IVM Institute for Environmental Studies

Interlaboratory Study on the Analysis of Chlorinated Paraffins in Environmental Matrices

Phase II

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Summary

The second phase of an international interlaboratory study (ILS) on chlorinated paraffins (CPs) in environmental matrices was organised by the Institute for Environmental Studies (IVM) in cooperation with the QUASIMEME proficiency testing organisation. This assessment was initiated as an outcome of a workshop on the analysis of CPs in Ostend, Belgium, organised by QUASIMEME in March 2010, where a clear need for an ILS, preferably designed in a step-wise way, was concluded. The first objective of the study was to assess the intercomparability of the data produced and secondly to initiate improvements where possible.

In total 11 laboratories participated in the present study. The participants were requested to quantify the total concentration of CPs in a cleaned fish extract. A solution containing CPs in known concentrations was provided to the participants for calibration purposes. Participants used their in-house quantification methods and techniques. The results were collected and statistically evaluated using the Cofino statistics. Between-laboratory coefficients of variation (CV) and z-scores were appointed to the laboratory's results as an expression of accuracy.

For the total-CP analysis a between-laboratory CV of 137% was found. This CV and the assigned value (AV) were calculated after the removal of one extreme outlier.

No other intercomparison studies are known for the analysis of CPs in a cleaned fish extract. The present study showed that the overall performance of participants in the analysis of total CPs was better than that in the ILS of Pellizzato *et al*. (2009) in which a raw industrial soil extract was analysed.

1 Introduction

Chlorinated paraffins (CPs), also known as polychlorinated alkanes (PCAs), are complex mixtures of linear chlorinated n-alkanes with carbon chain lengths of 10 to 30 and a chlorination degree between 30% and 70% by mass. Characterization of CPs is performed by their alkane chain lengths. They are divided into three groups: short-chain (C10-13) (SCCP), medium-chain (C14-17) (MCCP) and long chain (C18-32) (LCCP) CPs.

CPs are used in several industrial applications such as flame retardants in the rubber industry, high temperature lubricants and cutting fluids in the metalworking industry and additives in liquids, paints and textile. The analysis of CPs is highly complicated. There are tens of thousands of congeners which make separation by gas chromatography (GC) and even by comprehensive two-dimensional GC (GCxGC) difficult. Alternative methods are scarce and may suffer from false positive results. Therefore, data reported on CPs include very high uncertainties (100% or more).

There is much pressure on analyzing CPs. They are being produced in high volumes, they are under discussion in the United Nations Environmental Programme (UNEP) for possible listing as a persistent organic pollutant (POP), and they are a mandatory determinand in the European Water Framework Directive. However, their analysis is subject to very large variation, as the CP patterns are extremely complex. In March 2010, QUASIMEME organized a workshop on the analysis of CPs in Ostend, Belgium. A number of critical issues in the analysis of these CPs were discussed. It was generally agreed that there was a clear need for an interlaboratory study (ILS), preferably designed in a step-wise way and accompanied by expert comments.

The first phase of the ILS on the analysis of CPs, organised in 2011/2012 (Van der Veen *et al.*, 2012), focused on the analysis of SCCPs in a solution of undisclosed concentration, to assess the possibility of producing precise and accurate data for a range of CPs in a mixture. In total 15 laboratories participated, of which 11 submitted data. Participants used their in-house quantification methods and techniques. The majority of the laboratories (57-71%) obtained satisfactory z-scores for the analysis of three individual CPs and the model between lab coefficients of variation (CVs) varied between the compounds from 22 to 46%. For total CPs a between lab CV of 56% was obtained.

The second phase of the ILS on the analysis of CPs, described in this report, focuses on the analysis of SCCPs in a cleaned fish extract. In total 20 laboratories subscribed for this study, of which 11 submitted data. The ILS study focussed on the total SCCPs.

This study was carried out by the Institute for Environmental Studies (IVM) in collaboration with QUASIMEME (www.QUASIMEME.org).

2 Materials and methods

2.1 Study design

Laboratories were asked to quantify, in triplicate, the total level of CPs in a cleaned fish extract, using the provided standard solution (Chapter 2.2).

The first invitation for participation in the study was sent out in October 2012 and the samples were distributed in March 2013.

2.2 Material preparation

Ampoule A
Ampoule A contained a cleaned fish extract in iso-octane, prepared from a herring from the Western Scheldt (The Netherlands).

Ampoule B
Ampoule B contained a technical mixture of SCCPs (C10-C13, 51.5% Cl), 66.8231 µg/g, in iso-octane.

2.3 Methods used by participants

A short description of the methods reported by each individual participant is provided in Appendix 6. One participant (CPP15) handed in two data sets, obtained with two different analysis methods (marked with m1 and m2). Although, participants used different detection methods for the analysis of CPs, all participants used a GC method for the separation (Figure 2.1). The preferred method was GC-mass spectrometry (GC-MS) (67%), and a few laboratories used GS-MS/MS (25%). Only one participant did not use an MS for the detection. A GCxGC-electron capture detector (ECD) system was used instead. The ion sources used by the participants who did use an MS technique varied. The majority of the participants (64%) used chemical ionization (CI), while 18% used electron impact (EI) and 18% used electron capture chemical ionization (ECNI) (Figure 2.2). All participants, except one, used a negative polarity.

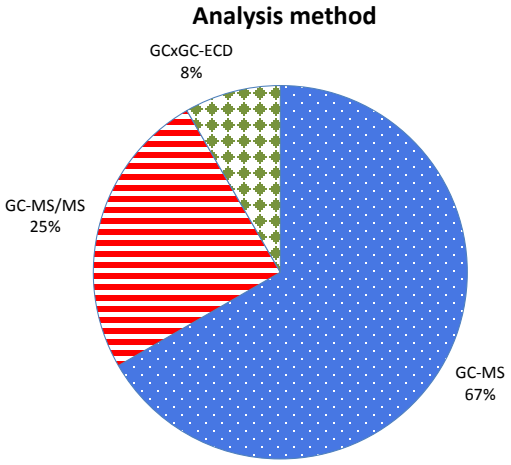

Figure 2.1 Methods used for analysis of CPs (for details see Appendix 6).

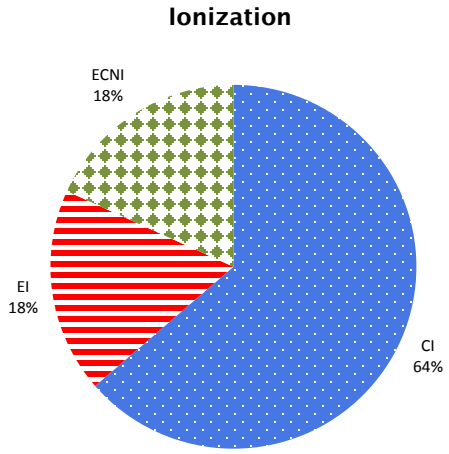

Figure 2.2 Ionization techniques of the MS methods used.

For the GC separation a variety of columns is used. Often a DB-5MS column was used (25%), but also other columns were utilised, such as HP5-MS and Rtx-5MS (Figure 2.3). Splitless injection was used (50%) as well as pulsed splitless (25%), programmed temperature vaporization injection (PTV) (17%) and on-column injections (8%).

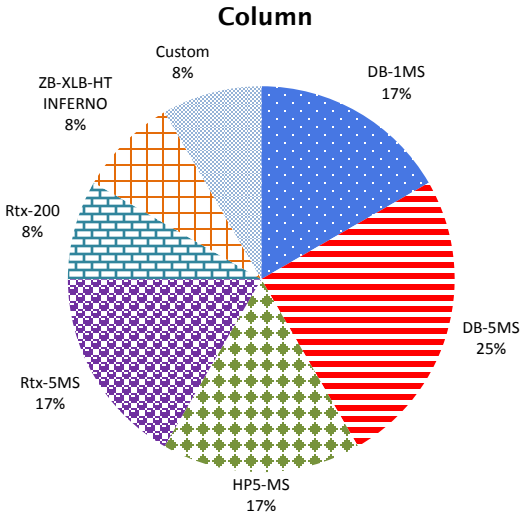

Figure 2.3 Columns used for analysis of CPs on a GC.

