standard. There is not much experience (internationally) with the analysis of this analyte. Although in the study the support was offered for obtaining an analytical standard (see instruction letter, Annexes 4 and 5), only 4 laboratories submitted results. While for all matrices acceptable HorRat values were obtained, except for fish oil (3.6) the number of laboratories with acceptable results is lower than 5. Therefore, the standard is not validated for this compound. The grand mean values for particularly the pig feed, fish meal and vegetable oil are close to the theoretical (spiked) values. For the fish oil overestimation occurs compared to the spiked levels (Annex 1), most likely due to the presence of these contaminants in the material prior to spiking.

The analysis of HCB and HCHs by the standard showed good HorRat values (≤2.5) in 18 out of 20 analyte-matrix combinations, based on ≥10 accepted results in each case. Particularly the fish oil sample turned out to be challenging for the participants, possibly due to the high levels (>200 ng/g). For HCB and gamma-HCH the Horrat values were >2.5. The grand mean values for particularly the feeds and vegetable oil are close to the theoretical (spiked) values. For the fish meal and fish oil some overestimation occurs compared to the spiked levels (Annex 1), most likely due to the presence of these contaminants in the material prior to spiking.

The cis- and trans-nonachlor results show the standard could not be evaluated for these compounds because of an insufficient number of laboratories producing acceptable results.

Below the results of the collaborative study are presented by each individual compound.

Aldrin - Results summary of the collaborative trial.

Material name	**Chicken feed**	**Pig feed**	**Veg oil**	**Fish oil**	**Fish meal**
Analyte	**Aldrin**	**Aldrin**	**Aldrin**	**Aldrin**	**Aldrin**
Year of inter-laboratory study	**2016**	**2016**	**2016**	**2016**	**2016**
Number of laboratories	10	10	10	10	10
Number of samples	2	2	2	2	2
Number of laboratories considered as non-compliant	4	3	4	4	3
Number of outlying laboratories	0	0	0	0	1
Number of accepted results	12	14	12	12	12
Mean value, x, µg/kg	9,6	10,6	31,9	34,4	27,8
Repeatability standard deviation sr, µg/kg	1,8	1,8	4,5	4,5	1,7
Repeatability relative standard deviation, RSDr, %	19,1	16,6	14,1	13,2	6,2
Repeatability limit r [r = 2,8 × sr], µg/kg	5,1	4,9	12,6	12,7	4,9
Reproducibility standard deviation sR, µg/kg	3,3	3,1	10,4	11,3	8,7
Reproducibility relative standard deviation, RSDR, %	34,7	29,0	32,5	32,8	31,2
Reproducibility limit R [R = 2,8 × sR], µg/kg	9,3	8,6	29,1	31,6	24,3
HorRat value	1,6	1,3	1,5	1,5	1,4

Dieldrin - Results summary of the collaborative trial.

Material name	Chicken feed	Pig feed	Veg oil	Fish oil	Fish meal
Analyte	**Dieldrin**	**Dieldrin**	**Dieldrin**	**Dieldrin**	**Dieldrin**
Year of inter-laboratory study	**2016**	**2016**	**2016**	**2016**	**2016**
Number of laboratories	8	8	10	10	10
Number of samples	2	2	2	2	2
Number of laboratories considered as non-compliant	1	1	3	2	2
Number of outlying laboratories	1	1	0	1	1
Number of accepted results	10	11	14	14	14
Mean value, x, µg/kg	13,2	12,4	70,4	97,6	28,6
Repeatability standard deviation sr, µg/kg	1,6	2,1	4,1	12,1	1,2
Repeatability relative standard deviation, RSDr, %	12,2	16,7	5,8	12,4	4,3
Repeatability limit r [r = 2,8 × sr], µg/kg	4,5	5,8	11,5	33,8	3,4
Reproducibility standard deviation sR, µg/kg	6,3	6,1	32,7	30,4	12,4
Reproducibility relative standard deviation, RSDR, %	55,7	53,7	46,5	31,1	43,2
Reproducibility limit R [R = 2,8 × sR], µg/kg	17,7	17,1	91,6	85,0	34,6
HorRat value	2,5	2,4	2,1	1,4	2,0

Endrin - Results summary of the collaborative trial.

