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Brandsma, S.H.

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2.1: Organophosphorus flame retardant and plasticizer analysis - challenges and recommendations based on the first worldwide interlaboratory study

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Sicco H. Brandsma¹, Jacob de Boer¹, Wim P. Cofino², Adrian Covaci³, Pim E.G. Leonards¹

¹ Institute for Environmental Studies, VU University, De Boelelaan 1087, 1081 HV Amsterdam, The Netherlands

² Wageningen University and Research Centre, P.O. Box 8130, NL-6700 EW, Wageningen, The Netherlands

³ Toxicological Center, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk-Antwerp, Belgium

Abstract

The first worldwide interlaboratory study on organophosphorus flame retardants (PFRs) was organized to improve the quality of the data reported in literature. The study involved standard solution, dust, fish oil and sediment sample. The differences in coefficients of variation (CV) between the samples were related more to the PFR concentration (with high blanks being reported by some laboratories) and less to the matrix type. Not all participating laboratories suffered from blank problems, which indicate that it was possible to control the blanks. We include recommendations on how to improve analytical performance, especially to reduce contamination of blanks.

Introduction

Brominated flame retardants (BFRs) such as polybrominated diphenyl ethers (PBDEs), hexabromocyclododecane (HBCD) and tetrabromobisphenol A (TBBPA) are used in various products to enhance fire safety. Yet, the usage patterns of flame retardants are changing in light of environmental hazard and exposure data and new regulations. Due to their persistence, bioaccumulative and toxic properties, the EU banned Penta- and Octa-BDE in 2004 (BSEF, 2010). US BFR producers have started to phase-out these mixtures (BSEF, 2010), while some US states have already banned the production of the Penta- and Octa-BDE commercial mixtures (BSEF, 2010). In 2009 the EU banned the use of Deca-BDE in electric and electronic products and the US EPA announced a phase out of DecaBDE by the end of 2013 (USEPA, 2009). The phase-out of several high production volume BFRs has led to an increase in production and application of alternative flame retardants (FRs), such as organophosphorus flame retardants (PFRs) (CEFIC, 2011). In 2004, PFRs accounted for 14% of the global FR production volume, compared to 21% for BFRs (CEFIC, 2011). In many products, such as furniture, textiles, cables, building materials, insulation materials, paints, floor polishes, hydraulic fluids and electronic appliances, PFRs are also used as plasticizers or anti-foaming agents (CEFIC, 2011; Marklund et al., 2003; Reemtsma et al., 2008). In most applications, PFRs are used as additive chemicals and thus not covalently bound to the polymeric structure (WHO, 2000). The toxicity data for some PFR chemicals to date shows evidence of neurotoxicity and carcinogenicity, although the number of studies is limited (WHO, 2000; WHO, 1998; Dishaw et al., 2011; Meekers and Stapleton, 2010). During use or after their disposal, PFRs can leach into the environment (Reemtsma et al., 2008).

As far as environmental exposure is concerned, PFRs were reported by Sheldon & Hites, (1978) in the late 1970s in sediments from the Delaware River. Muir and co-workers (Muir et al., 1980 and 1981) were the first to study the fate, bioaccumulation and biodegradation of PFRs. Increasing production volumes of PFRs, as a consequence of the phase out of the commercial Penta- and Octa-BDE mixtures, have led to renewed interest in both environmental and indoor levels of PFRs (Van der Veen and De Boer, 2012). PFRs have been recently detected in dust and indoor air and other environmental compartments, such as river water, groundwater, waste water, sediment and different fish species (Marklund et al., 2003; Reemtsma et al., 2008; Van der Veen and De Boer, 2012; Marklund Sundkvist

et al., 2010; Kim et al., 2011; Chen et al., 2012; Meyer et al., 2004; Brommer et al., 2012). The concentrations detected in house dust are comparable, or in some cases exceed the PBDE, HBCD and TBBP-A concentrations in house dust (Van den Eede et al., 2011; Stapleton et al., 2009). Different analytical and cleanup methods have been reported by several labs to analyze PFRs in various matrices like dust, sediment and biota samples (Marklund Sundkvist et al., 2010; Kim et al., 2011; Chen et al., 2012; Meyer et al., 2004; Brommer et al., 2012; Van den Eede et al., 2011). Accelerated solvent extraction (ASE) was mainly used for the extraction of the PFRs from fish, egg and sludge samples (Marklund Sundkvist et al., 2010; Kim et al., 2011; Chen et al., 2012; Meyer et al., 2004), whereas extraction of dust was performed with ultrasonication (Brommer et al., 2012; Van den Eede et al., 2011) and liquid-liquid extraction (LLE) with dichloromethane was used for wastewater (Marklund et al., 2005). Various cleanup methods were described in literature like gel permeation chromatography (GPC) using a glass column containing Biobeads SX-3 for biota and sludge samples (Marklund Sundkvist et al., 2010; Marklund et al., 2005), silica gel columns used for fish samples (Kim et al., 2011) and amino-propyl silica columns for eggs (Chen et al., 2012). Wastewater samples were only filtered before LLE (Marklund et al., 2005). Quantification was carried out with gas chromatography (GC coupled to mass spectrometry (MS), GC coupled to nitrogen phosphate detector (NPD), GC-high resolution (HR)-MS and liquid chromatography (LC) coupled to MS/MS (Marklund Sundkvist et al., 2010; Kim et al., 2011; Chen et al., 2012; Meyer et al., 2004; Brommer et al., 2012; Van den Eede et al., 2011; Marklund et al., 2005). No standardized method for the extraction and cleanup of PFRs from various matrices is described in the literature. Blank problems, ion suppression and lack of adequate standards were reported by various laboratories and these topics were also discussed at the workshop that was held prior the interlaboratory study (ILS) organized within the framework of the NORMAN network [WHO, 2000; Marklund et al., 2005; Rodil et al., 2005; Rodriguez et al., 2006; Zhang et al., 1996; LeBel et al., 1983; Fries et al., 2011; Bergh et al., 2012; Bergh, 2011).