2.3.1 Quantification

For the quantification of the concentration of total CPs in the cleaned fish extract in ampoule A participants were requested to use ampoule B. One of the participants (CPP17) did not use Ampoule B, but used an in-house mixture instead because the chlorination degree of the CPs in that mixture was more similar to the chlorination degree of the CPs in the fish. In addition to this, two participants (CPP4 and CPP18) handed in two data sets. The first data set, marked with m1, was obtained by quantification of total CPs with ampoule B and the second data set, marked with m2, was obtained with quantification with in-house mixtures.

The majority of the participants (58%) used in addition an internal standard, like cis-chlordane, d10 anthracene, 1,2,5,5,6,9,10-Heptachlordecane, 13C10 1,5,5,6,6,10-Hexachlorodecane, and 13C-PCB 180 for the quantification of CPs.

Almost half of the participants (45%) were able of quantify the separate alkane groups (C10, C11, C12, C13).

2.4 Data Assessment

The data assessment was carried out according to the principles employed in the data assessment of the QUASIMEME proficiency testing organisation (www.quasimeme.org). All data received from the participants were entered into a database and assessed using a standard procedure enabling direct comparison between participants. The approach of the assessment is based on the standard, ISO 13528 (2005), the IUPAC International Harmonised Protocol for Proficiency Testing (Advanced Draft) by Thompson *et al.* (2006). Additions or differences in the assessment from these standards are given or referred to in this report. However, the assigned value (AV), the between-lab CV values and the laboratory assessment using z-scores are based on Cofino Model (Cofino *et al.*, 2000). In Table 3-1 the so-called 'Inclusion rate' is shown. This value is a percentage that reflects how many of the data are included in the 'Between-lab CV', shown in the column left from the Inclusion rate column. The higher

the inclusion rate, the lower the number of outliers. A higher inclusion rate also tells that the Between lab CV is more representative for the entire group of participants that produced that specific matrix-determinand combination.

The Cofino model provides a highly reliable estimate of the measurement relating to the method. It is generally acknowledged that robust statistics cannot cope with more than 10 % extreme values, particularly with a skewed distribution. The Cofino model is able to routinely cope with these types of distribution and provide the best estimate of the consensus value, which may be used as the AV.

The Cofino model has been developed for the routine QUASIMEME assessments. The Cofino model uses a Normal Distribution Assumption (NDA). The AV is based on the Cofino NDA model without any trimming of the data. This approach includes all data in the evaluation and no subjective truncation or trimming is made. This model has been further developed to include Left Censored Values (LCV)[1]. The development of these models has been fully documented and published (Cofino *et al.*, 2000; Cofino *et al.*, 2005; Wells *et al.*, 2004). An overview of the assessment with explanation and examples is given in the Assessment Rules for the Evaluation of the QUASIMEME LP Studies Data (Wells and Scurfield, 2004).

The details of the Cofino Model were provided elsewhere (Wells *et al.*, 2004, Wells and Scurfield, 2004), but in summary the approach is as follows:

- All data included in the assessment
- No data trimmed or down weighted
- AV based on Cofino NDA model
- All LCV are also included, provided certain criteria are met (Chapter 2.4.2).

2.4.1 Plots

The performance of the laboratories in this assessment is illustrated in the z-score histogram. Where the AV for a determinand is indicative, the values are plotted as their original reported concentrations. The rules for confirming whether the consensus value should be an AV or an indicative value are given in the Assessment Rules for the Evaluation of the QUASIMEME LP Studies Data (Wells and Scurfield, 2004) with relevant examples.

Normally, four plots are given for each determinand (Figure 2.4). The upper left plot provides an impression of the probability density function (PDF) model for all data (black) and for the first mode (PMF1) model of the data (blue dotted). Superimposed on these PDFs is a histogram of the individual measurements (grey bars). This plot shows the distribution of the data as a whole, and of the data in the main mode (PMF1) model on which the AV is based (inclusion rate in Table 3.1).

[1] Left Censored Values is the correct nomenclature for “less than” values

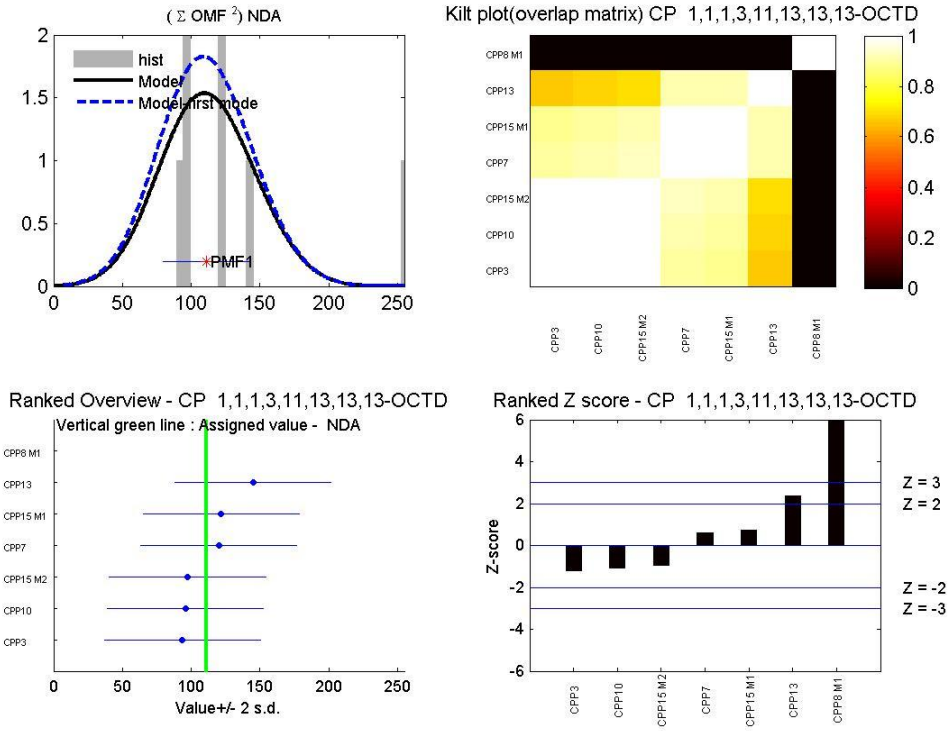

Figure 2.4 Examples of the graphical output of the Cofino Model statistics.

The "Kilt Plot" (Overlap Matrix) (upper right plot) provides an overview of the degree of overlap of each pair of data. It gives a clear indication of the degree of homogeneity of the data. As a key, the white areas indicate maximum overlap of the PDFs and, therefore, highest agreement (an overlap of one implies that the two laboratories of the pair report exactly the same results), while the black area show the pairs in poor agreement.

The lower left plot is a ranked overview of all data with an error bar of ± 2 SD. The numerical values are given in blue and the LCVs are given in red.

The ranked z-score plot (lower right) is based on the mean of the data, which is normally also the AV. However, if there is any adjustment required to the AV as a result of the assessment, *e.g.,* use of the nominal concentration or a trimmed value, then the final z-score given in the z-score histograms will reflect these changes. In this assessment, no such adjustments are made and therefore, the z-score plot (lower right) is the definite plot for obtaining the individual lab z-scores.

For each matrix-determinand combination a set of these four graphs is available. They can be found in Appendix 5.

2.4.2 The assigned values and indicative values

The AV is obtained from the main mode model of the data using the Cofino Model (bleu dotted line in upper left panel in Figure 2.4), and is centred around the highest density of values. Unless otherwise stated, the AV is based on this consensus value of *all* data. Although *all* data are included in the assessment, those values that lie some distance from AV contribute less to the mean than values which occur at or near the mean.