Material name	Chicken feed	Pig feed	Veg oil	Fish oil	Fish meal
Analyte	**Endrin**	**Endrin**	**Endrin**	**Endrin**	**Endrin**
Year of inter-laboratory study	**2016**	**2016**	**2016**	**2016**	**2016**
Number of laboratories	7	7	9	9	9
Number of samples	2	2	2	2	2
Number of laboratories considered as non-compliant	0	1	1	1	1
Number of outlying laboratories	0	1	1	0	1
Number of accepted results	14	10	14	16	13
Mean value, x, µg/kg	14,9	13,1	63,6	88,1	28,6
Repeatability standard deviation sr, µg/kg	2,1	1,1	3,2	10,8	3,5
Repeatability relative standard deviation, RSDr, %	14,3	8,4	5,0	12,2	12,2
Repeatability limit r [r = 2,8 × sr], µg/kg	6,0	3,1	8,9	30,2	9,7
Reproducibility standard deviation sR, µg/kg	6,1	1,8	29,3	31,6	13,8
Reproducibility relative standard deviation, RSDR, %	40,5	13,9	46,1	35,8	49,0
Reproducibility limit R [R = 2,8 × sR], µg/kg	16,9	5,1	82,2	88,4	38,7
HorRat value	1,8	0,6	2,1	1,6	2,2

Endrin ketone - Results summary of the collaborative trial.

Material name	Chicken feed	Pig feed	Veg oil	Fish oil	Fish meal
Analyte	**Endrin ketone**	**Endrin ketone**	**Endrin ketone**	**Endrin ketone**	**Endrin ketone**
Year of inter-laboratory study	**2016**	**2016**	**2016**	**2016**	**2016**
Number of laboratories	5	4	5	5	5
Number of samples	2	2	2	2	2
Number of laboratories considered as non-compliant	3	2	3	4	4
Number of outlying laboratories	0	2	0	0	0
Number of accepted results	4	0	4	2	2
Mean value, *x*, µg/kg	14,9	16,0	97,5	15,1	3,9
Repeatability standard deviation s_r, µg/kg	1,2	0,3	162,0	3,0	0,8
Repeatability relative standard deviation, RSD_r, %	8,2	1,8	166,2	19,7	21,8
Repeatability limit *r* [$r = 2,8 \times s_r$], µg/kg	3,4	0,8	453,6	8,3	2,4
Reproducibility standard deviation s_R, µg/kg	17,1	15,3	162,0	[a]	[a]
Reproducibility relative standard deviation, RSD_R, %	115,0	76,4	968,7	[a]	[a]
Reproducibility limit *R* [$R = 2,8 \times s_R$], µg/kg	48,0	42,8	453,6	[a]	[a]
HorRat value	5,2	3,5	44	[a]	[a]

[a] No evaluation possible.

cis-chlordane - Results summary of the collaborative trial.

Material name	**Chicken feed**	**Pig feed**	**Veg oil**	**Fish oil**	**Fish meal**
Analyte	**cis chlordane**	**cis chlordane**	**cis chlordane**	**cis chlordane**	**cis chlordane**
Year of inter-laboratory study	**2016**	**2016**	**2016**	**2016**	**2016**
Number of laboratories	8	8	10	10	10
Number of samples	2	2	2	2	2
Number of laboratories considered as non-compliant	0	1	3	4	3
Number of outlying laboratories	1	1	1	0	1
Number of accepted results	14	12	12	12	12
Mean value, x, µg/kg	8,9	7,3	23,9	23,8	16,1
Repeatability standard deviation sr, µg/kg	0,3	0,4	1,4	20,6	0,9
Repeatability relative standard deviation, RSDr, %	2,9	5,2	5,7	86,5	5,7
Repeatability limit r [r = 2,8 × sr], µg/kg	0,7	1,1	3,8	57,6	2,6
Reproducibility standard deviation sR, µg/kg	5,0	5,0	12,8	20,6	5,2
Reproducibility relative standard deviation, RSDR, %	56,7	68,3	53,5	128,2	32,1
Reproducibility limit R [R = 2,8 × sR], µg/kg	14,1	14,0	35,8	57,6	14,4
HorRat value	2,6	3,1	2,4	5,8	1,5

trans-chlordane - Results summary of the collaborative trial.

Material name	**Chicken feed**	**Pig feed**	**Veg oil**	**Fish oil**	**Fish meal**
Analyte	**trans-chlordane**	**trans-chlordane**	**trans-chlordane**	**trans-chlordane**	**trans-chlordane**
Year of inter-laboratory study	**2016**	**2016**	**2016**	**2016**	**2016**
Number of laboratories	8	8	8	8	10
Number of samples	2	2	2	2	2
Number of laboratories considered as non-compliant	0	1	1	1	2
Number of outlying laboratories	0	0	0	0	1
Number of accepted results	16	14	14	14	14
Mean value, x, µg/kg	7,7	7,3	22,7	25,4	18,9
Repeatability standard deviation sr, µg/kg	0,6	0,6	4,8	4,4	1,3
Repeatability relative standard deviation, RSDr, %	7,9	8,6	21,3	17,3	7,0
Repeatability limit r [r = 2,8 × sr], µg/kg	1,7	1,8	13,5	12,3	3,7
Reproducibility standard deviation sR, µg/kg	3,5	3,2	8,3	6,5	8,6
Reproducibility relative standard deviation, RSDR, %	45,9	44,1	36,6	25,4	45,6
Reproducibility limit R [R = 2,8 × sR], µg/kg	9,9	9,0	23,2	18,1	24,1
HorRat value	2,1	2,0	1,7	1,1	2,1

Oxychlordane – Results summary of the collaborative trial.