The aim of this study was to evaluate the quality of the results of the participants and improve the quality by supplying feedback to the participants and propose general precautions which need to be taken prior or during the analysis of the PFRs. This will improve the quality of the PFR data reported in literature.

Design of interlaboratory study

Within this ILS, the following test materials were sent around; sediment, house dust, fish oil and a test solution with PFRs in undisclosed concentrations. The sediment was a sterilized freeze-dried, sieved (90 µm) and homogenized sediment collected from the Western Scheldt (The Netherlands). Its homogeneity has been tested extensively for BDE47, BDE99, BDE100, BDE154, BDE153 and BDE209. The house dust was a reference material from NIST, coded SRM2585, which was certified for thirty three polycyclic aromatic hydrocarbons (PAHs), thirty polychlorinated biphenyl congeners (PCBs), four chlorinated pesticides, fifteen PBDE congeners, but not for PFRs. This sample was a sterilized, freeze-dried and sieved (< 100 µm) house dust from vacuum cleaner bags collected from US homes, motels,

and hotels (NIST, 2010). The fish oil from menhaden was supplied by Sigma-Aldrich Chemie B.V. (Zwijndrecht, the Netherlands) and was spiked for the ILS with ten PFRs in the range of 20 to 140 ng/g. Two test solutions were prepared, one for GC, in toluene, and one for LC in methanol. The test solutions contained ten PFRs listed in Table 1, and were provided to check for possible calibration errors. The PFR concentrations were in the range of 40-140 ng/g. The test solution could be analyzed directly by GC and LC without any dilution or concentration steps to avoid handling errors. The test solution did not contain internal standards. The structures and abbreviations are listed in Figure 1.

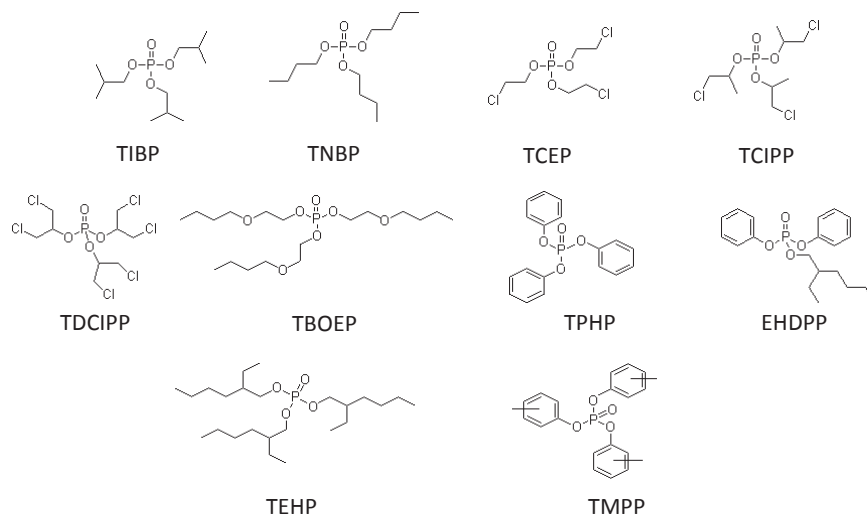


Figure 1. Ten PFRs that were studied during this inter-laboratory study.

The samples should be analyzed using routinely applied and validated methods and procedures. The use of internal standards (such as TNBP_{-d27} and TPHP_{-d15}) was recommended. The use of more than two labeled standards was encouraged. Each sample must be determined in three independent replicates to check for the intra-laboratory variation. The participants were also asked to include three independent blanks for each method and report the blank levels. The blank levels were used to investigate the blank performances of the participants. Before performing the statistical evaluation, results were corrected for the blank levels. The statistical evaluation of this study was carried out using the Cofino model (Cofino et al., 2005; Wells and De Boer, 2006; De Boer and Wells, 2006). The mean of the three independent replicates was used for the statistical evaluation. An underlying normal distribution of the data was assumed and a proportional error of 12.5% and a constant error of 0.025 ng/g were used for the analysis of all determinands. The Cofino model was developed for the QUASIMEME assessments and is used for many ILS (Well and De Boer, 2006; De Boer and Well, 2006; Van Leeuwen et al., 2006; Staaf et al., 2005).

Table 1. The compound name, CAS number, acronym and vendor of the PFRs that were studied during this interlaboratory study.

Compound	Acronym	CAS no.
Tris(isobutyl) phosphate ¹	TIBP	126-71-6
Tris(butyl) phosphate ²	TNBP	126-73-8
Tris(chloroethyl) phosphate ²	TCEP	115-96-8
Tris(2-chloroisopropyl) phosphate ³	TCIPP	13674-84-5
Tris(1,3-dichloroisopropyl)phosphate ²	TDCIPP	13674-87-8
Tris(2-butoxyethyl) phosphate ²	TBOEP	78-51-3
Tris(phenyl) phosphate ²	TPHP	115-86-6
2-Ethylhexyl diphenyl phosphate ²	EHDPP	1241-94-7
Tris(2-ethylhexyl) phosphate ²	TEHP	78-42-2
Tris (methylphenyl) phosphate ²	TMPP (TCP)	1330-78-5

¹Merck, Darmstadt, Germany; ²Sigma-Aldrich Chemie B.V. Zwijndrecht, the Netherlands. ³Dr. Ehrenstorfer Laboratories Augsburg, Germany.