In some instances it is not possible to set an AV, and an indicative value is given. No assessment of laboratory performance is given where an indicative value is set. An overview of the assessment, with explanation, decision flowcharts and examples, is given in the paper *Assessment Rules for the evaluation of the QUASIMEME Laboratory Performance Studies Data*, available on the QUASIMEME website (www.quasimeme.org). A summary of the categories is given below:

Category 1

For data with the number of numerical observations ≥ 7

An AV is based on the mean when ≥ 33% of values have a z-score of $|Z| < 2$. Where < 33% of the data has $|Z| < 2$ the value is indicative. *i.e.* at least 33% must be in good agreement.

Category 2

For data with the number of numerical observations > 3 and < 7

An AV is based on the mean when ≥ 70% of values have a z-score of $|Z| < 3$ and a minimum of 4 observations have $|Z| < 2$. Otherwise the value is indicative. i.e. for small datasets, $n > 3$ and $n < 7$, there need to be very good agreement and a maximum of one extreme value before an AV can be given.

Category 3

For data with the number of numerical observations < 4

No AV is given. Normally the median value is given as an indicative value.

Category 4

For data with the high Total Error% >100% in combination with bad performance

No AV is given.

2.4.3 The Z-score Assessment

A z-score (Thompson and Wood, 1993) is calculated for each participant's data for each matrix / determinand combination which is given an AV. The z-score is calculated as follows:

$$z\text{-score} = \frac{\text{Mean from Laboratory} - \text{Assigned Value}}{\text{Total Error}}$$

It is emphasized that in many ILSs the between-laboratory standard deviation obtained from the statistical evaluation of the assessment is used as 'total error' in the formula above. In the QUASIMEME data assessment, the total error is estimated independently taking the needs of present-day international monitoring programs as starting point. For each determinand in a particular matrix, a proportional error (PE) and a constant error (CE) have been defined. The total error depends on the magnitudes of these errors and on the AV:

$$\text{Total Error} = \frac{\text{Assigned Value} \times \text{Proportional Error (\%)}}{100} + 0.5 \times \text{Constant Error}$$

The values for the PE and CE were developed by QUASIMEME. The values are based on the following criteria:

- Consistency of the required standard of performance to enable participating laboratories to monitor their assessment over time.
- Achievable targets in relation to the current state of the art and the level of performance needed for national and international monitoring programmes.

The assessment is based on ISO 43 and z-scores. The QUASIMEME model is designed to provide a consistent interpretation over the whole range of concentration of analytes provided, including an assessment where LCVs are reported.

The PE in this assessment was set at 12.5 %. The CE has been set for each determinand or determinand group. This value was initially set to reflect the limit of determination, but is at present more closely related to the overall laboratory performance. The magnitude of the CE is set to provide a constant assessment in terms of z-score regardless of concentration. Therefore, at low concentrations the level of accuracy required to obtain a satisfactory z-score is less stringent than at a high concentrations.

Following usual practices *e.g.* ISO 43, the z-scores can be interpreted as follows to assure the quality of their data:

$\|Z\| < 2$	Satisfactory performance
$2 < \|Z\| < 3$	Questionable performance
$\|Z\| > 3$	Unsatisfactory performance
$\|Z\| > 6$	Frequently points to gross errors (mistakes with units during reporting, calculation or dilution errors, etc.).

Figure 2.5 illustrates the interpretation of the z-scores:

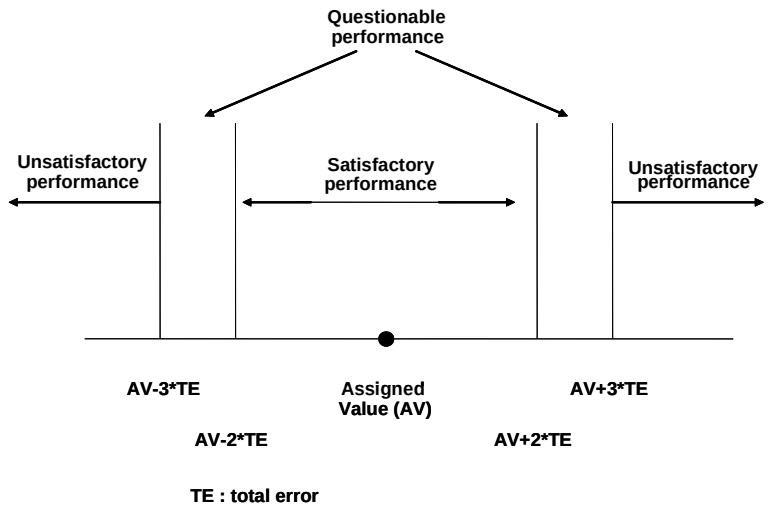

Figure 2.5 Interpretation of z-scores.

It is not possible to calculate a z-score for LCVs as LCVs represent a cut-off value rather than continuous data. However, Quasimeme provides a simple quality criterion:

LCV/2 < (concentration corresponding to |z|=3): LCV consistent with AV.

LCV/2 > (concentration corresponding to |z|=3): LCV inconsistent with AV, i.e. LCV reported by laboratory much higher than numerical values reported by other laboratories.

Z-score key:	S – Satisfactory
	Q – Questionable
	U – Unsatisfactory
LCV key:	C – Consistent
	I – Inconsistent
No data:	B - Blank

3 Results

The submitted results have been evaluated statistically and whenever the data met the requirements (as described in Chapter 1), an AV was established. Z-scores were calculated based on the AV. Due to a huge variation in the results of the participants, it was only possible to calculate an AV and z-scores after removal of one extreme outlier (CPP12). Summary statistics are presented in Table 3-1 and Table 3-3. A summary of the AVs and the percentage of satisfactory to unsatisfactory z-scores are presented in Table 3-2 and Table 3-4. Whenever less than values (LCV) were submitted, the percentage of consistent and inconsistent LCVs with the AV is given. The submitted data is presented in Appendix 2. Tables with individual z-scores are presented in Appendix 3, consistencies of the individual results are presented in Appendix 4 and z-score plots in Appendix 5. An example of the chromatograms obtained for the cleaned extract is given in Figure 3.1.

Table 3-1 Summary of results of CPs in the cleaned fish extract (results in µg/g).

Determinand	n	Assigned value	Indicative value	Model Mean	Median	Min*	Max**	Between Lab CV (%)	Inclusion rate (%)	n>LOQ
Total CP	14	NA	0.352	0.353	0.352	0.041	82.69	140	51	11

* Min: lowest value submitted > LOQ

** Max: highest value submitted > LOQ

Table 3-2 Summary of laboratory performance for CPs in the cleaned fish extract (results in µg/g).

Deter-minand	Assigned value	Indi-cative value	% of the data received	% of z-scores |Z|<2 Satisfactory	% of z-scores 3>|Z|>2 Questionable	% of z-scores 6>|Z|>3 Unsatisfactory	% of z-scores |Z|>6 Extreme	% of con-sistent LCV	% of incon-sistent LCV
Total CP	NA	0.352	61	NA	NA	NA	NA	NA	NA

Table 3-3 Summary of results of CPs in the cleaned fish extract after removing the data set of CPP12 (results in µg/g).

Determinand	n	Assigned value	Model Mean	Median	Min*	Max**	Between Lab CV (%)	Inclusion rate (%)	n>LOQ
Total CP	13	0.191	0.191	0.292	0.041	1.762	137	50	10

* Min: lowest value submitted > LOQ

** Max: highest value submitted > LOQ

Table 3-4 Summary of laboratory performance for CPs in the cleaned fish extract after removing the data set of CPP12 (results in µg/g).