Material name	**Chicken feed**	**Pig feed**	**Veg oil**	**Fish oil**	**Fish meal**
Analyte	**Oxychlor-dane**	**Oxychlor-dane**	**Oxychlor-dane**	**Oxychlor-dane**	**Oxychlor-dane**
Year of inter-laboratory study	**2016**	**2016**	**2016**	**2016**	**2016**
Number of laboratories	6	6	7	7	7
Number of samples	2	2	2	2	2
Number of laboratories considered as non-compliant	5	4	5	5	5
Number of outlying laboratories	0	0	0	0	0
Number of accepted results	2	4	4	4	4
Mean value, *x*, µg/kg	5,9	10,7	11,6	12,8	10,2
Repeatability standard deviation s_r, µg/kg	0,0	1,6	0,8	0,6	1,0
Repeatability relative standard deviation, RSD_r, %	0,0	14,7	6,7	4,9	10,1
Repeatability limit *r* [$r = 2,8 \times s_r$], µg/kg	0,0	4,4	2,2	1,8	2,9
Reproducibility standard deviation s_R, µg/kg	[a]	5,9	2,9	4,9	5,9
Reproducibility relative standard deviation, RSD_R, %	[a]	54,9	25,2	38,3	57,2
Reproducibility limit *R* [$R = 2,8 \times s_R$], µg/kg	[a]	16,5	8,2	13,8	16,4
HorRat value	[a]	2,5	1,1	1,7	2,6

[a] No evaluation possible.

p,p'-DDT - Results summary of the collaborative trial.

Material name	Chicken feed	Pig feed	Veg oil	Fish oil	Fish meal
Analyte	**p,p'-DDT**	**p,p'-DDT**	**p,p'-DDT**	**p,p'-DDT**	**p,p'-DDT**
Year of inter-laboratory study	**2016**	**2016**	**2016**	**2016**	**2016**
Number of laboratories	10	10	11	10	10
Number of samples	2	2	2	2	2
Number of laboratories considered as non-compliant	3	2	3	2	3
Number of outlying laboratories	1	1	1	0	0
Number of accepted results	12	13	14	16	14
Mean value, x, µg/kg	19,2	19,3	174,8	199,6	44,6
Repeatability standard deviation sr, µg/kg	1,5	2,1	144,3	47,4	7,7
Repeatability relative standard deviation, RSDr, %	7,8	10,8	82,5	23,7	17,2
Repeatability limit r [r = 2,8 × sr], µg/kg	4,2	5,8	403,9	132,6	21,5
Reproducibility standard deviation sR, µg/kg	10,5	12,8	144,3	73,7	15,8
Reproducibility relative standard deviation, RSDR, %	54,5	60,7	93,5	36,9	35,5
Reproducibility limit R [R = 2,8 × sR], µg/kg	29,3	35,9	403,9	206,3	44,4
HorRat value	2,5	2,8	4,4	1,8	1,6

o,p'-DDT - Results summary of the collaborative trial.

Material name	Chicken feed	Pig feed	Veg oil	Fish oil	Fish meal
Analyte	**o,p'-DDT**	**o,p'-DDT**	**o,p'-DDT**	**o,p'-DDT**	**o,p'-DDT**
Year of inter-laboratory study	**2016**	**2016**	**2016**	**2016**	**2016**
Number of laboratories	7	7	9	9	6
Number of samples	2	2	2	2	2
Number of laboratories considered as non-compliant	1	1	3	3	3
Number of outlying laboratories	0	0	1	0	0
Number of accepted results	12	11	10	12	11
Mean value, x, µg/kg	9,1	8,3	67,9	87,2	21,1
Repeatability standard deviation sr, µg/kg	0,5	0,5	1,5	19,3	4,0
Repeatability relative standard deviation, RSDr, %	5,3	6,6	2,1	22,1	18,8
Repeatability limit r [r = 2,8 × sr], µg/kg	1,4	1,5	4,1	53,9	11,2
Reproducibility standard deviation sR, µg/kg	3,1	3,6	14,1	35,6	11,0
Reproducibility relative standard deviation, RSDR, %	33,8	44,9	20,7	40,9	54,4
Reproducibility limit R [R = 2,8 × sR], µg/kg	8,6	10,0	39,4	99,7	30,9
HorRat value	1,5	2,0	0,9	1,9	2,5

p,p'-TDE - Results summary of the collaborative trial.