Blanks

Background contamination is a major problem in the analysis of PFRs. Therefore, the participants were asked to report their blank levels found for each matrix. Almost each laboratory reported blank levels with different patterns and concentrations (Figure 2). For example, lab 3 reported TNBP and TBOEP as the two dominated PFRs in the blank, lab 8 reported TDCIPP and TCEP, lab 2 reported TBOEP and EHDPP and lab 5 TNBP and TIBP. Each laboratory seems to have their own blank problem for different PFRs most probably due to different sources in the lab. Differences in concentrations were also observed. The labs 2, 3, 4, 5 and 8 reported higher levels compared to the other labs. Similar variations in blanks were observed for the sediment and the fish oil methods. Overall, TNBP, TIBP, TBOEP and total-TCIPP were the most predominated PFRs reported in the blanks (Table 2).

The variety of the methods (extraction and cleanup) used could explain the different blank patterns and levels reported by the laboratories. However, some laboratories using the same extraction and cleanup method also showed different concentrations and patterns. Therefore, it is more likely that the blank problems are related to the experience of the laboratory to minimize the blank values, but also to its refurbishing and the activities.

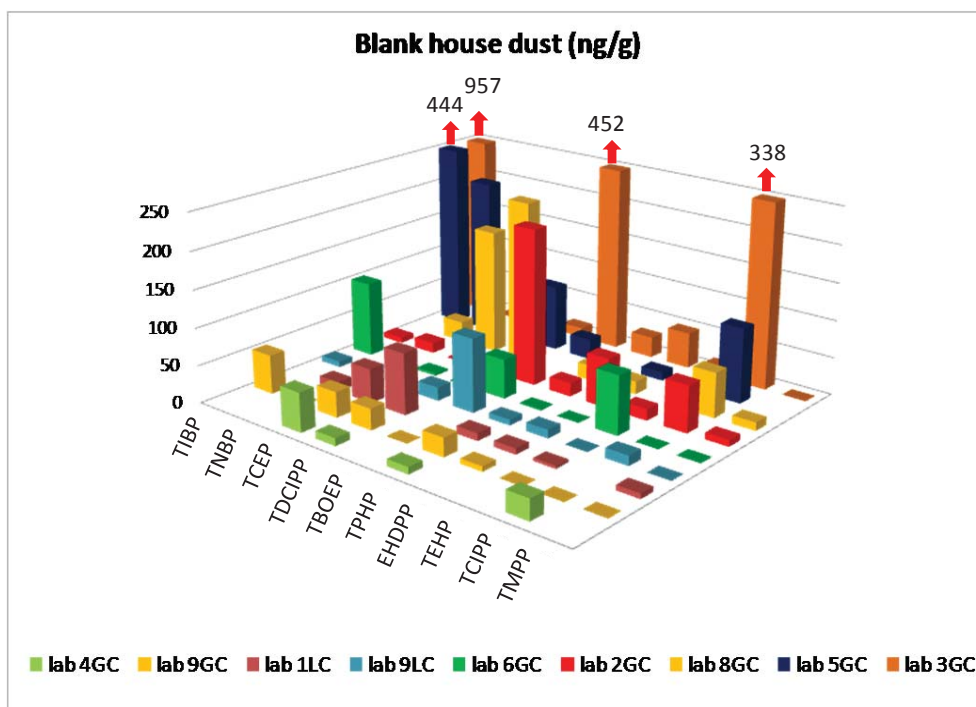


Figure 2. Mean (n=3) blank concentrations of the laboratories reported for the house dust method.

Table 2. The mean PFR blank levels reported by the participated laboratories for sediment, fish oil and house dust in ng/g.

ng/g	Sediment			Fish oil			House dust		
	mean	std	n	mean	std	n	mean	std	n
TIBP	19	34	17	114	175	8	262	345	16
TNBP	3.0	4.1	20	7.9	14	15	36	67	20
TCEP	4.7	7.0	20	11	12	18	45	51	20
TDCIPP	3.5	5.5	23	9.4	14	21	51	67	23
TBOEP	8.6	15	17	54	95	11	140	155	16
TPHP	1.8	1.9	20	4.8	6.5	18	15	8.6	20
EHDPP	2.9	4.1	15	4.7	7.6	17	21	22	20
TEHP	1.9	3.0	14	11	24	13	19	28	16
TCIPP	13	26	23	41	89	18	75	105	20
TMPP	0.2	0.4	17	4.5	11	14	3.8	4.5	17

There are different ways to minimize blank contamination. Minimizing surface contact during sampling handling by reducing cleanup, extraction and evaporation steps is important. PFRs have been detected in high levels in dust ($\mu\text{g/g}$) and indoor air (ng/m^3) (Marklund et al., 2003 and 2005; Staaf and Ostman, 2005; Bergh et al., 2011; Kanazawa et al., 2010). TBOEP was the major PFR in dust followed by TCIPP, TPHP and TDCIPP (Brommer et al., 2012; Van den Eede et al., 2011 and 2012). In

