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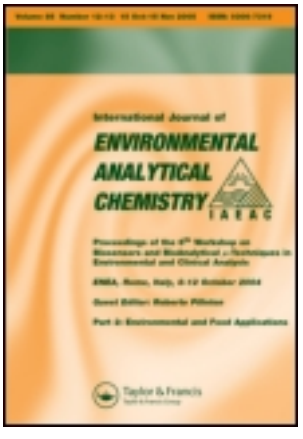

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An improved, rapid method for polychlorobiphenyl (PCB) extraction from lipid rich matrices and determination by GC-MS is presented. PCBs accumulate in the environment, can be extremely persistent, and health complications including neurotoxic effects have been reported. Thus, monitoring these persistent organic pollutants seems prudent. The analytical procedure for PCB assessment from environmental samples typically consists of the three steps extraction, purification and analysis by GC coupled with electron-capture detection or mass spectrometry. The aim of the present study was the implementation of a protocol allowing for determining selected indicator PCBs ($n = 6$) following a single extraction/purification step. Pressurised liquid extraction (PLE) combined with different silica layers directly in the extraction cell allowed efficient removal of lipids and direct analysis by GC-MS. Accuracy was determined by comparison with a reference standard (SRM 1946), and the equivalence of PLE to an established extraction method (soxhlet) was verified. Mean recovery of the combined procedure from trout spiked with a mixture of 50 ng of each PCB was 87 ± 8 (range 74–94)%, and results of ASE were comparable to soxhlet (difference total PCBs <16%). In addition, the protocol showed higher throughput (20 min/extraction cycle) and required less organic solvents (90 mL/sample). This method was then applied for monitoring PCBs in a variety of Luxembourgish fresh water fish (trout, eels, roaches, $n = 38$). The PCB profile was dominated by congeners 153 and 138, with maximum concentrations of 30 and 21 ng g^{-1} trout (fresh weight), respectively, highlighting that PCB concentrations might vary considerably in fish depending on species, eating habits and weight.

Keywords: PCBs; pressurised liquid extraction; fish; persistent organic pollutants

1. Introduction

Polychlorobiphenyls (PCBs) correspond to a category of chlorinated aromatic hydrocarbons, encompassing 209 congeners, differing in the number and position of chlorine atoms bound. The basic structure of PCBs is given in Figure 1 [1–3]. Taking into account thermodynamic aspects and spatial configuration constraints, the number of existing congeners can be estimated to range between 130 and 150 [4].

PCBs were synthesised for the first time in 1881 in Germany, and produced industrially since 1929. Since then, over 2 million tons of PCBs [5] have been produced as commercial

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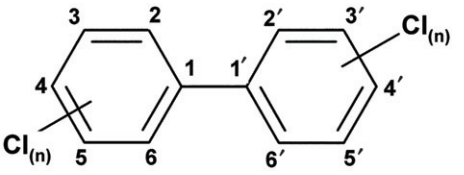

Congener	Number of chlorine atoms	Molecular mass ($g\,mol^{-1}$)	Position of chlorine atoms
18	3	256.0	2-2′-5
28	3	256.0	2-4-4′
31	3	256.0	2-4′-5
44	4	289.9	2-2′-3-5′
52	4	289.9	2-2′-5-5′
101	5	323.9	2-2′-4-5-5′
118	5	323.9	2-3′-4-4′-5
138	6	357.8	2-2′-3-4-4′-5′
149	6	357.8	2-2′3-4′-5′-6
153	6	357.8	2-2′-4-4′-5-5′
170	7	391.8	2-2′-3-3′-4-4′-5
180	7	391.8	2-2′-3-4-4′-5-5′
194	8	425.8	2-2′-3-3′-4-4′-5-5′-6
209	10	493.7	2-2′-3-3′-4-4′-5-5′-6-6′

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mixtures such as Aroclor, Clophen or Pyralène. PCBs present the capacity to store electrostatic energy and have therefore been used in transformers, condensers and electromagnets. They have in addition been widely used as oil bath radiators, heat transfer fluids, hydraulic fluids, lubrication oils and in paints and textiles for their adhesive qualities [5].

The long-term stability of PCBs proved to be a major problem. Once in the environment, congeners do not readily break down and therefore may remain for long periods of time cycling between air, water and soil. PCB degradation depends largely on their degree of chlorination, as the persistence increases with the degree of chlorination [6]. Half-lives for PCBs undergoing photo-degradation range from approximately 10 d to 1.5 y [7]. Thus, their chemical and physical properties favour long-range transport; PCBs have been detected in Arctic air, water and organisms [8], especially within species that rank high in the food chain [9]. Consequently, organisms living in polluted waters, especially fish and organisms consumed as seafood, can store PCBs in their fatty tissues, representing a risk for human consumption [10].