Material name	Chicken feed	Pig feed	Veg oil	Fish oil	Fish meal
Analyte	**p,p'-TDE**	**p,p'-TDE**	**p,p'-TDE**	**p,p'-TDE**	**p,p'-TDE**
Year of inter-laboratory study	**2016**	**2016**	**2016**	**2016**	**2016**
Number of laboratories	6	6	6	8	8
Number of samples	2	2	2	2	2
Number of laboratories considered as non-compliant	0	2	2	2	3
Number of outlying laboratories	0	1	0	0	0
Number of accepted results	12	10	12	12	10
Mean value, x, µg/kg	8,8	7,5	96,4	103,5	16,9
Repeatability standard deviation sr, µg/kg	0,4	0,4	5,1	13,4	2,2
Repeatability relative standard deviation, RSDr, %	4,3	5,1	5,3	13,0	12,8
Repeatability limit r [r = 2,8 × sr], µg/kg	1,1	1,1	14,4	37,6	6,0
Reproducibility standard deviation sR, µg/kg	4,6	5,0	49,3	55,1	9,7
Reproducibility relative standard deviation, RSDR, %	53,1	66,3	51,2	53,3	57,4
Reproducibility limit R [R = 2,8 × sR], µg/kg	13,0	13,9	138,1	154,3	27,2
HorRat value	2,4	3,0	2,3	2,4	2,6

p,p-DDE - Results summary of the collaborative trial.

Material name	Chicken feed	Pig feed	Veg oil	Fish oil	Fish meal
Analyte	**p,p'-DDE**	**p,p'-DDE**	**p,p'-DDE**	**p,p'-DDE**	**p,p'-DDE**
Year of inter-laboratory study	**2016**	**2016**	**2016**	**2016**	**2016**
Number of laboratories	8	8	10	10	10
Number of samples	2	2	2	2	2
Number of laboratories considered as non-compliant	0	0	0	0	0
Number of outlying laboratories	1	1	0	1	1
Number of accepted results	12	14	20	18	18
Mean value, x, µg/kg	21,7	21,4	226,3	263,6	49,4
Repeatability standard deviation sr, µg/kg	0,9	1,7	56,6	38,8	6,4
Repeatability relative standard deviation, RSDr, %	4,2	8,1	25,0	14,7	13,0
Repeatability limit r [r = 2,8 × sr], µg/kg	2,5	4,8	158,4	108,5	18,0
Reproducibility standard deviation sR, µg/kg	4,2	6,5	58,9	38,8	10,9
Reproducibility relative standard deviation, RSDR, %	18,9	30,4	26,1	14,7	22,0
Reproducibility limit R [R = 2,8 × sR], µg/kg	11,7	18,2	165,1	108,5	30,4
HorRat value	0,9	1,4	1,3	0,7	1,0

α-endosulfan - Results summary of the collaborative trial.

Material name	Chicken feed	Pig feed	Veg oil	Fish oil	Fish meal
Analyte	**α-endosulfan**	**α-endosulfan**	**α-endosulfan**	**α-endosulfan**	**α-endosulfan**
Year of inter-laboratory study	**2016**	**2016**	**2016**	**2016**	**2016**
Number of laboratories	8	8	10	9	10
Number of samples	2	2	2	2	2
Number of laboratories considered as non-compliant	1	2	1	3	1
Number of outlying laboratories	0	0	1	1	1
Number of accepted results	14	12	16	10	16
Mean value, x, µg/kg	15,7	15,1	165,4	24,4	35,5
Repeatability standard deviation sr, µg/kg	1,6	1,5	45,4	2,3	3,3
Repeatability relative standard deviation, RSDr, %	10,3	9,8	27,5	9,4	9,4
Repeatability limit r [r = 2,8 × sr], µg/kg	4,5	4,1	127,1	6,4	9,3
Reproducibility standard deviation sR, µg/kg	5,4	4,8	71,6	12,8	11,2
Reproducibility relative standard deviation, RSDR, %	34,0	31,8	46,4	52,4	31,5
Reproducibility limit R [R = 2,8 × sR], µg/kg	15,0	13,5	200,5	35,9	31,3
HorRat value	1,5	1,4	2,2	2,4	1,4

β-endosulfan - Results summary of the collaborative trial.