indoor air, TCIPP, TCEP, TNBP and TIBP were dominating (Marklund et al., 2003 and 2005, Staaf and Ostman, 2005; Bergh et al., 2011; Kanazawa et al., 2010) . Variation in PFR levels have been observed between buildings and between rooms within one building (Bergh et al., 2011; Kanazawa et al., 2010). This indicates that indoor PFR levels are influenced by various sources, like electronic equipment, furniture or isolation material (Marklund et al., 2003 and 2005; Bergh et al., 2011). TBOEP is used in floor polish which could be another source of blank contamination in laboratories (WHO, 2000). The high PFR levels observed in dust and indoor air can easily contaminate samples and glassware. Therefore, pre-cleaning of all glassware with a polar and non-polar solvent like hexane, acetone or methanol is essential. Especially glass frits in Soxhlet extraction thimbles and metal frits of accelerated solvent extraction (ASE) cells are sources of blank contamination (Quintana et al., 2008). Working in a clean room is recommended. If that is not available, it is necessary to cover all samples and glassware with aluminum foil to avoid dust contamination. Rodriguez et al., (2006) reported absorption of TEHP to glass wall during spiking of water samples. Rinsing the glass wall with an organic solvent solves this problem. Adsorption to glassware was also observed for TBOEP (Staaf and Ostman, 2005; Calsson et al., 1997). In this ILS water samples were not included. Therefore, losses of PFRs by adsorption to the glass wall would probably not take place. Because all the participants used organic solvents during extraction and cleanup (instead of water) the PFRs will be extracted from the glass wall. However, low recoveries have been observed for TBOEP during Soxhlet extraction with dichloromethane (Carlsson et al., 1997). Silica, florisil, amino-propyl silica or alumina columns or SPE cartridges used for cleanup may also contain PFRs. Therefore, pre-cleaning is needed with a solvent that is strong enough to extract the PFRs from the sorbents like dichloromethane, methanol, ethyl acetate or a mixture of these solvents. TBOEP contamination from Teflon layered silicon septa have been reported by Rodriguez et al. (2006). In the same paper the authors reported TBOEP contamination of biota samples by rubber stoppers (Zhang et al., 1996) and of water samples from taps with rubber O-ring and seals (LeBel et al., 1983). Fries et al. (2011) used tap water instead of ultra pure water taken from a Sartorius Arium 611VF purification system because of the four times lower blank levels for TNBP. Bergh et al. (2011) investigated in detail the PFR blank contamination of polyethylene SPE cartridges containing polypropylene frits used for air sampling and dust cleanup and no blank contamination was observed. However, this may be different for other SPE brands and batches. Overall plastic and rubber material should be avoided during sample handling.

Total-TCIPP and TNBP contamination can also come from the detection system itself, such as from the LC-MS/MS if necessary, a guard-column should be installed between the pump and the injector to trap this contamination. This is commonly used for the analyses of perfluoroalkyl substances (PFASs) with LC (Flaherty et al., 2005).

Extraction, cleanup and analytical methods

Suggested sample intakes were given in the instruction protocols sent to the participants and were as follows: 50 to 100 mg for the dust material, 1.5 to 2 g for the sediment, and 100 to 200 mg for the fish

oil. Still the sample intakes of the participants varied as 10-200 mg for house dust, 0.5 to 2 g for sediment and 75-200 mg for the fish oil. Different methods were used for the extraction of PFRs from sediment, house dust and fish oil sample (see Table 3). Ultrasonication and ASE was the most common extraction method used for sediment and house dust, followed by Soxhlet extraction. Five participants did not use an extraction method for the fish oil, but instead treated the fish oil as an extract and went directly to the cleanup. However, three participants still used ASE for the extraction and one used liquid-liquid extraction (LLE) for fish oil.

In Table 3, the various extraction solvent and cleanup methods are listed. Almost each participant used a different solvent mixture for the extraction of the sediment, house dust and fish oil sample. A mixture between non-polar and a polar solvent was most commonly used (e.g. hexane-acetone mixture). A wide range of cleanup methods were reported by the participants, in total eight different cleanup methods (see Table 3). Florisil columns/SPE was most common used to remove matrix interferences. Three participants used methods that they had already published (Kim et al., 2011; Chen et al., 2012; Van den Eede et al., 2012). Not all the participants provided information on the elution solvent used for their cleanup method. In general, hexane was used as a wash step for the Florisil SPE/columns and dichloromethane for the elution of the PFRs. Hexane-dichloromethane was used for the wash step for the amino propyl silica SPE columns and ethyl acetate for the elution of the PFRs. The use of at least one labeled PFR standards was mandatory. Nine of the fourteen participants corrected their result for the recovery of the internal standards. Two participants used only TNBP-d₂₇ as internal standard and six participants used TNBP-d₂₇ and TPHP-d₁₅, two participants used four labeled standards TNBP-d₂₇, TPHP-d₁₅ and respectively TCEP-d₁₂ and TDCIPP-d₁₅, three labs used five labeled standards TIBP-d₂₇, TPHP-d₁₅, TBOEP-d₆, TCEP-d₁₂ and TDCIPP-d₁₅, and one participants had six labeled internal standards TNBP-d₂₇, TCEP-d₁₂, TPHP-d₁₅, TEHP-d₅₁, TPrP-d₂₁ and TMPP-d₂₁. Only TNBP-d₂₇ and TPHP-d₁₅ are commercially available all the other labeled standards are probably synthesized by, or on behalf of the participant.