PCB congeners can be divided into two major groups, based on their biological activity and toxicity: the dioxin-like PCBs (4 non-ortho and 8 mono-ortho) and congeners with 'non-dioxin-like' toxicity. The dioxin-like PCBs exert a wide range of toxic responses especially on the endocrine system, while PCBs with two or more ortho chlorine atoms seem to produce neurotoxic effects [11]. In the middle 1980s, following decades of massive use and cases of poisoning in Japan (1968) and Taïwan (1979), toxicological studies about PCBs were published leading to their restriction of use and to the prohibition of their production [12,13]. Data on the occurrence and the distribution of non-dioxin-like PCBs in ecological systems, however, has remained limited [14]. Nevertheless, several studies have meanwhile shown that PCB 153 (IUPAC) and PCB 138, both non-dioxin-like, can be predominant in fish and seafood samples in significant concentrations, up to 40 $ng\,g^{-1}$

fresh weight [15–18]. Thus, with respect to their long persistence there is an ongoing need for methods to allow monitoring a number of environmental or food samples for their non-dioxin-like PCB concentrations, such as in Luxembourg where few data are available.

The analytical procedure for PCB assessment in food matrices consists, in general, of three steps: extraction, purification, separation and quantification by GC coupled with electron-capture detection (GC-ECD) or mass spectrometry (GC-MS). Soxhlet extraction [15] and matrix solid-phase dispersion [19] have been the traditional methods used for extraction of PCBs from environmental samples. However, soxhlet extraction usually requires large amounts of solvent (c. 200 mL) and is often carried out for 18 h. A variation to soxhlet is ultrasound-assisted extraction, which can reduce the total time of extraction, to about two hours, due to closer contact between tissue and solvent [20]. In order to minimise solvent consumption and reduce extraction time, new extraction techniques have been investigated including supercritical fluid extraction [21], micro-scale method using ultrasonication [22], microwave-assisted extraction [23] and pressurised liquid extraction (PLE) [24]. However, all these extraction procedures tend to be of limited selectivity and thus extract a wide range of undesirable organic compounds including lipids, which tend to be problematic for PCB detection from lipid-rich samples. Consequently, prior to chromatographic determination of PCBs, these matrix compounds have to be removed by additional purification steps to avoid excessive background noise within chromatograms and to prevent column contamination, to result in lower detection limit and improved repeatability. Depending on the complexity of the sample matrix, different cleanup techniques can be applied. Classical liquid adsorption chromatography is still the dominant technique, involving alumina, silica gel and florisil [25]. For extracts containing considerable amounts of lipid, sulphuric acid can be used prior to adsorption chromatography in order to destroy fatty acids [26]. Despite their efficiency, these treatments can be time-consuming, lead to decrease of the overall analytical throughput, and increase the risk of additional PCB losses and analytical costs.

The aim of the current investigation was to set up an improved protocol for PCB analysis, allowing the determination of congeners following a single extraction step. We describe a rapid method consisting of an in-cell cleanup procedure with PLE allowing simultaneous extraction and purification of samples, followed by direct analyses with GC-MS. This method was then applied for the detection of PCBs in a variety of fish samples, especially trout, which is most frequently caught for consumption in Luxembourg.

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2. Experimental

2.1 *Reagents and standards – target compounds*

All reagents used for the analysis of PCBs were of trace analysis grade. Sulphuric acid (98%), silica gel 60 and anhydrous sodium sulphate were supplied by Merck (Darmstadt, Germany). n-Hexane was purchased from Biosolve (Valkenswaard, The Netherlands). Standards of PCBs, d_{12}-chrysene and d_{10}-phenanthrene (internal standards for injection) were supplied by Sigma-Aldrich (Seelze, Germany) and LGC Promochem (Molsheim, France). Before use, sand was dried overnight at 200°C and washed with n-hexane. Silica gel was deactivated during 12 h at 200°C. Impregnated acid-silica was prepared by mixing deactivated silica gel with sulphuric acid 98% (40% w/w). All adsorbents were stored in hermetical flasks until use.

Table 1. Pressurised liquid extraction* parameters used for fish extraction (ASE100, Dionex).

	Lipid extraction	PCB extraction
Extraction temperature (°C)	125	125
Pressure (Psi)	1500	1500
Heat up time (min)	5	5
Static time (min)	5	3
Flush volume (%)	60	60
Purge time (s)	60	60
Number of static cycles	3	3
Solvent	n-Hexane	n-Hexane
Sorbent	None	Silica + acid impregnated silica

*The sample cell was heated in the oven and extraction was performed by direct contact of the sample with the hot solvent in both static and dynamic modes.

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A set of 14 PCBs (including internal standard PCB209) was analysed, including the six congeners considered as indicators for food contamination monitoring by the French Food Safety Agency (AFSSA): PCB-28, 52, 101, 138, 153 and 180 [1]. The number and position of chlorine atoms bound to the biphenyl rings for these six congeners are shown in Figure 1. These congeners are present in commercial mixtures such as AROCLOR, relatively persistent in the environment, and have been reported to account for approximately 50% of all PCBs present in food items of animal origin and in human fat tissue [1]. In addition, a 'dioxin-like' congener (PCB 118), to which a toxic equivalency factor has been assigned by the World Health Organization (WHO-TEF), and six other congeners relevant due to their presence in the environment (PCB-18, 31, 44, 149, 170 and 194) as listed in the DIN EN Norm 12766 [27] were also measured. PCB 209 was used as internal standard to monitor PCB extraction and check potential leakage during PLE.