Deter-minand	Assigned value	% of the data received	% of z-scores |Z|<2 Satisfactory	% of z-scores 3>|Z|>2 Questionable	% of z-scores 6>|Z|>3 Unsatisfactory	% of z-scores |Z|>6 Extreme	% of con-sistent LCV	% of incon-sistent LCV
Total CP	0.191	59	31	0	15	31	15	8

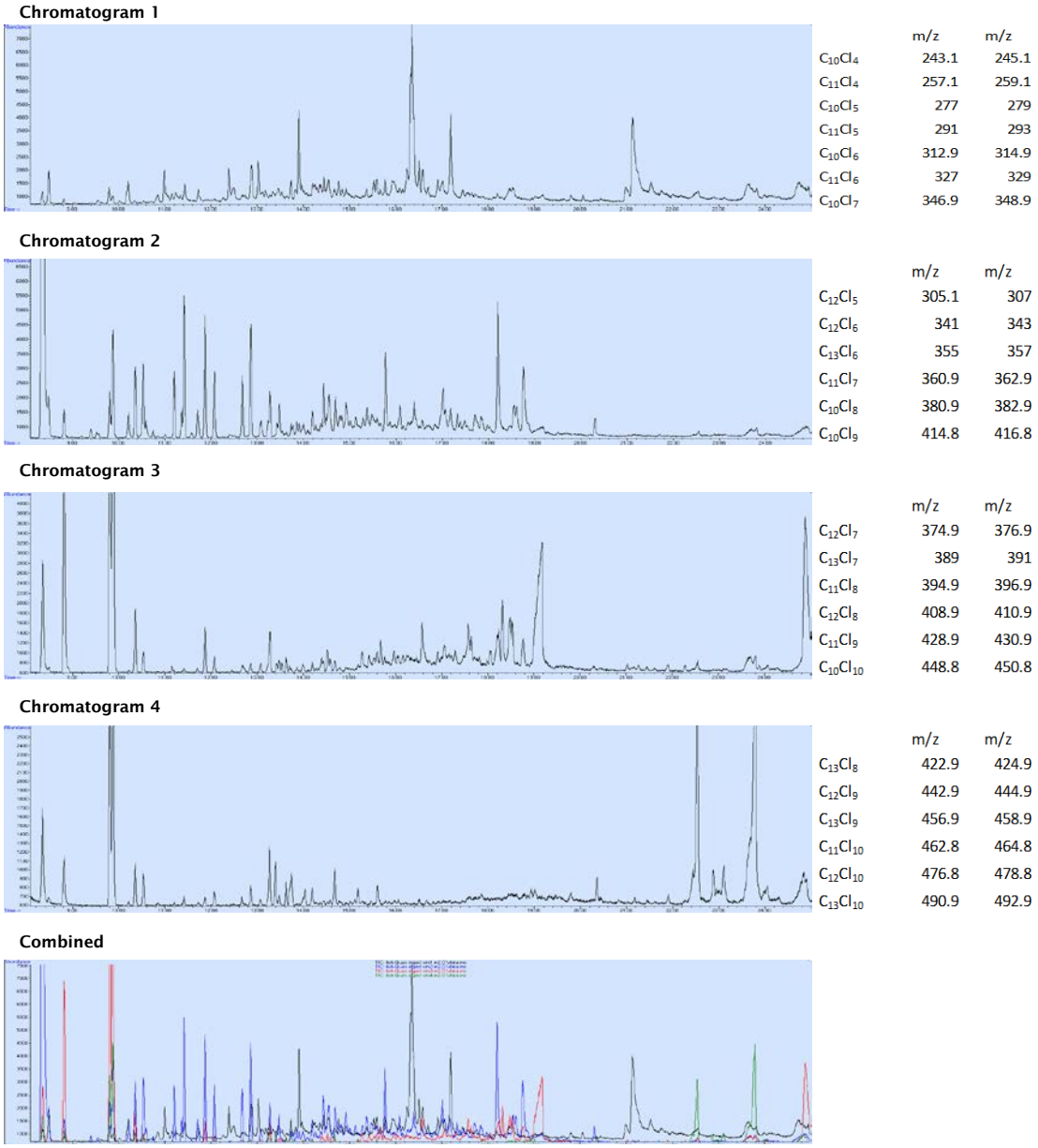

Figure 3.1 Chromatograms of the cleaned fish extract obtained by GC-MS with negative CI.

4 Discussion

In total 20 laboratories from all over the world participated in the present assessment. Of those laboratories, 11 submitted data, of which three laboratories handed in two data sets obtained with either two different analyses methods (Chapter 2.3), or with different calibration solutions (Chapter 2.3.1). Five participants were already working on CP analysis for over 3 years, while five other laboratories were analysing CPs between 1 and 3 years. Only one laboratory had experience in CP analysis of shorter than one year. No significant difference is observed between the reported concentrations of the less experienced participants and the more experienced participants (Figure 4.1).

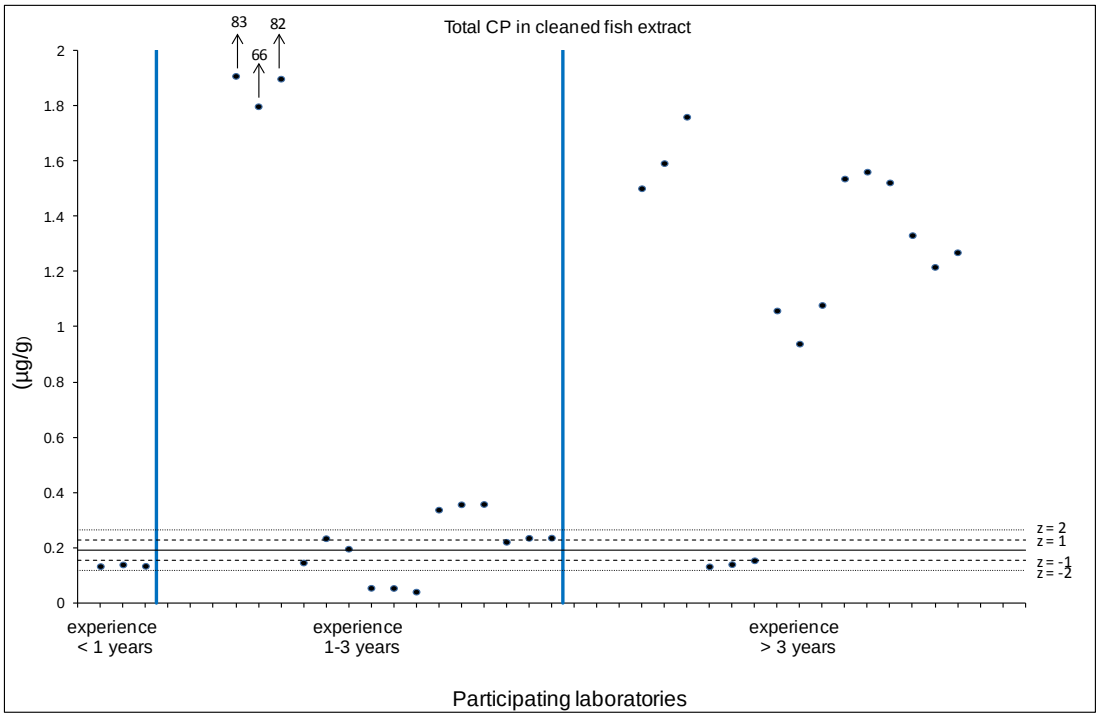

Figure 4.1 Experience of participant vs total CP concentration reported.

4.1 Laboratory performance

As shown in Table 3-1, the model between lab CV for total CPs in the fish extract is 140% which is very high, showing that the analysis of CPs is still very complex. Due to the high variation between the participants it was not possible to calculate an AV and to determine z-scores. However, after removal of the data set of CPP12 it was possible to calculate an AV and z-scores. The lines for the mean and mean ± 2z in 5Appendix 2 and Figure 4.1 t/m Figure 4.4 are based on the AV calculated after removal of data set CPP12 (0.191 µg/g) .

In Figure 4.2 a comparison is shown between the different ionization techniques used and the reported concentration of total CPs. Since only limited data is available per method no firm conclusions can be made. Only one of the participants, marked with an asterisk inFigure 4.2 used High Resolution (HR) MS.