PFRs can be analyzed by liquid chromatography (LC) or gas chromatography (GC) coupled to mass spectrometry (MS). GC-MS in the electron-impact (EI) mode was used by nine participants. This is a sensitive and selective method for most PFRs included in this exercise with the exception of TNBP, TIBP, TEHP and the labeled internal standard TNBP-d₂₇. These PFRs undergo three consecutive McLafferty rearrangements, resulting in a GC-EI-MS spectrum with a base peak at m/z 99 (Björklund et al., 2004; Quintana et al., 2007). Quantification of TNBP, TIBP, TNBP-d₂₇ and TEHP using m/z 99 in a standard solution is no problem. However, when more complex matrices are analyzed, like dust, sediment and fish oil, the matrix may introduce noise in the low mass region that interferes with the m/z 99 (Björklund et al., 2004; Quintana et al., 2007) resulting in overestimated concentrations of these compounds. Quantification of TNBP, TIBP, labeled TNBP-d₂₇ and TEHP can therefore only be performed with the less sensitive ions m/z 211 or 212 for TNBP and TIBP, m/z 211 or 113 for TEHP and m/z 167 or 231 for TNBP-d₂₇, if GC-MS is used in the EI mode. Another disadvantage of the use of GC-MS is the decrease of response in time for TCEP, TDCIPP and TBOEP due to the degradation on the

column, injector and/or liner of the GC (Bergh et al., 2012; Bergh, 2011). The short chain alkyl PFRs TIBP and TNBP show tailing peaks with time (Bergh et al., 2012; Bergh, 2011). This is of course depending on the matrix and sample cleanup. It is therefore imported to check the stability of GC-MS during a run by randomly injecting a control standard through the sequence. The use of GC-MS in positive ion chemical ionization mode (PICl) with iso-butane as ionization gas may solve this problem (Bergh et al., 2012b).

Table 3. Extraction and cleanup methods used by the participating laboratories for the analysis of PFRs in sediment, house dust and fish oil.

Sediment	<i>n</i>	House dust (SRM 2585)	<i>n</i>	Fish oil	<i>n</i>
Extraction methods					
Ultrasonication	5	Ultrasonication	4	Liquid liquid extraction (LLE)	1
ASE	4	ASE	4	ASE	3
Soxhlet	2	Soxhlet	2	None	5
Total	11	Total	10	Total	9
Extraction solvents					
Hexane:Acetone (1:1, v/v)	2	Hexane:Acetone (1:1, v/v)	3	Hexane:Acetone (1:1, v/v)	2
Hexane:Acetone (3:1,v/v)	2	Hexane:Acetone (3:1,v/v)	2	ACN:Hexane (1:1, v/v)	1
DCM:Hexane (1:1, v/v)	1	DCM:Hexane (1:1, v/v)	1	DCM:Acetone (1:1, v/v)	1
DCM:Acetone (1:1, v/v)	1	DCM	1	None	5
DCM	1	MeOH:Acetone (6:4, v/v)	1	Total	9
MeOH:Acetone (6:4, v/v)	1	Ethyl Acetate	1		
Ethyl Acetate	1	Ethyl Acetate:Cyclohexane (5:2, v/v)	1		
Ethyl Acetate:Cyclohexane (5:2, v/v)	1	Total	10		
Ethyl Acetate:ACN (1:1, v/v)	1				
Total	11				
Cleanup methods					
Florisil columns/SPE	5	Florisil columns/SPE	4	Florisil columns/SPE	3
Florisil + Envi carb SPE	1	Florisil + Envi carb SPE	1	Florisil + Envi carb SPE	1
HLB SPE	1	HLB-SPE	1	NH ₂ -SPE	2
NH ₂ -SPE	1	Filtration sodium sulphate	1	GPC + NH ₂ -SPE	1
GPC + NH ₂ -SPE	1	GPC + NH ₂ -SPE	1	GPC	1
SiO ₃ /AgNO ₃ columns + deactivated alumina column	1	SiO ₃ /AgNO ₃ columns + deactivated alumina column	1	GPC + SiO ₃ column	1
Unkown	1	deactivated alumina column	1	Total	9
Total	11	Total	10		

n= participating laboratories

An alternative for quantifying the PFR levels is the use of LC-MS/MS, which has been chosen by six laboratories. A disadvantage of LC-MS/MS is the signal suppression or enhancement due to matrix effects. Rodil et al. (2005) observed strong matrix effects for TBOEP (enhancement), TCIPP

(enhancement) and TEHP (suppression) in treated wastewater samples. For TCEP, TDCIPP, TPHP, TNBP and TIBP, the matrix effects remained below 20%. However, changes of the LC-MS/MS signal due to matrix effects may be significantly different for each LC-MS/MS system due to the differences in source design and operation parameters. No internal standards were available at that time and therefore Rodil et al. (2005) concluded that quantification could only be performed by standard addition methods. Nowadays, three internal standards are commercially available: TNBP-d₂₇, TPHP-d₁₅ and tris(ethyl) phosphate (TEP-d₁₅). The PFR levels resulted from GC-MS were statistically compared with those resulted from LC-MS/MS. For all matrices no significant differences in mean concentrations between GC and LC were found (Anova, P>0.05), except for total-TMPP in dust where LC was significantly lower than GC (P<0.05). This indicates that matrix effects were less important or that the labeled internal standards corrected for suppression or enhancement. It should be mentioned that some participants using LC-MS/MS employed more than two labeled standards which compensated for matrix effects and improved the reliability of the results.