2.2 *Analytical procedure*

2.2.1 *Sample preparation and lipid determination*

Fish fillets, including skin, were prepared, mixed and blended with a food processor (La Moulinette (Moulinex, Ecully Cedex, France)), then freeze-dried for 24 h and stored at −20°C until treatment. The lipid content was determined by pressurised liquid extraction (PLE) on an ASE100 (Dionex, Sunnyvale, CA) and was carried out in duplicate. The equivalent of 15 g of homogenised fresh tissue was mixed in a mortar with the same amount of washed sand (Eggert Luchterhand GmbH, Achim, Germany) and introduced into a 34 mL cell (Dionex ASE cell). The parameters used for the lipid determination are given in Table 1. Following PLE, the resulting extract containing the lipids was evaporated until dryness by rotary evaporation (40°C, 300 mbar, Laborata 4000, Heidolph GmbH & Co, Schwabach, Germany). Lipid content was determined gravimetrically and expressed as percentage of wet weight.

2.2.2 *PCB extraction*

The sample preparation scheme is outlined in Figure 2. Extractions were performed by PLE. Prior to extraction, a 34 mL cell was prepared by placing one cellulose filter

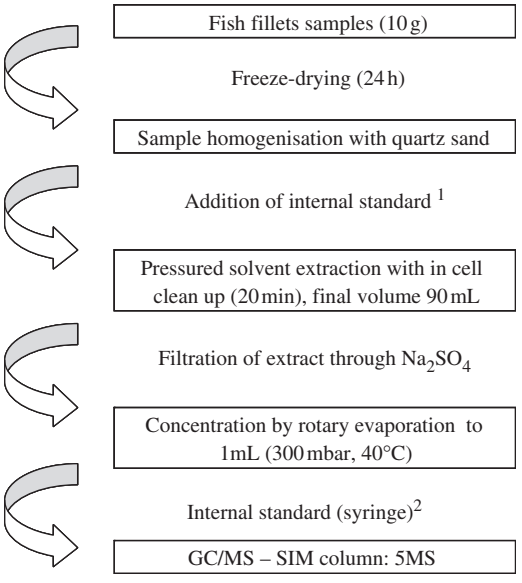

Figure 2. Major preparation and analysis steps used for determining PCBs in fish tissue based on pressured solvent extraction ([1]: PCB 209 was added as internal standard. [2]: D-10 phenanthrene and D-12 chrysene were added as internal standards).

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(30 mm, 1.2–1.3 μm pore size, Dionex) at the outlet and two successive layers of silica and acid-impregnated silica (a total of c. 4 g). Three grams of freeze-dried sample (amount depending on lipid content, containing approximately 500 mg lipids) was mixed with about 10 g of washed sand in a mortar and added to the top of the cell. Preceding the extraction, 50 ng of PCB 209 were added. Following extraction (Table 1), the resulting c. 90 mL extract was filtered through anhydrous sodium sulphate. The filtrate was then concentrated by rotary evaporation to a final volume of 1 mL. Prior to GC-MS analysis, 1 μg of the internal standards of injection (chrysene, phenanthrene) were added. Temperature, solvent, and adsorbents were manipulated in order to verify for optimal extraction procedure. Completeness of extraction was verified by a second extraction of the same cell with identical analytical conditions.

2.2.3 *Limit of detection, recovery, accuracy, precision of method*

(a) *Recovery and precision*: To determine the quality of the method, recovery investigations were performed on a pool of spiked trout fillet ($n = 18$), with an initial content below the detection limit to be determined (i.e. no PCBs could be determined), following sample processing and GC-MS analysis. Trout was chosen as it was expected to be the most frequently analysed fish in the future. Trout fillet was also used to compare PLE ($n = 3$) to the established soxhlet method ($n = 6$). For recovery experiments, trout samples were spiked with 200 μL of a mixture containing PCB-28, 52, 101, 153, 138,180 and 209 at 200 ng mL^{-1} for each PCB prior to extraction.

(b) *Limit of detection*: The detection and quantification limits were determined by the method detection limits (MDLs) according to EPA standard procedures [28], injecting seven times spiked trout samples containing the six indicator PCBs, with three times the

SD of this series been defined as the limit of detection (LOD) and six times the SD defined as the limit of quantification (LOQ). For this purpose, samples were spiked with 100 µL of a mixture containing PCB-28, 52, 101, 153, 138,180 and 209 at 200 ng mL^{-1} for each PCB prior to extraction (around 4 ng g^{-1} of each PCB per fresh weight).

(c) *Accuracy*: Analyses were carried out with a standard reference material (SRM 1946, Lake Erie trout) in order to assess PLE method's performance and accuracy. For these purposes, c. 5 g of the reference material were processed in quintuples.