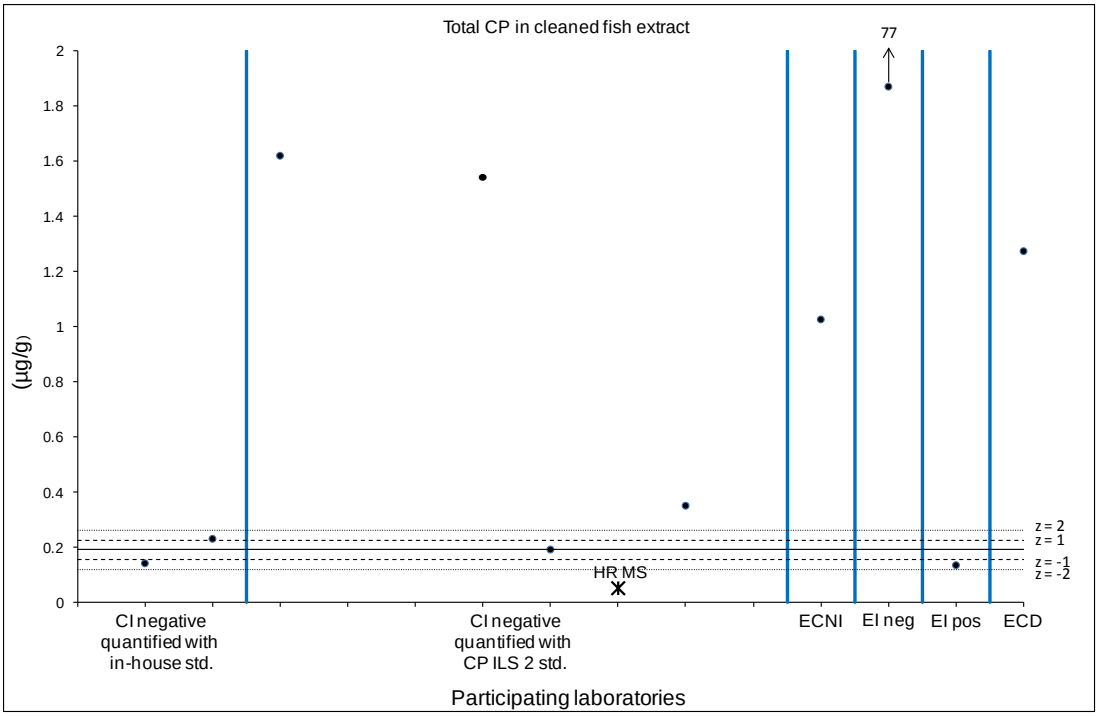

Figure 4.2 Methods used by participant vs total CP concentration reported.

In Figure 4.3 a comparison is shown between the reported results of participants using external calibration and the results of participants using internal calibration. In general Figure 4.3 shows that participants using external calibration report higher concentrations than participants using internal calibration.

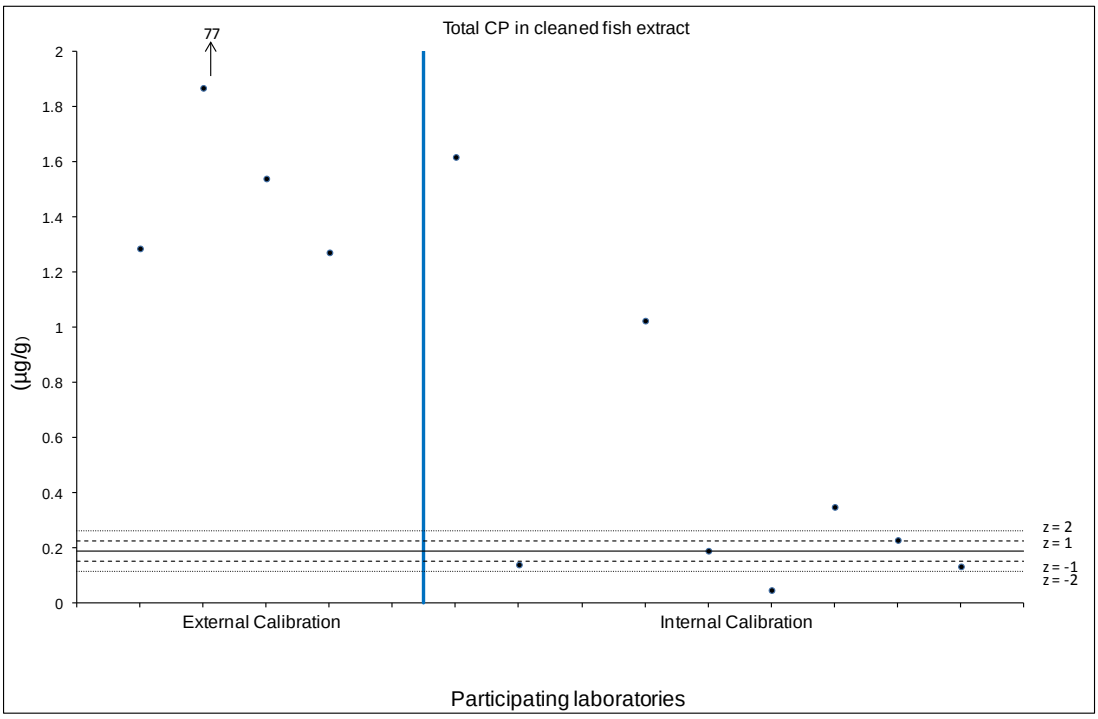

Figure 4.3 Type of calibration used by participants vs total CP concentration reported.

Three participants reported total CP concentrations based on in-house standards (Chapter 2.3.1). Two of those participants also quantified the CP ILS 2 standard solution of ampoule B, which contained 66.8231 µg/g CPs (Chapter 2.2), with their in-house standard solution and found respectively 5.69 µg/g (CPP4m2) and 66.53 µg/g (CPP17). Two of the three reported CP concentrations quantified with in-house standard solutions were within |Z| < 2 (Figure 4.4), while the third was not far off. This may indicate the importance of a standard that mimics the pattern in the sample as much as possible. It is unclear why lab CPP4m2 obtained such a deviating value after analysing the standard solution that was sent by using their own standard.

The CP concentration that had to be analysed was not very high. Due to the multitude of peaks the signal is also dived over all these peaks, which makes it even more difficult. Nevertheless, a concentration of 0.2 mg/kg is actually relatively high compared to levels of many other persistent organic pollutants. So, laboratories are expected to be able analysing this without too big errors. In this round we have not paid specific attention to the possible presence of toxaphene, which can be interfering with the masses of the CPs monitored. This will be an additional point of attention for the next round of this study.

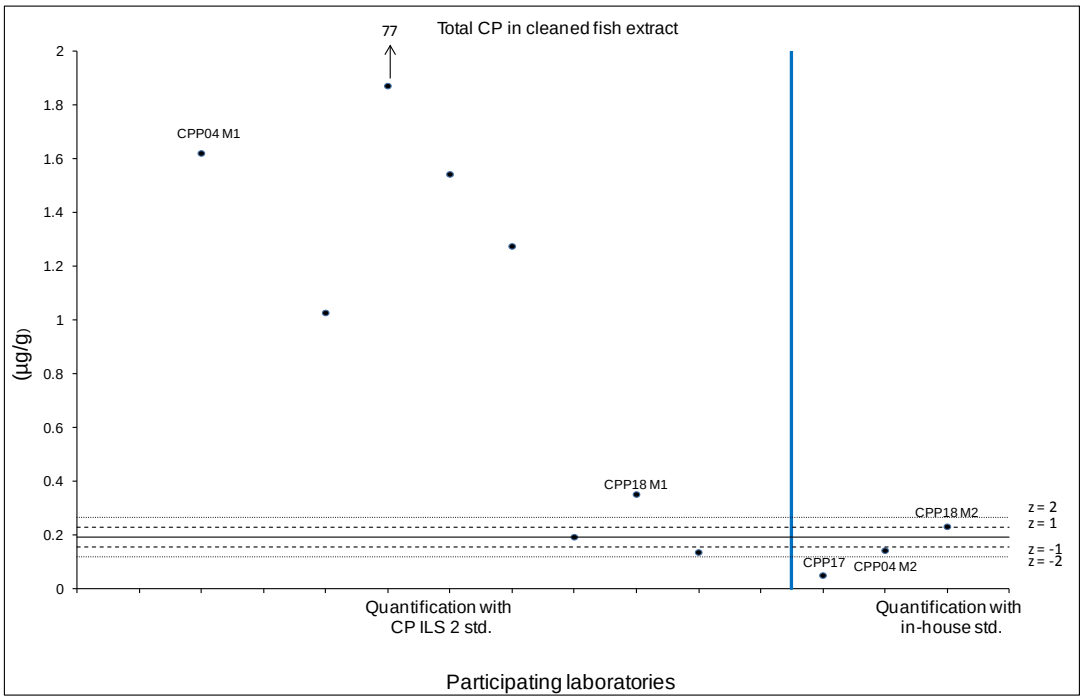

Figure 4.4 Quantification solution used by participants vs total CP concentration reported.