Material name	Chicken feed	Pig feed	Veg oil	Fish oil	Fish meal
Analyte	**β-endosulfan**	**β-endosulfan**	**β-endosulfan**	**β-endosulfan**	**β-endosulfan**
Year of inter-laboratory study	**2016**	**2016**	**2016**	**2016**	**2016**
Number of laboratories	5	6	10	7	10
Number of samples	2	2	2	2	2
Number of laboratories considered as non-compliant	0	1	2	2	2
Number of outlying laboratories	0	0	1	0	0
Number of accepted results	10	10	14	10	16
Mean value, x, µg/kg	27,5	26,4	331,2	33,3	70,1
Repeatability standard deviation sr, µg/kg	3,3	3,1	8,4	6,2	7,5
Repeatability relative standard deviation, RSDr, %	11,8	11,8	2,5	18,8	10,7
Repeatability limit r [r = 2,8 × sr], µg/kg	9,1	8,7	23,5	17,5	21,1
Reproducibility standard deviation sR, µg/kg	7,5	7,0	72,9	10,8	17,7
Reproducibility relative standard deviation, RSDR, %	27,4	26,7	22,0	32,4	25,2
Reproducibility limit R [R = 2,8 × sR], µg/kg	21,1	19,7	204,2	30,2	49,5
HorRat value	1,2	1,2	1,2	1,5	1,1

Endosulfan sulphate - Results summary of the collaborative trial.

Material name	**Chicken feed**	**Pig feed**	**Veg oil**	**Fish oil**	**Fish meal**
Analyte	**Endosulfan-sulphate**	**Endosulfan-sulphate**	**Endosulfan-sulphate**	**Endosulfan-sulphate**	**Endosulfan-sulphate**
Year of inter-laboratory study	**2016**	**2016**	**2016**	**2016**	**2016**
Number of laboratories	10	9	9	8	10
Number of samples	2	2	2	2	2
Number of laboratories considered as non-compliant	3	3	1	4	4
Number of outlying laboratories	1	1	0	0	0
Number of accepted results	12	10	16	8	12
Mean value, x, µg/kg	38,5	36,9	372,0	29,3	61,1
Repeatability standard deviation s_r, µg/kg	4,9	3,1	24,4	5,6	9,4
Repeatability relative standard deviation, RSD_r, %	12,8	8,4	6,6	19,0	15,5
Repeatability limit r [$r = 2,8 \times s_r$], µg/kg	13,7	8,7	68,4	15,6	26,4
Reproducibility standard deviation s_R, µg/kg	21,5	26,7	249,2	9,0	34,3
Reproducibility relative standard deviation, RSD_R, %	56,0	59,7	67,0	30,7	56,1
Reproducibility limit R [$R = 2,8 \times s_R$], µg/kg	60,3	74,9	697,8	25,1	96,0
HorRat value	2,5	2,7	3,6	1,4	2,5

Heptachlor - Results summary of the collaborative trial.

Material name	**Chicken feed**	**Pig feed**	**Veg oil**	**Fish oil**	**Fish meal**
Analyte	**Heptachlor**	**Heptachlor**	**Heptachlor**	**Heptachlor**	**Heptachlor**
Year of inter-laboratory study	**2016**	**2016**	**2016**	**2016**	**2016**
Number of laboratories	10	10	10	10	10
Number of samples	2	2	2	2	2
Number of laboratories considered as non-compliant	3	2	3	3	1
Number of outlying laboratories	1	1	1	0	0
Number of accepted results	12	14	12	13	18
Mean value, x, µg/kg	15,1	15,3	279,9	365,4	31,4
Repeatability standard deviation sr, µg/kg	2,1	1,0	14,8	46,5	3,8
Repeatability relative standard deviation, RSDr, %	13,8	6,7	5,3	12,7	12,1
Repeatability limit r [r = 2,8 × sr], µg/kg	5,8	2,9	41,5	130,1	10,6
Reproducibility standard deviation sR, µg/kg	7,7	6,8	126,7	135,0	14,3
Reproducibility relative standard deviation, RSDR, %	51,4	44,8	45,3	39,1	45,6
Reproducibility limit R [R = 2,8 × sR], µg/kg	21,7	19,2	354,9	378,1	40,1
HorRat value	2,3	2,0	2,3	2,1	2,1

Heptachlor epoxide - Results summary of the collaborative trial.