2.2.4 *GC-MS analysis*

Determination of PCBs was carried out by GC coupled to mass spectrometry. Ions were obtained by electron impact at 70 eV. Analyses were performed on a HP6890-GC system with a 5973 MSD detector (Agilent, Germany). Samples were injected in splitless mode (1 µL; injector temperature: 280°C) on a HP-5MS capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness, Agilent). The oven temperature program was elevated from 80°C to 280°C at 5°C min^{-1}, with a final 5 min hold. Helium was used as carrier gas at a constant flow rate of 1.3 mL min^{-1}. Transfer line, source and quadrupole temperatures were set at 300°C, 230°C and 150°C, respectively. Analyses were accomplished in single ion monitoring (SIM) mode, using homologue retention time windows to increase sensitivity wherever possible. The identification of target compounds was based simultaneously on retention time, resulting fragment ions and on the maintenance of ratio among these fragments, in order to reduce the error of chromatogram interpretation due to the possible coelutions [29]. However, due to similarity of several PCB congeners and limited resolving power of the presented method, it is expected that several additional, albeit in general somewhat less frequently abundant PCBs, could co-elute with the indicator congeners. Table 2 summarises the detection parameters for target compounds and internal standards.

Quantification was achieved by external calibration curves. The internal standards, d_{12} chrysene (for PCBs 170, 180, 194 and 209) and d_{10} phenanthrene (for PCBs below congener 170), were used to compensate the variability of the injection. Calibration curves using standard solutions were recorded in the 10–150 ng mL^{-1} range, at six levels: 10-20-40-80-100 and 150 ng mL^{-1}. For all compounds, the linear regression was accepted if the correlation coefficient (r^2) was above 0.99.

Lipid carry-over between runs was verified by injection of blank samples every two fish samples. Additional method blanks were run to determine background noise and potential interference by the chemicals used.

2.2.5 *Collection of caught fish*

The sample collection area was located in the northern part of the Grand-Duchy of Luxembourg with its more intact ecosystem. Fish caught at $n = 11$ fishing stations distributed along five different rivers (Haute-Sûre, Our, Wiltz, Wark and Troine) were analysed. Trout (*Salmo trutta*), roaches (*Rutilus rutilus*) and eels (*Anguilla anguilla*) were caught by electric fishing between August and September 2007. These fish are typically the most frequently caught and consumed varieties in Luxembourg. Fish that were caught were stored in a coolbox for a maximum of 6 h, and were kept frozen at −20°C until further analysis. All fish samples ($n = 38$) were analysed in triplicate and all data were corrected to the internal standards of injection.

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Table 2. Mass spectrometry acquisition parameters and ratio between quantification ion and qualifier ions from GC-MS analysis of PCB in fish.

	Target ion (m/z)	Qualifier 1 (m/z)	%[a]	Qualifier 2 (m/z)	%[a]	Qualifier 3 (m/z)	%[a]
d_{10} Phenanthrene[b]	188	–	–	–	–	–	–
PCB 18	256	258	90	260	30	–	–
PCB 31	256	186	50	258	90	260	25
PCB 28	256	186	50	258	100	260	30
PCB 52	292	220	80	290	75	294	50
PCB 44	292	220	85	290	75	294	45
PCB 101	326	256	50	324	60	328	60
PCB 149	362	290	80	360	100	358	60
PCB 118	326	256	30	328	60	324	60
PCB 153	360	290	55	362	80	358	50
PCB 138	360	290	60	362	90	358	60
d_{12} Chrysene[b]	240	–	–	–	–	–	–
PCB 180	394	324	60	396	95	398	50
PCB 170	396	324	70	394	100	392	45
PCB 194	430	358	60	428	90	426	30
PCB 209[c]	498	426	40	500	80	496	70

[a]Ratio between qualifier area and target ion area.
[b]Used as internal standard of injection. d_{10} phenanthrene for PCB-18, 31, 28, 52, 44, 101, 149, 118, 153 and 138. d_{12} chrysene for PCB-180, 170, 194 and 209.
[c]Used as internal standard of extraction.

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2.2.6 *Statistical analyses*

Statistical analyses were carried out using SPSS 16.0 (Chicago, IL). Normal distribution of data (PCB content in fish, PCB recovery from spiking experiments, and comparison of soxhlet versus PLE) was tested with Q-Q plots and Kolmogorov-Smirnoff tests, equality of variance by Box-plots and Levene's test. Comparison of recovery and concentrations between different rivers was carried out by 1-way ANOVA followed by post-hoc tests (Tukey's). *P* values below 0.05 (2-sided) were considered as significantly different. For correlations between weight and PCB content of the caught fish from the rivers, Spearman correlation coefficients were obtained as data were not entirely normally distributed. All other data was shown to be normally distributed. Unless otherwise stated, all values represent mean ± SD (standard deviation).

3. Results

3.1 *Extraction, recovery, limit of detection, and precision*

Extraction: Parameters including solvent, time, and temperature of ASE extraction, as well as the choice of adsorbents (e.g. silica, florisil and neutral alumina, all VWR, Fontenay sous Bois, France) were optimised according to reduction of interfering compounds in the final chromatogram (data not shown). For example, dichlormethane was tried as an extractive solvent, but resulted in inferior chromatographic purity. In addition, a larger extraction cell (100 mL) was tried; however, this did not improve further the signal to noise (S/N) ratio. The final PLE protocol applied was rapid and could be performed within 20 min per sample. Method blanks including only used chemicals did not result in the

Table 3. Results of PCB recovery experiments for pressurised solvent extraction with in cell cleanup from trout spiked with six indicator PCBs.[1]