4.2 Comparison with other ILSs

In the first phase of the ILS on the analysis of CPs in Environmental Matrices (Van der Veen *et al.*, 2012) a between lab CV of 56% was obtained for the analysis of total SCCPs in a solution of undisclosed concentration. In an ILS of Tomy *et al.* (1999), in which all participants used the same analyses method, lower CVs (20 and 44%) were obtained for quantification of two undisclosed solutions, but concentrations of those solutions were 6-10-fold higher than the concentration of the solution in the first phase of the study of Van der Veen *et al.* (2012), in which participants all used their in-house methods.

Results of the present study show a much higher CV (137%). Possibly, the relatively low total CP concentration in the cleaned fish extract (AV= 0.191 µg/g) compared to the concentration of total CPs in the solution of the first phase (12 µg/mL) is the main reason for this relatively poor result. In addition, due to the use of a fish extract, the pattern of CPs was different than in a standard solution. A mismatch between the mixture in fish and the standards is an obvious second explanation of the results.

Unfortunately no intercomparison studies are known on the analysis of CPs in a cleaned fish extract, but in an intercomparison study of Pellizzato *et al*. (2009) the total SCCP concentration was determined in an extract of industrial soil. Concentrations were 24-9,000 times higher in the soil extract than in the cleaned fish extract, but the CV was also higher (209%). The better performance in the present study compared to the study of Pellizzato *et al*. (2009) is most likely due to the clean extract in the present study compared to the raw extract used in the study of Pellizzato *et al.* (2009).

5 Conclusions

For total SCCP analysis in a cleaned fish extract carried out by 11 laboratories the between-lab CV was 137%. A larger number of laboratories subscribed for the second phase of the ILS than for the first phase, showing an increasing interest in the quantitative analysis of CPs, as well as the intention to evaluate laboratory performance so as to improve the data produced in the field. This study showed that a variety of quantification techniques are used in the analysis of SCCPs, but that the CVs are still very high.

The present study suggests that the overall performance of participants in the analysis of total CPs has improved compared to the ILS of Pellizzato *et al*. (2009), although the present study only dealt with a cleaned extract, while the study of Pellizzato *et al*. dealt with a raw soil extract.

Obviously, further developmental work in laboratories and more interlaboratory comparison exercises are needed to improve the analysis of CPs. Analytical standards of individual CP congeners that occur in environmental samples would be a great help. Although these are difficult to synthesize, they are badly needed.

The next round of this study will focus on the differences between a clean and an uncleaned sample extract, so the effect of clean up can be evaluated. Then, attention will also be paid to the possible interference of toxaphene.

Acknowledgements

Prof. Dr. Wim Cofino is acknowledged for making his statistical expertise available.

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Appendices

1. List of participants.
2. Results and graphical representation
3. Numerical z-score values per matrix
4. Consistency of data
5. Graphical output of the Cofino Model statistics
6. Additional method information

Appendix 1 Participants

Laboratory	Contact person	Delivery address	Postal code and city	Country	E-mail
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Laboratory	Contact person	Delivery address	Postal code and city	Country	E-mail
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Appendix 2 Results and graphical presentation

Cleaned fish extract (µg/g)	Assigned value	Model Mean	Median	MIN	MAX	Model Between Lab CV%	Model percentage in PMF1	n>LOQ
Total CP	NA	0.353	0.352	0.041	82.69	140	51	11
ND: not detected NA: not analyzed								

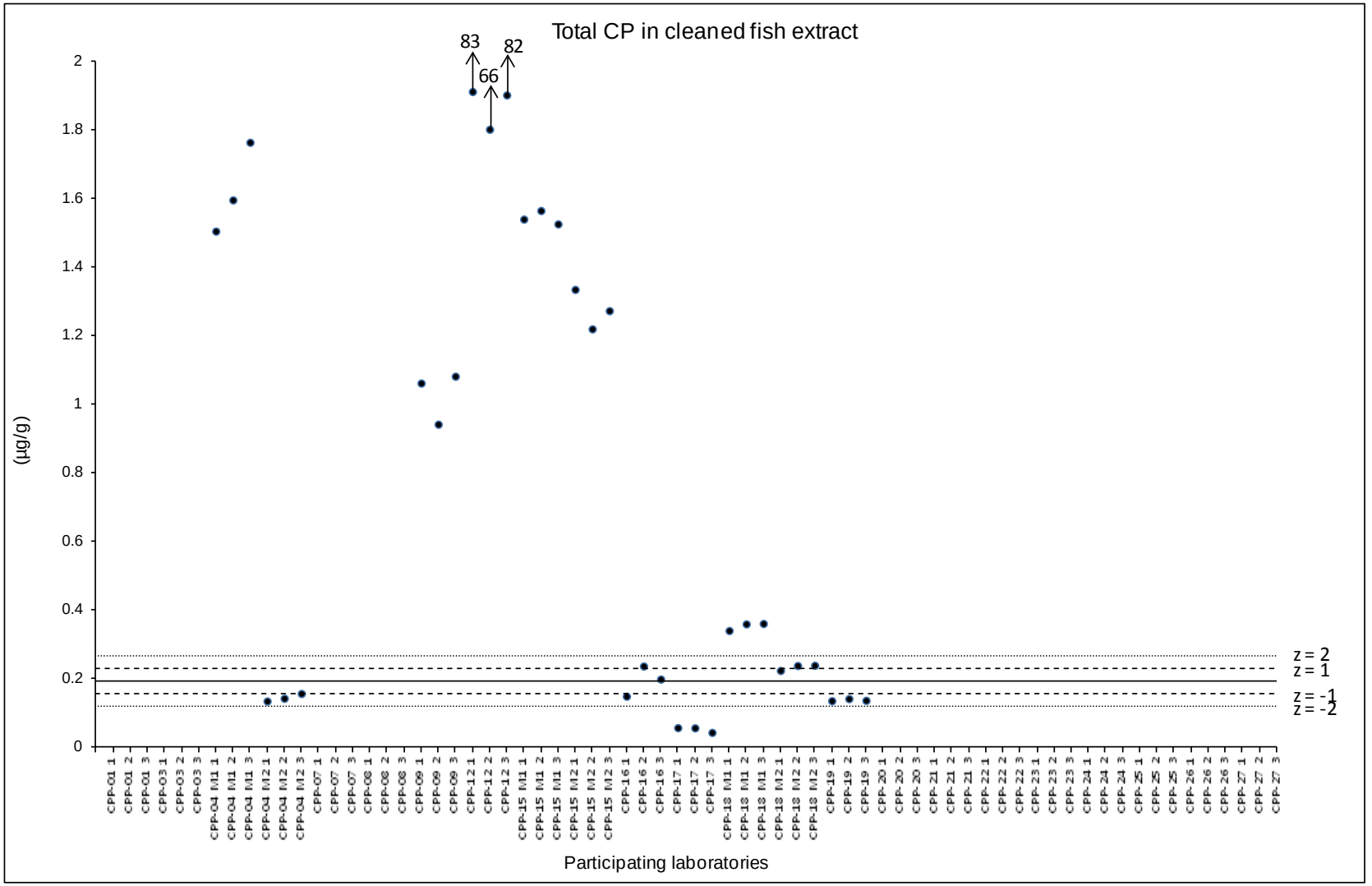
Participant code:	CPP1 1	CPP1 2	CPP1 3	CPP3 1	CPP3 2	CPP3 3	CPP4 m1 1	CPP4 m1 2	CPP4 m1 3	CPP4 m2 1	CPP4 m2 2	CPP4 m2 3	CPP7 1	CPP7 2	CPP7 3
Date Samples Received:	NA			26-03-2013			07-03-2013			07-03-2013			NA		
Date Analyzed:	NA			02-05-2013			10-05-2013			10-05-2013			NA		
Weight Received (g):	NA			7.2633			7.251			7.251			NA		
Weight Analyzed (g):	NA			7.2641			0.0718			0.0718			NA		
Cleaned fish extract (µg/g)															
Total CP	NA	NA	NA	< 0.1	< 0.1	< 0.1	1.503	1.594	1.762	0.133	0.141	0.155	NA	NA	NA
ND: not detected NA: not analyzed															