Material name	**Chicken feed**	**Pig feed**	**Veg oil**	**Fish oil**	**Fish meal**
Analyte	**Heptachlor-epoxide**	**Heptachlor-epoxide**	**Heptachlor-epoxide**	**Heptachlor-epoxide**	**Heptachlor-epoxide**
Year of inter-laboratory study	**2016**	**2016**	**2016**	**2016**	**2016**
Number of laboratories	10	10	10	10	10
Number of samples	2	2	2	2	2
Number of laboratories considered as non-compliant	1	1	1	0	1
Number of outlying laboratories	0	1	0	1	1
Number of accepted results	18	16	18	17	15
Mean value, x, µg/kg	15,5	16,5	335,1	382,4	36,2
Repeatability standard deviation sr, µg/kg	1,4	2,1	47,0	56,1	2,9
Repeatability relative standard deviation, RSDr, %	9,0	12,8	14,0	14,7	8,1
Repeatability limit r [r = 2,8 × sr], µg/kg	3,9	5,9	131,7	157,1	8,2
Reproducibility standard deviation sR, µg/kg	4,3	4,4	67,1	82,0	13,5
Reproducibility relative standard deviation, RSDR, %	27,4	26,4	20,0	21,2	36,3
Reproducibility limit R [R = 2,8 × sR], µg/kg	11,9	12,2	187,9	229,5	37,8
HorRat value	1,2	1,2	1,1	1,1	1,6

Photo heptachlor - Results summary of the collaborative trial.

Material name	**Chicken feed**	**Pig feed**	**Veg oil**	**Fish oil**	**Fish meal**
Analyte	**Photo heptachlor**	**Photo heptachlor**	**Photo heptachlor**	**Photo heptachlor**	**Photo heptachlor**
Year of inter-laboratory study	**2016**	**2016**	**2016**	**2016**	**2016**
Number of laboratories	4	4	4	4	4
Number of samples	2	2	2	2	2
Number of laboratories considered as non-compliant	1	1	2	1	1
Number of outlying laboratories	0	0	0	0	0
Number of accepted results	6	6	4	6	6
Mean value, x, µg/kg	16,2	11,0	253,8	405,0	9,4
Repeatability standard deviation s_r, µg/kg	2,7	1,8	30,9	138,8	4,1
Repeatability relative standard deviation, RSD_r, %	16,8	16,4	12,2	34,3	43,6
Repeatability limit r [$r = 2,8 \times s_r$], µg/kg	7,6	5,0	86,4	388,6	11,5
Reproducibility standard deviation s_R, µg/kg	8,3	4,9	30,9	269,3	4,1
Reproducibility relative standard deviation, RSD_R, %	51,1	44,9	12,2	66,5	43,6
Reproducibility limit R [$R = 2,8 \times s_R$], µg/kg	23,1	13,8	86,4	754,0	11,5
HorRat value	2,3	2,0	0,6	3,6	2,0

HCB - Results summary of the collaborative trial.

Material name	**Chicken feed**	**Pig feed**	**Veg oil**	**Fish oil**	**Fish meal**
Analyte	**HCB**	**HCB**	**HCB**	**HCB**	**HCB**
Year of inter-laboratory study	**2016**	**2016**	**2016**	**2016**	**2016**
Number of laboratories	9	9	10	10	9
Number of samples	2	2	2	2	2
Number of laboratories considered as non-compliant	1	0	1	1	0
Number of outlying laboratories	0	1	1	1	1
Number of accepted results	16	16	16	15	18
Mean value, x, µg/kg	10,5	8,5	169,7	251,6	19,0
Repeatability standard deviation sr, µg/kg	2,6	1,4	39,8	11,7	3,9
Repeatability relative standard deviation, RSDr, %	24,6	16,1	23,5	4,7	20,7
Repeatability limit r [r = 2,8 × sr], µg/kg	7,2	3,8	111,6	32,8	11,0
Reproducibility standard deviation sR, µg/kg	4,5	2,1	82,8	144,0	6,9
Reproducibility relative standard deviation, RSDR, %	42,9	24,8	48,8	57,7	36,2
Reproducibility limit R [R = 2,8 × sR], µg/kg	12,6	5,9	231,8	403,1	19,3
HorRat value	1,9	1,1	2,3	2,9	1,6

a-HCH - Results summary of the collaborative trial.