PCB	Molecular formula	Mean amount PCB spiked (ng)	Mean amount PCB recovered (ng)	Recovery (%) ± SD[1]
PCB 28	$C_{12}H_7Cl_3$	10 ± 1	7 ± 3	$75 \pm 19^{A(2)}$
PCB 52	$C_{12}H_6Cl_4$	11 ± 1	9 ± 3	91 ± 13^{B}
PCB 101	$C_{12}H_5Cl_5$	11 ± 1	9 ± 3	91 ± 11^{B}
PCB 138	$C_{12}H_4Cl_6$	11 ± 1	10 ± 3	94 ± 12^{B}
PCB 153	$C_{12}H_4Cl_6$	11 ± 1	10 ± 3	93 ± 11^{B}
PCB 180	$C_{12}H_3Cl_7$	11 ± 1	9 ± 3	88 ± 10^{AB}
PCB 209	$C_{12}Cl_{10}$	10 ± 1	7 ± 3	74 ± 14^{A}

[1]Recovery rate and standard deviation calculated for $n = 18$ for each individual PCB.
[2]Recoveries not sharing the same superscript indicate statistically significant differences (ANOVA, followed by Tukey's, $P < 0.05$).

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determination of any traces of PCBs, and blanks in between runs on fish matrices did not indicate significant carry-over of lipids.

Recovery: The recovery determination focused on the six PCB congeners (PCB-28, 52, 101, 153, 138 and 180), as potential target analytes, which are frequently the most abundant in various environmental matrices. The recovery rate of PCB 209 was investigated too, in order to use it as internal standard of extraction, i.e. to check potential leakage during PLE. The recovery rate of the congeners is detailed in Table 3. Mean recoveries ranged from 74–94% with standard deviations below 19%, and with an average (global) recovery rate of 87 ± 8%. PCBs 28 and 209 had significant lower recovery rates of 75 ± 19% and 74 ± 14% when compared to the other PCBs ($P < 0.0001$, ANOVA F-test, followed by Tukey's post-hoc test).

Limit of detection: LOD was determined to be 20 ng g^{-1} lipid, LOQ 40 ng g^{-1} lipid, using spiked trout as a matrix, and was comparable for all individual PCBs.

Precision: Precision of the overall method, as determined from the standard deviation following injection of the spiked trout at 100 ng g^{-1} lipid was found to vary between 10 and 19% for individual PCBs, with higher values in principle for the lighter PCBs (Table 3).

3.2 *Reference standard and comparison with soxhlet*

Accuracy, as verified by comparing the results of the presented method with results of a standard reference material suggested a sufficient accordance. Recovery from the standard reference material (1946, trout fish) was 54, 50, 76, 62, 78 and 72%, for predominant PCBs 52, 101, 118, 153, 138 and 180, respectively (average all PCBs 68%).

Comparison of PLE with soxhlet revealed similar values between the two extraction methods, with no statistical difference between either individual PCBs or mean PCB content (Table 4).

3.3 *Occurrence and distribution of PCBs in fish samples*

Considering the number of fish caught, this environmental study focused on the results obtained from trout. The mean size and weight for the 31 trout caught was 23 ± 4

Table 4. Comparison of the concentration of six indicator PCBs obtained following soxhlet and pressurised liquid extraction (PLE), employing a contaminated trout sample caught in Luxembourg.

	Concentration obtained by Soxhlet $n=6$	Concentration obtained by PLE $n=3$	P value
PCB 28	<LoQ**	<LoQ	n.d.
PCB 52	<LoQ	<LoQ	n.d.
PCB 101	2 ± 1	3 ± 1*	NS^{+}
PCB 138	12 ± 4	15 ± 1	NS
PCB 153	13 ± 4	15 ± 1	NS
PCB 180	6 ± 2	7 ± 1	NS
ΣPCBs	33 ± 8	39 ± 3	NS

*All values in ng g^{-1} fresh weight, mean ± SD.
**LoQ: Limit of quantification (40 ng g^{-1} lipid weight).
$^{+}$NS: not significantly different, $p>0.05$ (Student's paired t-test, 2-tailed).

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(range: 4–34)cm and 151 ± 79 (range: 30–405)g. For the five roaches caught, means of 17 ± 3 (range: 15–17)cm and 71 ± 15 (range: 57–87)g were obtained. Only two eels were caught, one of 78 cm and 1102 g and the other one of 71 cm and 615 g. The amount of lipid showed a weak variation between trout and roaches, being 3.9 ± 2.0 (range: 0.6–10.3)% and 4.3 ± 2.6 (range: 2.2–8.3)%, respectively, while a mean amount of lipids of 34.3% (range: 32.6–36.0%) was found for eels.