Within-laboratory performance

Almost each participating laboratory analyzed each sample in three independent replicates and, therefore, the within laboratory variation could be calculated. The within laboratory variation obtained for the LC standards solution was between 1 and 13% for all participating laboratories. For the GC standard solution, 91% of the participating laboratories obtained a within laboratory variation between 1 and 7%. The exceptions were TPHP (14%), TBOEP (2 labs; 10 en 23%), TDCIPP (11%), total-TMPP (19%), EHDPP (13%) en TEHP (22%). The within laboratory variation increased for the fish oil, house dust and sediment samples. For house dust, 89% of the participating laboratories obtained a within laboratory variation < 16% and 95% < 26%. For six PFRs, the variation was higher than 26%, namely total-TMPP (38%), TNBP (48%), EHDPP (42%), TEHP (58%), TPHP (25%) and TIBP (49%). For the fish oil, the within laboratory variation slightly increased to <24% for 86% of the participating laboratories with the exception of TPHP (51%), TIBP (2 labs; 29 and 46%), EHDPP (2 labs; 34 en 43%), TEHP (33%), TBOEP (27%), total-TMPP (29 and 35%) and total-TMPP (26%). The highest within laboratory variation was observed for the sediment. This is related to the relatively high blank levels observed for the sediment, close to the reported values (see Table 2). 61% of the labs obtained a within laboratory variation of <20%, 77% <30% and 81% <50%. The highest values were observed for total-TMPP (63%), EHDPP (63%), TPHP (67%), TBOEP (56 en 65%), TCEP (73 and 75%) and TDCIPP (87%).

Results of 14 laboratories from ten different countries were received. The results are summarized in Table 4. One laboratory reported two data sets, one analyzed with GC-MS and one analyzed with LC-MS/MS. Twelve laboratories reported results for the sediment sample, eleven for the house dust and ten laboratories reported data for the fish oil. Nine laboratories used GC-MS and six used LC-MS/MS. The assigned value is the mean value of all participants obtained from the Cofino Model (Cofino et al., 2000 and 2005).

Table 4. Results of the interlaboratory study.

GC solution						LC solution					
Determinand	Assigned value	Indicative Value	Between lab CV%	<i>p</i>	NObs	Determinand	Assigned value	Indicative Value	Between lab CV%	<i>p</i>	NObs
TIBP	113	NA	18	77	6	TIBP	NA	59	27	65	3
TNBP	51	NA	17	75	8	TNBP	NA	75	25	63	6
TCEP	141	NA	11	70	9	TCEP	143	NA	10	66	6
TDCIPP	119	NA	19	73	9	TDCIPP	NA	129	23	64	6
TBOEP	87	NA	25	69	7	TBOEP	140	NA	13	71	6
TPHP	50	NA	9	65	9	TPHP	NA	65	25	81	6
EHDPP	48	NA	21	75	7	EHDPP	NA	59	15	59	5
TEHP	122	NA	32	84	8	TEHP	139	NA	17	85	5
TCIPP total	101	NA	23	77	8	TCIPP total	NA	83	29	77	5
TMPP total	NA	128	43	77	6	TMPP total	105	NA	25	73	6

Fish oil						SRM 2585 house dust					
Determinand	Assigned value	Indicative Value	Between lab CV%	<i>p</i>	NObs	Determinand	Assigned value	Indicative Value	Between lab CV%	<i>p</i>	NObs
TIBP	NA	42	123	64	3	TIBP	NA	17	91	65	3
TNBP	37	NA	20	71	6	TNBP	269	NA	7	62	10
TCEP	130	NA	23	69	10	TCEP	792	NA	16	61	11
TDCIPP	57	NA	26	65	8	TDCIPP	1556	NA	34	64	11
TBOEP	107	NA	13	60	7	TBOEP	73464	NA	44	66	8
TPHP	26	NA	30	77	9	TPHP	1104	NA	9	53	11
EHDPP	47	NA	37	83	8	EHDPP	963	NA	21	76	9
TEHP	NA	46	68	87	7	TEHP	265	NA	42	61	10
TCIPP total	NA	57	50	73	7	TCIPP total	944	NA	28	79	10
TMPP total	NA	130	38	70	7	TMPP total	NA	843	48	80	10

Sediment					
Determinand	Assigned value	Indicative Value	Between lab CV%	<i>p</i>	NObs
TIBP	NA	2.9	121	57	4
TNBP	3.2	NA	62	64	10
TCEP	4.2	NA	33	66	11
TDCIPP	NA	1.9	96	59	8
TBOEP	7.0	NA	35	68	11
TPHP	NA	4.2	86	62	11
EHDPP	NA	3.6	39	62	8
TEHP	18	NA	15	65	9
TCIPP total	26	NA	41	67	11
TMPP total	NA	3.5	53	78	10

NA=not analyzed; *P*=probability factor; NObs=Total numbers of observations from each laboratories.

In some instances it is not possible to set an assigned value, because the data set is too small (<4), or the total error% >100% in combination with bad performance, and an indicative value is given. No assessment of laboratory performance is calculated where an indicative value is set. Besides the assigned, indicative value and the between lab coefficient of variation (CV), also a *p* value is listed in Table 4. This *p* factor is the percentage of data on which the mean and associated uncertainty (CV value) is based. The Cofino model automatically corrects for outliers, by selecting the most probable data distribution (probability density function) to calculate the assigned value. This *p* factor ranges

between 59 and 87% for the standards solutions, house dust, fish oil and sediment, which indicates a high reliability of the assigned values and associated uncertainties. The total number of observations reported by each laboratory (NObs) was also given in Table 4. Each number represents an independent triplicate analysis with the exception of one lab that did only a duplicated measurement.