Material name	**Chicken feed**	**Pig feed**	**Veg oil**	**Fish oil**	**Fish meal**
Analyte	**α-HCH**	**α-HCH**	**α-HCH**	**α-HCH**	**α-HCH**
Year of inter-laboratory study	**2016**	**2016**	**2016**	**2016**	**2016**
Number of laboratories	10	11	10	10	7
Number of samples	2	2	2	2	2
Number of laboratories considered as non-compliant	3	2	3	2	2
Number of outlying laboratories	0	1	1	0	1
Number of accepted results	14	16	12	15	14
Mean value, x, µg/kg	20,9	21,8	164,3	246,8	49,0
Repeatability standard deviation sr, µg/kg	1,8	7,3	7,2	32,6	4,1
Repeatability relative standard deviation, RSDr, %	8,6	33,3	4,4	13,2	8,3
Repeatability limit r [r = 2,8 × sr], µg/kg	5,0	20,3	20,1	91,2	11,3
Reproducibility standard deviation sR, µg/kg	11,5	11,6	53,4	113,9	23,5
Reproducibility relative standard deviation, RSDR, %	55,2	55,1	32,5	47,3	47,9
Reproducibility limit R [R = 2,8 × sR], µg/kg	32,3	32,4	149,5	318,9	65,7
HorRat value	2,5	2,5	1,5	2,4	2,2

β-HCH - Results summary of the collaborative trial.

Material name	**Chicken feed**	**Pig feed**	**Veg oil**	**Fish oil**	**Fish meal**
Analyte	**β-HCH**	**β-HCH**	**β-HCH**	**β-HCH**	**β-HCH**
Year of inter-laboratory study	**2016**	**2016**	**2016**	**2016**	**2016**
Number of laboratories	8	10	10	10	10
Number of samples	2	2	2	2	2
Number of laboratories considered as non-compliant	4	5	4	3	4
Number of outlying laboratories	0	0	0	1	1
Number of accepted results	11	10	10	11	10
Mean value, x, µg/kg	6,5	7,9	77,5	84,3	18,3
Repeatability standard deviation sr, µg/kg	0,8	1,7	2,6	13,9	2,9
Repeatability relative standard deviation, RSDr, %	12,6	21,4	3,4	16,5	15,7
Repeatability limit r [r = 2,8 × sr], µg/kg	2,3	4,7	7,3	38,9	8,1
Reproducibility standard deviation sR, µg/kg	2,8	2,9	6,8	15,2	4,2
Reproducibility relative standard deviation, RSDR, %	47,5	36,9	8,8	18,0	22,9
Reproducibility limit R [R = 2,8 × sR], µg/kg	7,8	8,1	19,1	42,5	11,7
HorRat value	2,2	1,7	0,4	0,8	1,0

Gamma-HCH - Results summary of the collaborative trial.

Material name	**Chicken feed**	**Pig feed**	**Veg oil**	**Fish oil**	**Fish meal**
Analyte	**γ-HCH**	**γ-HCH**	**γ-HCH**	**γ-HCH**	**γ-HCH**
Year of inter-laboratory study	**2016**	**2016**	**2016**	**2016**	**2016**
Number of laboratories	8	10	10	8	10
Number of samples	2	2	2	2	2
Number of laboratories considered as non-compliant	4	4	5	3	4
Number of outlying laboratories	1	0	0	0	1
Number of accepted results	10	12	10	10	10
Mean value, x, µg/kg	16,8	24,1	186,3	310,5	45,5
Repeatability standard deviation sr, µg/kg	1,6	2,8	21,2	30,3	4,6
Repeatability relative standard deviation, RSDr, %	9,2	11,4	11,4	9,7	10,0
Repeatability limit r [r = 2,8 × sr], µg/kg	4,3	7,7	59,3	84,7	12,8
Reproducibility standard deviation sR, µg/kg	4,6	12,6	31,1	194,5	13,4
Reproducibility relative standard deviation, RSDR, %	27,3	52,1	16,7	62,6	29,5
Reproducibility limit R [R = 2,8 × sR], µg/kg	12,8	35,2	87,1	544,5	37,5
HorRat value	1,2	2,4	0,8	3,3	1,3

cis-nonachlor - Results summary of the collaborative trial.

Material name	**Chicken feed**	**Pig feed**	**Veg oil**	**Fish oil**	**Fish meal**
Analyte	***cis*-nonachlor**	***cis*-nonachlor**	***cis*-nonachlor**	***cis*-nonachlor**	***cis*-nonachlor**
Year of inter-laboratory study	**2016**	**2016**	**2016**	**2016**	**2016**
Number of laboratories	5	5	5	5	5
Number of samples	2	2	2	2	2
Number of laboratories considered as non-compliant	1	2	1	1	0
Number of outlying laboratories	0	0	1	0	0
Number of accepted results	8	6	6	8	9
Mean value, x, µg/kg	7,8	9,2	26,5	28,5	17,8
Repeatability standard deviation s_r, µg/kg	0,6	1,4	0,4	2,4	1,0
Repeatability relative standard deviation, RSD_r, %	8,0	15,1	1,4	8,4	5,8
Repeatability limit r [r = 2,8 × s_r], µg/kg	1,7	3,9	1,1	6,7	2,9
Reproducibility standard deviation s_R, µg/kg	2,8	1,6	8,8	12,8	11,5
Reproducibility relative standard deviation, RSD_R, %	36,0	17,7	33,1	45,0	69,5
Reproducibility limit R [R = 2,8 × s_R], µg/kg	7,9	4,6	24,6	35,8	32,1
HorRat value	1,6	0,8	1,5	2,0	3,2

trans-nonachlor - Results summary of the collaborative trial.