Participant code:	CPP8 1	CPP8 2	CPP8 3	CPP9 1	CPP9 2	CPP9 3	CPP12 1	CPP12 2	CPP12 3	CPP15 m1 1	CPP15 m1 2	CPP15 m1 3	CPP15 m2 1	CPP15 m2 2	CPP15 m2 3
Date Samples Received:	25-03-2013			NA			NA			05-03-2013			05-03-2013		
Date Analyzed:	09-05-2013			21-05-2013			30-05-2013			09-03-2013			09-03-2013		
Weight Received (g):	7.3168			7.3368			7.238			7.2498			7.2498		
Weight Analyzed (g):	500 µL			NA			NA			7.2508			7.2508		
Cleaned fish extract (µg/g)															
Total CP	< 2	< 1	< 2	1.06	0.94	1.08	82.69	66.32	82.09	1.538	1.563	1.524	1.333	1.218	1.271
ND: not detected NA: not analyzed															

Participant code:	CPP16 1	CPP16 2	CPP16 3	CPP17 1	CPP17 2	CPP17 3	CPP18 m1 1	CPP18 m1 2	CPP18 m1 3	CPP18 m2 1	CPP18 m2 2	CPP18 m2 3	CPP19 1	CPP19 2	CPP19 3
Date Samples Received:		NA			24-03-2013			07-03-2013			07-03-2013			08-03-2013	
Date Analyzed:		NA			30-05-2013			11-04-2013			11-04-2013			15-04-2013	
Weight Received (g):		NA			7.23484			7.2632			7.2632			NA	
Weight Analyzed (g):		NA			1 mL			NA			NA			NA	
Cleaned fish extract (µg/g)															
Total CP	0.147	0.235	0.197	0.055	0.055	0.041	0.338	0.358	0.359	0.222	0.236	0.237	0.134	0.140	0.135
ND: not detected NA: not analyzed															

Participant code:	CPP20 1	CPP20 2	CPP20 3	CPP21 1	CPP21 2	CPP21 3	CPP22 1	CPP22 2	CPP22 3	CPP23 1	CPP23 2	CPP23 3	CPP24 1	CPP24 2	CPP24 3
Date Samples Received:		NA			NA			NA			07-03-2013			NA	
Date Analyzed:		NA			NA			NA			13-03-2013/ 30-04-2013			NA	
Weight Received (g):		NA			NA			NA			7.2131			NA	
Weight Analyzed (g):		NA			NA			NA			7 mg/140 mg			NA	
Cleaned fish extract (µg/g)															
Total CP	NA	NA	NA	NA	NA	NA	NA	NA	NA	< 0.02	< 0.02	< 0.02	NA	NA	NA
ND: not detected NA: not analyzed															

Participant code:	CPP25 1	CPP25 2	CPP25 3	CPP26 1	CPP26 2	CPP26 3	CPP27 1	CPP27 2	CPP27 3
Date Samples Received:		NA			NA			NA	
Date Analyzed:		NA			NA			NA	
Weight Received (g):		NA			NA			NA	
Weight Analyzed (g):		NA			NA			NA	
Cleaned fish extract (µg/g)									
Total CP	NA	NA	NA	NA	NA	NA	NA	NA	NA
ND: not detected NA: not analyzed									


Total CP in cleaned fish extract
83
82
66
(µg/g)
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
z = 2
z = 1
z = -1
z = -2
Participating laboratories

Appendix 3 Numerical z-score values per matrix after removal of CPP12

Cleaned fish extract

Determinand	CPP1	CPP3	CPP4 m1	CPP4 m2	CPP7	CPP8	CPP9	CPP15 m1	CPP15 m2	CPP16	CPP17
Total CP	NR	NR	39.33	-1.31	NR	NR	23.01	37.18	29.81	0.06	-3.86

Determinrnd	CPP18 m1	CPP18 m2	CPP19	CPP20	CPP21	CPP22	CPP23	CPP24	CPP25	CPP26	CPP27
Total CP	4.43	1.13	-1.50	NR	NR	NR	NR	NR	NR	NR	NR

NR = Not Reported

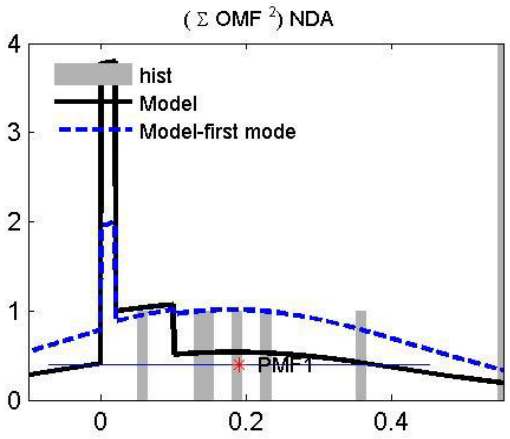
Appendix 4 Consistency of data after removal of CPP12

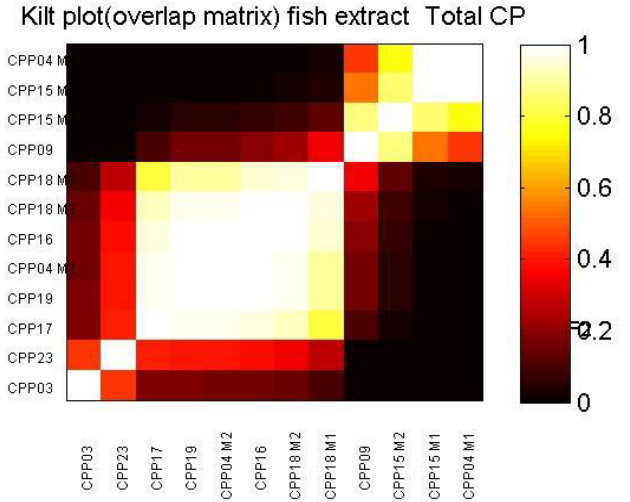
Cleaned fish extract

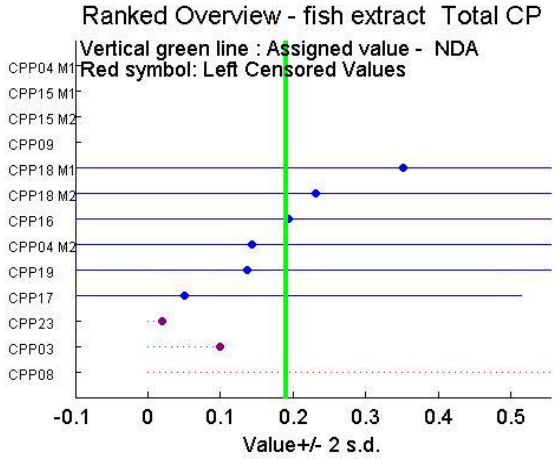
Determinand	CPP1	CPP3	CPP4 m1	CPP4 m2	CPP7	CPP8	CPP9	CPP15 m1	CPP15 m2	CPP16	CPP17
Total CP	B	I	U	S	B	I	U	U	U	S	U