Between-laboratory performance

Standard solutions

The CV values obtained for both standard solutions were acceptable (<25%) for most PFRs (9-25%) with exception of TIBP (27%), total-TCIPP (29%), TEHP (32%) and total-TMPP (43%) measured with GC-MS (see Table 4 and Figure 3). TMPP can consist of ten possible structural isomers and can only be separated by GC (Nola et al., 2008). The technical mixture of TMPP (CAS number 1330-78-5) contains four different isomers. For this study, the total-TMPP levels are reported. Potential sources of error could be response differences between the isomers. Erratic integration, sometimes including various isomers, may be one of the reasons for the higher CV values in GC/MS (43%) compared to LC-MS/MS (25%). However, it is more likely that the CV value is influenced by one of the participating laboratories, which used a cresyl diphenyl phosphate mixture from Fluka (OEKANAL, lot, SZBA203X) instead of the technical mixture of TMPP (CAS number 1330-78-5) for quantification. In this mixture, fewer congeners of TMPP are present compared to the technical mixture of TMPP, which explains the lower concentration (43 ng/g) reported by this participant compared to the indicative value (128 ng/g). TCIPP consist of three isomers which can be separated with GC (Rodriquez et al., 2006). In this study the total-TCIPP levels are reported. In general, the participants encountered no problems measuring the PFRs in both test solutions with the exception of total-TMPP.

House Dust, fish oil and sediment

Figure 3 shows that the CV values increase from house dust < fish oil < sediment and are probably more related to the concentration level than the matrix. The assigned PFR levels ranged from 1.9-26 ng/g in the sediment, from 26-130 ng/g in the fish oil and from 265-73464 ng/g in the house dust, with the exception of TIBP (17 ng/g) which was much lower compared to the other nine PFRs observed in house dust. Acceptable CV values (7-28%) are obtained for TNBP, TCEP, TPHP, EHDPP and total-TCIPP in house dust, for TNBP, TCEP, TDCIPP, TBOEP and TPHP (13-30%) in fish oil and only for TEHP (15%) in sediment. The CV values were somewhat higher for TDCIPP, TBOEP, TEHP and total-TMPP in house dust (34-48%), for EHDPP, total-TMPP and total-TCIPP in fish oil (37-50%) and for TCEP, TBOEP, EHDPP, total-TCIPP and total-TMPP in sediment (33-53%). Unacceptable CV values have been obtained for TIBP in house dust, TIBP and TEHP in fish oil and TIBP, TNBP, TDCIPP and TPHP in sediment.

The high CV values obtained for TIBP in all three matrices are influenced by the limited data points reported by the participating laboratories for TIBP in combination with high blank levels observed for some of the laboratories (see Table 2 and Figure 2). Also, the TIBP level (17 ng/g) in house dust was

more than ten times lower compared to the second lowest PFR detected in the house dust sample and close to the limit of detection, partly due the low sample intake (0.01-0.2 g).

CV values for total-TMPP in all three samples were comparable with the ones obtained from the standard solution. The CV values obtained for total-TMPP in the three samples was probably influenced by the integration error and the use of the different standard solutions which was already discussed for the standard solutions. Matrix residues interfering with m/z 99 used in some of the participating laboratories can have led to overestimation of the TEHP concentrations, resulting in higher CV values. This may explain the higher CV value for TEHP in the house dust and the unacceptable CV value for the fish oil. The relatively high CV values obtained for TDCIPP and TBOEP in house dust, fish oil and sediment may be influenced by the degradation of these PFRs in the GC (injector, liner or columns)(Bergh et al., 2012b; Bergh, 2011).

TNBP, TIBP, TBOEP and total-TCIPP were the most common blank values reported by the participating laboratories. Overall, the blank levels have the most influences on the results of this ILS. Especially for sediment which contains relatively the lowest PFR levels (1.9-26 ng/g). The blank values are often close to the reported values. The influences of the blank levels on the PFR levels in the sediment results in unacceptable CV values for almost all PFRs (see In Table 4 and Figure 3). The blank levels had less influence on the fish oil and the house dust results because the levels in the fish oil and house dust were much higher compared to the sediment. Figure 2 show that not all participating laboratories suffered from blank problems which means that it is possible to control the blanks.

Four of the participating laboratories used five or more labeled internal standards. The performances, based on the z-scores (data not shown), of these four laboratories were better compared to the laboratories using only one or two labeled standards. This indicated that there is a need for more commercially available labeled internal standards to improve the reliability of the PFR analysis. This shows that more internal standards should be available on a commercial scale.

PFRs versus PBDEs

In Figure 3 the results of this ILS have been compared with the results of the first worldwide ILS organized for PBDEs (De Boer and Cofino, 2002). The CV values for the PFRs in fish oil and house dust are comparable with those of the PBDEs. In both cases most of the CVs are lower than 40%, with the exception of TIBP and BDE209. BDE209 was more difficult to analyze because it is not stable at higher temperature, while no internal standard was available at the time and also blanks were a serious problem. The same problems have been observed for some of the PFRs, such as no available internal standards, relatively high blank levels for TIBP, TNBP, TBOEP and total-TCIPP and sensitivity for degradation in the GC for TDCIPP, TBOEP and TCEP.