Arithmetic mean, SD and range of PCB concentrations in ng g^{-1} fresh weight (ng g^{-1} fw) obtained for contaminated fish are presented in Table 5. Total PCB concentrations varied largely between rivers and fishing stations with values ranging from not determined (n.d.) to 94 ng g^{-1} fw. The Our and particularly Wallendorf showed highest concentrations (38 to 94 ng g^{-1} fw), followed by the Wiltz (8 to 28 ng g^{-1} fw). While from the Wiltz, all analysed fish were contaminated, only 70% from the Our contained PCBs. The Troine and the Haute-Sûre presented the lowest levels of PCB content (n.d. to 8 ng g^{-1} fw; n.d. to 14 ng g^{-1} fw, respectively). Statistical analyses showed a positive correlation between total PCB content and fish weight of trout ($r=0.816$, $P=0.003$) for the Our and for the Haute-Sûre ($r=0.910$, $P=0.001$). The values obtained for the sum of the six indicator congeners and the sum of 13 PCBs were interestingly to a large extent quite similar. Differences existed only for 2 fishing stations. For the station Syrbach, the variation of PCB concentration was very low, below 2 ng g^{-1} fw, whereas for the higher contaminated fish from Wallendorf, this variation was higher (c. 24 ng g^{-1} fw).

Mean concentrations, SD and range for the individual six European indicator congeners in trout are shown in Table 6. In almost all cases, with one exception, the six PCB indicator compounds accounted for the majority of all PCBs detected. PCB 28 and 52 were never detected in this study, while hexachlorobiphenyls, PCB 138 and 153, were detected in 74% and 68% of the analysed trout. In addition, they were accounting for 31% and 28%, on average, of the total PCB concentration, respectively. PCB 101 accounted for 24% of all PCB and PCB 180 for 17% of detected PCBs.

4. Discussion

In this study, we demonstrated the successful extraction and quantification of common PCBs from a complex, lipid-rich food matrix, using a single simultaneous extraction and

Table 5. PCBs residues in contaminated trout in various Luxembourg river locations.*

River	Fishing stations	n**		PCB content[a] (ng g^{-1} fw[b])	PCB content (ng g^{-1} lw[c])
Haute-Sûre	Moulin de Bigonville	2/3	Σ13PCBs[d]	8 (2–14)	850 (411–1288)
			Σ6PCBs[e]	8 (2–14)	850 (411–1288)
	Syrbach	3/3	Σ13PCBs	11 ± 2 (9–13)	293 ± 174 (92–399)
			Σ6PCBs	11 ± 1 (9–12)	277 ± 161 (92–386)
	Martelinville	1/2	Σ13PCBs	10	167
			Σ6PCBs	10	167
Our	Wallendorf	2/2	Σ13PCBs	66 (38–94)	1293 (1176–1410)
			Σ6PCBs	49 (29–70)	967 (886–1049)
	Stolzembourg	4/5	Σ13PCBs	12 ± 10 (3–27)	270 ± 185 (85–524)
			Σ6PCBs	12 ± 10 (3–27)	270 ± 185 (85–524)
	Moulin de Kalborn	1/3	Σ13PCBs	6	292
			Σ6PCBs	6	292
Wiltz	Winseler	3/3	Σ13PCBs	15 ± 10 (8–27)	372 ± 130 (222–459)
			Σ6PCBs	15 ± 10 (8–27)	372 ± 130 (222–459)
	Merckholtz	2/2	Σ13PCBs	20 (11–28)	377 (256–498)
			Σ6PCBs	18 (11–25)	344 (256–433)
Wark	Welscheid	3/4	Σ13PCBs	9 ± 3 (6–12)	274 ± 89 (186–364)
			Σ6PCBs	9 ± 3 (6–12)	274 ± 89 (186–364)
Troine	Neimillen	2/3	Σ13PCBs	6 (4–8)	183 (108–257)
			Σ6PCBs	6 (4–8)	183 (108–257)

*31 trout, 5 roaches and 2 eels were caught. For roaches, only one fish contained PCBs with Σ13PCBs = Σ6PCBs = 4 ng g^{-1} fw (183 ng g^{-1} lw). For eels, two positive samples were caught: Σ13PCBs = 173 (range: 170–176)ng g^{-1} fw (506(range: 473–540)ng g^{-1} lw) and Σ6PCBs = 159 (range: 149–170)ng g^{-1} fw (465 (range 457–473)ng g^{-1} lw).
**Number of contaminated trouts/number of trouts caught.
[a]Mean ± standard deviation (range). Only values above LOQ (40 ng g^{-1} lw) [28] are reported. Number of PCBs positive trout samples: 23.
[b]ng g^{-1} fresh weight.
[c]ng g^{-1} lipid weight.
[d]Σ13PCBs: sum of PCB-18, 28, 31, 44, 52, 101, 118, 138, 149, 153, 180, 170 and 194.
[e]Σ6PCBs: sum of PCB-28, 52, 101, 138, 153 and 180, corresponding to the six indicators [1].

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purification procedure, avoiding complex sample processing procedures prone to cause analyte losses. The mean, global recovery of 87 ± 8% of the six indicator PCBs from a spiked complex matrix indicated that the method was feasible for detecting the main PCB compounds with a reasonable amount of losses and variability. The method showed good correlation and slightly higher recovery compared to the established soxhlet method, and sufficient accuracy with less than 32% deviation from a certified fish reference material. In addition, this method was successfully implemented for a preliminary screening of PCBs in Luxembourgish fish species.