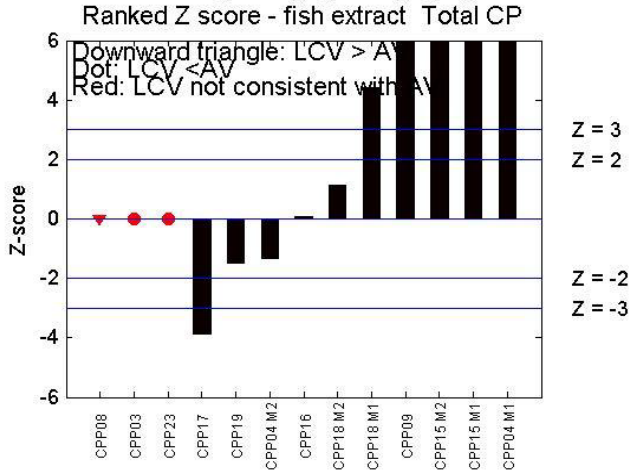
Determinand	CPP18 m1	CPP18 m2	CPP19	CPP20	CPP21	CPP22	CPP23	CPP24	CPP25	CPP26	CPP27
Total CP	U	S	S	B	B	B	I	B	B	B	B

Appendix 5 Graphical output of the Cofino Model statistics after removing CPP12









Appendix 6 Additional method information

		CPP-03	CPP-04	CPP-08	CPP-09
Instrument	Type	GC 6890N, Agilent	GC	Shimadzu GCMS-QP2010 Plus Series	Agilent GC-MS
	GC injector	pulsed Splitless	splitless	Pulsed splitless	on-column
	Detector type	ECNI-MS (Methane), MSD 5975, Agilent	MS	MSD	ECNI-MS
	Other		-		
	Column	DB-5MS, 15m x 0,250 mm x 0,10 µm, J&W (P/N 122-5511)	Rtx-200 (30m * 0.25mm; 0.25 µm film)	Rtx-5SiMS 30m x 0.25 mm x 0.25 µm	Agilent HP5-MS, 30m x 0.25 x 0.25
	Second column	N.A.	-	N.A.	n/a
	Pre-column	N.A.	-	N.A.	n/a
	Flow rate/ gas speed	1,8 mL/min	1.5 ml/min	0.88mL/min on column	constant pressure 15 psi
	Carrier gas	Helium 5.0	He	Helium	helium
	Injection volume	3 µl	2 µl	2µL	1ul
	Column temp. (°C)	120 °C (2min)->50 Grd/min->325 °C (3min)	90°C (1 min); 120 °C/min to 140 °C (0 min); 15 °C/min to 320 °C (10 min)	290°C	100
	Injector temp. (°C)	260	250 °C	245°C	120
	Interface temp. (°C)	280	290 °C	250°C	280
	Gradient/ temperature program	120 °C (2min)->50 Grd/min->325 °C (3min)		105 °C 1.0 min 34°C/min 190 °C 1.0 min 8 °C/min 250 °C 0.0 min 40 °C/min 290 °C 8.0	100°C held for 10 min 10°C/min to 260°C held at 260°C for 30 min
Detection	Type ((TOF) MS/ MSxMS/ECD/ etc)	ECNI-MS (Methane)	MS	Low resolution MS	MS
	Ionization mode (CI/ EI)	CI	Neg mode	Negative	EC
	Pos/Neg mode	neg	CI (CH4)	CI	NI
	Desolvation gas and setting	N.A.	-	N.A.	methane
	Temperatures (specify which)		-	200°C Source temperature	
	Source block temp. [°C]:	150	150°C	N.A.	230
	Desolvation temp. [°C]:		-	N.A.	
	Other (compound specific settings are to be given in form D)			Methane 8.4 10-4Pa	N.A.

		CPP-12	CPP-15 M1	CPP-15 M2	CPP-16
Instrument	Type	GC-MS-MS ION TRAP EI mode	GC	GC	GC 6890N; Agilent
	GC injector	Splitless	splitless	splitless	pulsed splittless
	Detector type	MS ion trap	MS-NCI	ECD	MSD 5973N; Agilent
	Other				
	Column	15 m x 0.18 mm ID RTX-5SILMS (0.25 µm film thickness)	DB5-MS; 15m * 0.25mm * 0,25 µm	DB1-MS, 30m*0,25mm*0,1um	DB5-MS (15 m x 250 µm x 0,1 µm); Agilent
	Second column			Innowax, 3m*0,25mm*0,1um	
	Pre-column			n.a.	
	Flow rate/ gas speed	1.5 ml/min	1.2 ml/min	first column: 0.4 ml/min, second column 30ml/min	1,3 ml/min
	Carrier gas	He	Helium	both columns Helium	Helium
	Injection volume	2 µL	1µl	1µl	2
	Column temp. (°C)	100°C 2 Min, 50°C/Min 300°C, 6 min	100°C	65°C	290
	Injector temp. (°C)	250°C	275°C	275°C	275
	Interface temp. (°C)	310°C	300°C		310
	Gradient/ temperature program		100°C (3min) -> 10°C/min -> 320°C	65°C (2min) -> 20°C/min -> 140°C-> 3°C/min -> 270°C	50 °C (1 min); 70 °C/min to 100 °C (1 min); 60°C/min to 290 °C (4 min)
Detection	Type ((TOF) MS/ MSxMS/ECD/ etc)	MS x MS/ion trap	MS	ECD	MS
	Ionization mode (CI/ EI)	EI	CI		CI
	Pos/Neg mode	negative mode	neg mode		Neg
	Desolvation gas and setting	0.3 ml/min			Methane
	Temperatures (specify which)				
	Source block temp. [°C]:	250°C	200°C (source)	270°C	150
	Desolvation temp. [°C]:		106°C (quadrupole)		150 (quadupole)
	Other (compound specific settings are to be given in form D)		methane as reagent gas for NCI	backup gas ECD: Nitrogen 115ml/min	

		CPP-17	CPP-18	CPP-19	CPP-23
Instrument	Type	GC	GC-MS/MS	GC-MS/MS	GC
	GC injector	splitless	PTV (Programmed Temperature Vaporising)	splitless	PTV
	Detector type	Thermo Finnigan MAT 95	MSD	Triple quad	MS (ENCI)
	Other				
	Column	glas column coated with DB-5 analogue stationary phase with a film thickness of 0.15 µm, 20m * 0.3 mm, self manufactured	HP-5MS UI; Length 15m; Diam. 0,250 mm; Film 0,25 µm; Agilent Technologies	30mx0.25mmx0.25µm ZB-XLB-HT INFERNO Phenomenex	DB-1MS
	Second column	n.a.	DB-5MS UI Restrictor; Length 0,55m; Diam. 0,150 mm; Film 0,15 µm; Agilent Technologies		/
	Pre-column		-		/
	Flow rate/ gas speed	40 kPa	Column 1 = 1,4 ml/min constant flow; Column 2 = 2,0 PSI constant pressure	1.4 ml/min	2.5 mL/min
	Carrier gas	H2	He	Helium	He
	Injection volume	2	5 µL	2	2
	Column temp. (°C)	110	60 °C	130	110?325
	Injector temp. (°C)	260	70 °C	275	150?280
	Interface temp. (°C)	280	280 °C	280	310
	Gradient/ temperature program	110 to 310 ° with 10°C/min)	60 °C (1 min); 20 °C/min to 300 °C (10 min); Total run time: 23 min	130 °C (4 min); 40 °C/min to 300 °C (4.25 min); Total run time: 12.5 min	110°C (0.5')?25°C/min?325°C (3')
Detection	Type ((TOF) MS/ MSxMS/ECD/ etc)	HRMS	GC-MS(MS)	MS/MS	MS (ENCI)
	Ionization mode (CI/ EI)	CI	CI	EI	CI
	Pos/Neg mode	Neg	Neg. mode	pos	neg
	Desolvation gas and setting	Methane			CH4
	Temperatures (specify which)				150°C Quadropol
	Source block temp. [°C]:	120	150	ion source: 230°C / quadrupoles: 150°C	150
	Desolvation temp. [°C]:				
	Other (compound specific settings are to be given in form D)		MS1 Quad Temp. 150 °C		