Material name	**Chicken feed**	**Pig feed**	**Veg oil**	**Fish oil**	**Fish meal**
Analyte	***trans*-nonachlor**	***trans*-nonachlor**	***trans*-nonachlor**	***trans*-nonachlor**	***trans*-nonachlor**
Year of inter-laboratory study	**2016**	**2016**	**2016**	**2016**	**2016**
Number of laboratories	5	5	5	5	5
Number of samples	2	2	2	2	2
Number of laboratories considered as non-compliant	2	0	1	4	1
Number of outlying laboratories	0	0	0	0	1
Number of accepted results	6	10	8	2	6
Mean value, x, µg/kg	24,0	13,0	52,2	107,0	29,5
Repeatability standard deviation s_r, µg/kg	0,7	22,3	10,1	7,1	1,7
Repeatability relative standard deviation, RSD_r, %	2,8	171,3	19,4	6,6	5,7
Repeatability limit r [$r = 2{,}8 \times s_r$], µg/kg	1,9	62,5	28,4	19,8	4,7
Reproducibility standard deviation s_R, µg/kg	9,8	22,3	29,3	[a]	14,1
Reproducibility relative standard deviation, RSD_R, %	40,8	665,4	56,2	[a]	47,6
Reproducibility limit R [$R = 2{,}8 \times s_R$], µg/kg	27,4	62,5	82,1	[a]	39,4
HorRat value	1,9	30,2	2,6	[a]	2,2

[a] No evaluation possible.

4 Conclusions

Within this collaborative study for PCBs and OCPs in feed and feed ingredients two prescribed revised standards were tested by several laboratories.

The revised standard for GC-ECD (EN15741) could not be evaluated due to insufficient number of participating laboratories (n=3). The extension of the standard with keto-endrin, photo-heptachlor and cis/trans-nonachlor is therefore not possible. Nevertheless the current standard remains intact for the OCPs that were included in the original standard.

The standard for GC-MS(/MS) (EN15742) was successfully revised.

- The method is suitable for analysis of nearly all ndl-PCBs in all tested matrices (29 out of 30 PCB matrix-analyte combinations), with HorRat values ≤2.0.
- The method is also suitable for the majority of OCPs with HorRat values ≤2.5.
- Due to HorRat values >2.5, or too low number of labs providing satisfactory results, the standard is not suitable for a number of matrix-analyte combinations, as detailed below.
- This concerns cis-chlordane in feeds and fish oil, pp-DDT in pig feed and vegetable oil, pp-TDE in pig feed and fish oil, endosulfan sulfate in pig feed and vegetable oil, HCB and γ-HCH in fish oil, cis-nonachlor in fish meal.
- For endrin keton, a newly added analyte in this revised standard, too limited data was submitted, and therefore, the standard is not validated for this analyte.
- For photoheptachlor, also a newly added OCP, the number of labs with valid data was too low and therefore the standard is not validated for this analyte.
- The suitability of the standard could also not be proven for cis- and trans-nonachlor because too limited number of laboratories provided valid results.
- For oxychlordane, also a too limited number of laboratories provided valid results. However, as this analyte was already included in the original standard, the validity of the standard for this analyte remains intact.
- For those matrix-analyte combinations where the validation data were regarded insufficient, the results obtained with this method can only be regarded as screening results, unless the laboratory performs an in-house validation to show that satisfactory results can be obtained.
- The method is not applicable to chlorocamphene (toxaphene), a complex mixture of polychlorinated camphenes. Chlorocamphene has a very distinctive chromatographic profile and is easily recognizable by GC/ECD. Positive identification of the toxaphene isomers can be performed by negative chemical ionization mass spectrometry (NCI-MS), electron impact tandem mass spectrometry (EI MS × MS) or electron impact high resolution mass spectrometry (EI-HRMS), which is not within the scope of this method.
- A limit of quantification (LOQ) for the mentioned organochlorine pesticides of 6 to 29 µg/kg should normally be obtained. For the ndl-PCBs an LOQ of 0,5 to 1,0 µg/kg should be obtained. The LOQs mentioned apply to the individual compounds (i.e. not the sum of two or more compounds).

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Annexes

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