Several sample extraction procedures have been previously suggested for PCB isolation from food matrices. De Azevedo e Silva *et al.* [15] performed sample extraction with soxhlet followed by lipid removal with sulphuric acid and an additional sample cleanup using a florisil column. Even though soxhlet has been shown to have a comparable extractive capacity for PCB compared to PLE [24], extraction times of 8–12 hrs are often considerably longer, even taking into account the possibility of simultaneous

Table 6. Summary of six PCB indicator compounds as determined in trout caught in Luxembourg rivers ($n = 31$).

Compound	Frequency of determination n, (% of all trout)	Concentration in positive samples[a]		Concentration over all samples[b]	
		Mean ± SD ($ng\,g^{-1}$ fw[c])	Range ($ng\,g^{-1}$ fw)	Mean ± SD ($ng\,g^{-1}$ fw)	Range ($ng\,g^{-1}$ fw)
PCB 28	-	n.d.	n.d.	n.d.	n.d.
PCB 52	-	n.d.	n.d.	n.d.	n.d.
PCB 101	2 (6)	5	2–9	0 ± 2	n.d.–9
PCB 138	23 (74)	6 ± 4	1–21	4 ± 5	n.d.–21
PCB 153	21 (68)	6 ± 6	1–30	4 ± 6	n.d.–30
PCB 180	11 (35)	4 ± 3	1–11	2 ± 3	n.d.–11
Σ6PCBs[d]	23 (74)	14 ± 14	2–70	10 ± 14	n.d.–70

n.d.: not detected (<LOD: $20\,ng\,g^{-1}$ lw).
[a]Only values above LOQ ($40\,ng\,g^{-1}$ lw) are reported.
[b]Calculated using values found in all samples. Values for all non-detected congeners as well as values below LOQ were assumed as equal to zero.
[c]$ng\,g^{-1}$ fresh weight.
[(d)]Σ6PCBs: sum of the six indicators: PCB-28, 52, 101, 153, 138 and 180.

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sample extraction. In addition, Soxhlet extraction typically requires further sample cleanup for PCBs, which could cause additional losses. In the case of De Azevedo e Silva *et al*., recoveries varied largely between 70% and 130%, indicating stronger discriminative effects of the soxhlet sample treatment procedure compared with results obtainable by PLE.

Another method, the traditional matrix solid-phase dispersion (MSPD) used for muscle extraction is interesting for its low solvent consumption (often below 15 mL), but it is not very suitable for wet or high lipid containing samples, adsorbent consumption is then relatively high. Furthermore, MSPD has been shown to be of limited selectivity, extracted a wide range of organic compounds and thus required additional cleanup steps [30]. For example, Bordajandi *et al*. [18] following MSPD used acid and basic impregnated silica gel multilayer columns, obtaining recoveries ranging between 60% and 120% for mono-ortho PCBs and European indicators, also indicating discriminative effects during sample processing. In the present study, no major disturbances were discovered during GC-MS analysis, which would impede chromatographic integration, indicating the effectiveness of the combined extraction/purification step for removal of lipids.

For a decade, new extraction techniques have become available for PCB isolation from fish, such as ultrasound facilitated extraction [31], supercritical fluid extraction [32] or microscale analytical methods based on acoustic energy aiding in the extraction [22], with recoveries ranging from 78 to 115%, 70 to 110% and 57 to 142%, respectively. These extraction techniques used heat, pressure and sonication to break down fish tissue in order to decrease time and solvent consumption compared to traditional methods, and typically consumed below 80 mL solvent and less than 20 min extraction time. The inconvenience of these methods consists of the necessity to proceed with a separate sulphuric acid treatment for removal of lipids, followed by liquid adsorption chromatography (florisil or silica gel).

With pressurised liquid extraction and in-cell cleanup within a single step, comparable recoveries were obtained for all PCBs (range: 74 to 94%) from spiked trout. Mean recovery of the described extraction ($87 \pm 8\%$) was close to that obtained in other studies, and results for PLE were similar to results obtained using the soxhlet extraction method. By taking into account the difficulty of the homogenisation of an environmental matrix, the reproducibility of this method was comparably good, with a maximum SD for the lighter PCBs, up to 19%. In addition, accuracy as determined by comparison with a standard reference material was sufficient, with a total recovery of 68%, albeit this is somewhat lower than the average recovery of the spiking experiments (87%), with a tendency for higher losses for the lighter PCBs, which might be explained by losses occurring during freeze-drying. However, freeze-drying was preferred, as done in previous published studies [33,34] as a water containing sample would be non-extractable by a non-polar solvent.

Compared to methods described above, in-cell cleanup thus avoided time-consuming additional processing steps, and only 20 min were needed for sample extraction and purification with this method, limiting further the cost of each experiment by decreasing solvent use to less than 100 mL. A similar method of in-cell cleanup has been used recently by Wiberg *et al.* [35] to detect several organic pollutants, among which were PCBs, from various food samples, employing sulphuric acid impregnated silica; however, the detection method employed high resolution GC-MS, being more sensitive and selective in detecting PCBs, albeit being also more cost-intensive. In another earlier study [34], in-cell cleanup with florisil was carried out to accomplish improved extraction of PCBs from seafood, but focused on slightly different PCBs than the indicator PCBs investigated in the present investigation. Also another, more selective and sensitive detector, GC combined with ion trap MS was employed, however, indicating the potential usefulness of in-cell cleanup.