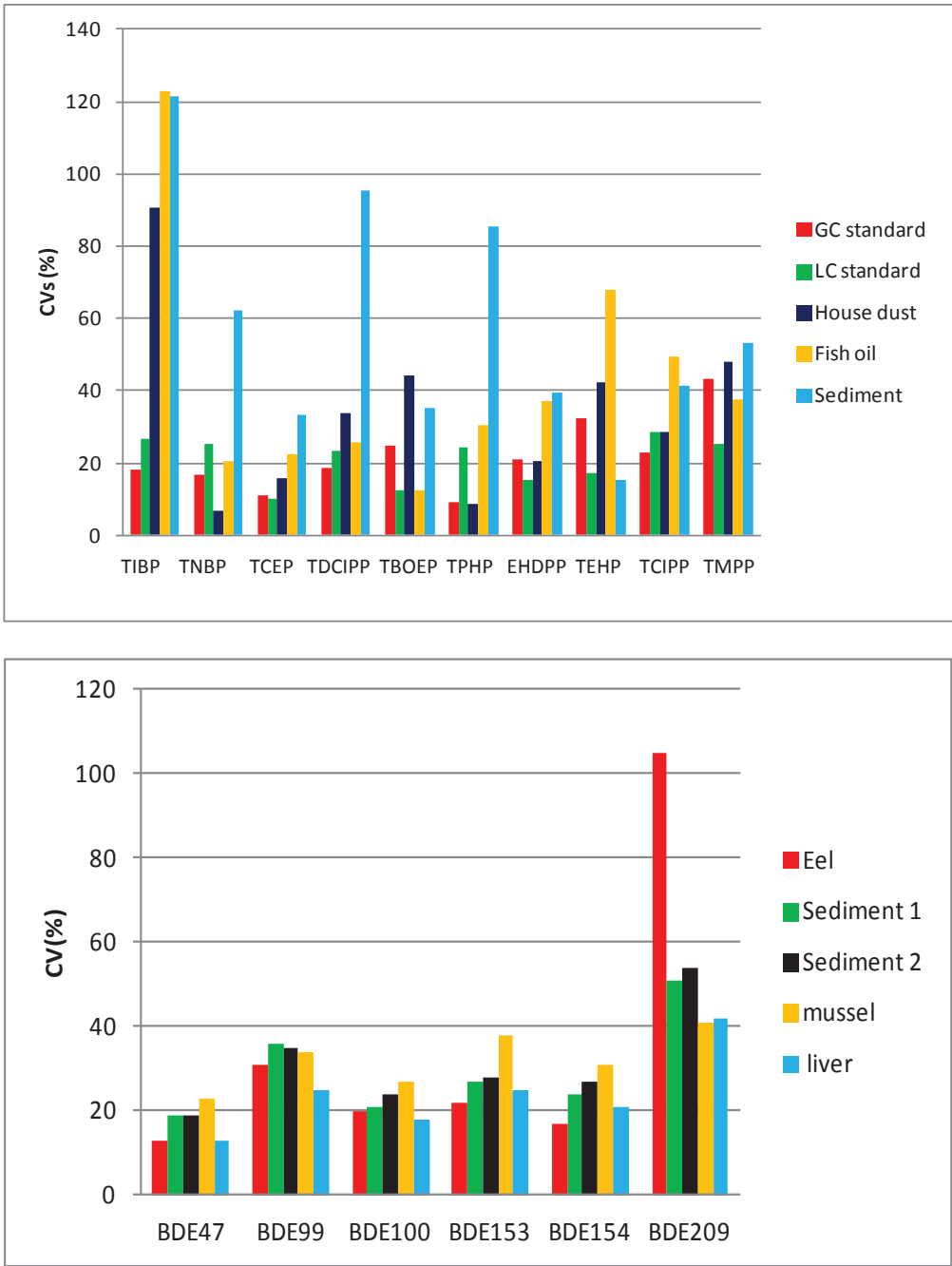


Figure 3. Group performance expressed as coefficient of variation (CV) between all laboratories for the ten PFRs in all matrices compared to the result of the first worldwide interlaboratory study organized for PBDEs.

Conclusion and recommendations

The first worldwide ILS for PFRs resulted in CV values for standard solutions of 9-25% for most PFRs with the exception of TIBP (27%), total-TCIPP (29%), TEHP (32%) and total-TMPP (43%) measured with GC-MS. The CV values increased for house dust, fish oil and sediment. Acceptable CV values (7-28%) were obtained for TNBP, TCEP, TPHP, EHDPP and total-TCIPP in house dust, for TNBP, TCEP, TDCIPP, TBOEP and TPHP (13-30%) in fish oil and only for TEHP (15%) in sediment. The CV values were somewhat higher for TDCIPP, TBOEP, TEHP and total-TMPP in house dust (34-48%), for EHDPP, total-TMPP and total-TCIPP in fish oil (37-50%) and for TCEP, TBOEP, EHDPP, total-TCIPP and total-TMPP in sediment (33-53%). The differences in results between the three samples are less related to the matrix type and more to the PFR concentrations in the test materials, in combination with the relatively high blanks reported by some of the participants. House dust contained the highest PFR levels followed by the fish oil and the lowest PFR levels were measured in the sediment sample. Because of the relatively high blank levels, in some instances close and, for some laboratories, higher than the reported level in the sediment, large variations in concentrations have been observed for the PFRs. This is also confirmed by the relatively high within-laboratory variation for sediment. Not all participating laboratories suffered from blank problems, which mean that it should be possible to control the blanks. Recommendations for a better analytical performance are given below.

- Minimizing the surface contact during sampling handling (reduce of blank).
- Solvent pre-cleaning of all glassware (reduce of blank).
- Working in a clean room if available (reduce of blank).
- Covering all sample and glassware with aluminum foil to avoid dust contamination (reduce of blank).
- Solvent pre-cleaning of the ASE and Soxhlet, especially the glass thimbles of the Soxhlet and the metal frits of the ASE cells (reduce of blank).
- Solvent pre-cleaning of the silica, florisil, amino-propyl silica or alumina SPE columns used for the cleanup (reduce of blank).
- Refraining from the use of Teflon layered silicon septa, rubber stops and floor polish in the laboratory (reduce of blank).
- Checking the LC-MS/MS for system blanks and if necessary installing a guard-column between the pump and the injector (reduce of blank).
- Checking TCEP, TDCIPP and TBOEP degradation in the GC column and injector liner (analytical performance).
- Selection of specific m/z values for the target PFRs (analytical performance).

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