One limitation of the present study is the LOQ of $40\,ng\,g^{-1}$ of lipid. One possible way to improve the LOQ without further analytical sample cleanup procedures and/or concentration would be the implementation of GC-MS/MS instrumentation [36]. This analytical device however was not available for our study and is usually a more cost-intensive approach. Another alternative rests in either using higher initial sample volumes or further concentration of the final extracts; however, both approaches have the drawback of also concentrating undesired, potentially interfering compounds, and could not be successfully implemented within our study.

Following successful implementation of the PCB detection, we monitored several fish species in various Luxembourgish rivers and streamlets for their PCB content. Especially predatory fish have been reported to be prone to PCB accumulation from the environment [37], as they are at the end of the food chain. However, PCB contamination has been reported to vary depending on local pollutant sources, as well as being dependent on species, age and size of the fish [38].

In the present examination, large variations were found in PCB concentrations between fish species, Σ6PCBs ranging from 149 to $170\,ng\,g^{-1}$ fw for eels, n.d. to $70\,ng\,g^{-1}$ fw for trout and was low in the single contaminated roach ($4\,ng\,g^{-1}$ fw). Concentrations between the six indicator PCB and the Σ13PCBs did not differ considerably, even though they have been reported to typically accompany the six indicator PCBs in concentrations of up to 10% total PCBs content [39], perhaps as the individual concentrations were just below detection limit. Trout, eels and roaches were investigated as these represent major commonly consumed fish species. The variations of concentrations in these species may be attributed to their different trophic positions and eating habits. Generally, eels are

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voracious predatory species and have higher trophic positions than other species sampled, resulting in elevated PCB levels. In addition, eels feed on organic detritus and stir up sediments during feeding, thus increasing organic pollutants in suspension render them prone to higher PCB exposure [37].

Data obtained within this preliminary monitoring study were higher than that observed by Bordajandi *et al*. [19] in the river Turia (Spain) who reported 44 ng g^{-1} fw (range: 9 to 126 ng g^{-1} fw for the sum of 20 PCBs) and 7 ng g^{-1} fw (range: 5 to 9) for trout.

However, similar concentrations of PCBs compared to our study have been reported earlier in Luxembourg. Perches and eels caught in the Sûre river had mean total PCB concentrations of 3.3 and 205 ng g^{-1} fw, respectively [38]. A previous study from 1993–1996 [40] showed variations between 50 and 3500 ng g^{-1} fw for the sum of 22 PCBs in different Luxembourg river fish. This high variation is due to the four fish species and to the 11 rivers studied. For eels caught in the Our a mean of 1706 ng g^{-1} fw (range: 1672 to 1771) was obtained while in 2007 we found a maximum concentration of 176 ng fw (Σ13PCB). In stone loaches caught in 1994 in the Wiltz river, a mean PCB content of 49 (range: 41 to 56) ng g^{-1} fw, respectively, was found. In the present study, for trout caught in the Wiltz the maximum contamination obtained was 33 ng g^{-1} fw. Thus, a higher contamination than those we obtained was observed, being in line with the postulation of slowly declining PCB concentrations in the environment [41].

The positive correlation found between PCB content and fish weight within trout from one river might indicate a link to the age of the animals and the time of accumulation of pollutants, even though other parameters such as food supply would also impact weight. A similar correlation was found by Vives *et al*. [42] in *Salmon trutta* from a mountain lake (Pyrenees) and Pandelova *et al*. [43] in Baltic fish. In the Pyrenees lake, an increase of 2–20 times of organochlorine compounds' (PCBs and pesticides) content between specimens aged one year and 15 years of age was observed.

Fish are able to bio-transform a large number of congeners and form hydroxylated PCBs, this biotransformation is largely based on chlorine substitution patterns [44]. Effectively, PCB 28 and PCB 52 (three and four chlorine atoms, respectively) were never detected in this study. In addition, trichlorobiphenyl and tetrachlorobiphenyl have a relatively high vapour pressure (range: 0.003 to 0.220 Pa) compared to higher chlorinated congeners (less than 0.002 Pa [7]) and thus are less likely to be retained in rivers and in fish.

In trout, the PCB profile was dominated by congeners 153 and 138, compounds which have been used in various industrial applications (aroclor 1260 and aroclor 1254). The persistence of these congeners is a direct consequence of their stereochemistry and degree of chlorination. Congeners with chlorine atoms in 2,4,5 in one ring, such as PCB 138 and 153, are particularly recalcitrant to degradation [45] and biodegradation [24].

5. Conclusion

A rapid extraction method based on in-cell cleanup and GC-MS was developed and used to determine PCB contamination in various fish samples. Pressurised liquid extraction combined with the use of different silica layers of various acidic properties directly in the extraction cell allowed an efficient removal of lipids and the direct analysis by GC-MS. This in-cell cleanup protocol showed a high sample throughput and required less organic solvents, compared with other established extraction protocols such as soxhlet, while obtaining equivalent analytical accuracy and variability. Future work will examine further

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applications of this method to analyse other types of biota samples in the presence of high level of lipids.

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