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ORIGINAL PAPER

GC×GC-ECD: a promising method for the determination of dioxins and dioxin-like PCBs in food and feed

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Abstract There is a need for cost-efficient alternatives to gas chromatography (GC)–high-resolution mass spectrometry (HRMS) for the analysis of polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) and dioxin-like polychlorinated biphenyls (PCBs) in food and feed. Comprehensive two-dimensional GC–micro electron capture detection (GC×GC-μECD) was tested and all relevant (according to the World Health Organisation, WHO) PCDD/Fs and PCBs could be separated when using a DB-XLB/LC-50 column combination. Validation tests by two laboratories showed that detectability, repeatability, reproducibility and accuracy of GC×GC-μECD are all statistically consistent with GC-HRMS results. A limit of detection of 0.5 pg WHO PCDD/F tetrachlorodibenzo-*p*-dioxin equivalency concentration per gram of fish oil was established. The reproducibility was less than 10%, which is below the recommended EU value for reference methods (less than 15%). Injections of vegetable oil extracts spiked with PCBs, polychlorinated naphthalenes and diphenyl ethers at concentrations of 200 ng/g showed no significant impact on the dioxin results, confirming in that way the robustness of the method. The use of GC×GC-μECD as a routine method for food and feed analysis is therefore recommended. However, the data evaluation of low dioxin concentrations is still laborious owing to the need for manual integration. This makes the overall analysis costs higher than those of GC-HRMS. Further developments of software are needed (and expected) to reduce the data evaluation time. Combination of the current method with pressurised liquid extraction with in-cell cleanup will result in further reduction of analysis costs.

Keywords Analysis · Comprehensive two-dimensional gas chromatography · Electron capture detection · Polychlorinated biphenyls · Polychlorinated dibenzo-*p*-dioxins and dibenzofurans · Food and feed

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Introduction

Polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs or 'dioxins') are a group of 210 highly toxic substances that can be formed as by-products during chemical and combustion processes. In organisms, they act by binding to the aryl hydrocarbon receptor (AhR), and

consequently give rise to a cascade of effects, including liver damage, weight loss, atrophy of the thymus gland and immunosuppression [1]. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) has the highest binding affinity to the AhR and has been classified as carcinogenic to humans by the International Agency for Research on Cancer [2]. Extensive studies on structure–activity relationships have shown that most planar halogenated aromatic compounds of approximately 0.3 nm×1 nm sizes, i.e. comparable to the size of TCDD, induce AhR-mediated effects [3]. Among the PCDD/Fs, all 17 2,3,7,8-substituted congeners are toxic, but the potency of individual isomers differs widely within this group. To facilitate risk assessment, the World Health Organisation (WHO) has established a TCDD equivalency (TEF) scheme that correlates individual congener toxicities to that of TCDD. This scheme includes TEFs for four non-*ortho*-substituted and eight mono-*ortho*-substituted polychlorinated biphenyls (PCBs) because these compounds can also adopt a planar configuration, bind to the AhR, and thus produce dioxin-like effects. These 12 PCBs are commonly referred to as the 'WHO-PCBs' or 'dioxin-like PCBs'. The structures and the TEFs assigned by the WHO in 1998 (WHO-TEFs) [4] for the 2,3,7,8-PCDD/Fs and WHO-PCBs are given in Table 1. These TEFs have been revised recently [5], but for practical purposes (all high-resolution mass spectrometry, HRMS, laboratories have calculated TEFs using the 1998 scale) we have not used the new values. Using the TEFs, one may calculate a TCDD equivalency concentration (TEQ); the TEQ is the sum of the products of each individual dioxin or PCB concentration and its TEF.

PCDD/Fs and WHO-PCBs are very persistent compounds. They accumulate in food webs and can reach high concentrations in top predators. As a direct consequence, food may become so heavily contaminated that human health could be compromised. Furthermore, dioxins have very long

Table 1 Structures, acronyms and toxic equivalency factors (*TEFs*) for polychlorinated dibenzo-*p*-dioxins (*PCDDs*), polychlorinated dibenzofurans (*PCDFs*) and World Health Organisation (WHO) polychlorinated biphenyls (*PCBs*) [4]

Species	Acronym	TEFs
PCDDs		
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin	4D	1
1,2,3,7,8-Pentachlorodibenzo-*p*-dioxin	5D	1
1,2,3,4,7,8-Hexachlorodibenzo-*p*-dioxin	6D1	0.1
1,2,3,6,7,8-Hexachlorodibenzo-*p*-dioxin	6D2	0.1
1,2,3,7,8,9-Hexachlorodibenzo-*p*-dioxin	6D3	0.1
1,2,3,4,6,7,8-Heptachlorodibenzo-*p*-dioxin	7D	0.01
Octachlorodibenzo-*p*-dioxin	8D	0.0001
PCDFs		
2,3,7,8-Tetrachlorodibenzofuran	4F	0.1
1,2,3,7,8-Pentachlorodibenzofuran	5F1	0.05
2,3,4,7,8-Pentachlorodibenzofuran	5F2	0.5
1,2,3,4,7,8-Hexachlorodibenzofuran	6F1	0.1
1,2,3,6,7,8-Hexachlorodibenzofuran	6F2	0.1
2,3,4,6,7,8-Hexachlorodibenzofuran	6F3	0.1
1,2,3,7,8,9-Hexachlorodibenzofuran	6F4	0.1
1,2,3,4,6,7,8-Heptachlorodibenzofuran	7F1	0.01
1,2,3,4,7,8,9-Heptachlorodibenzofuran	7F2	0.01
Octachlorodibenzofuran	8F	0.0001
Non-*ortho* PCBs		
3,3′,4,4′-Tetrachlorobiphenyl	CB77	0.0001
3,4,4′,5-Tetrachlorobiphenyl	CB81	0.0001
3,3′,4,4′,5-Pentachlorobiphenyl	CB126	0.1
3,3′,4,4′,5,5′-Hexachlorobiphenyl	CB169	0.01
Mono-*ortho* PCBs		
2,3,3′,4,4′-Pentachlorobiphenyl	CB105	0.0001
2,3,4,4′,5-Pentachlorobiphenyl	CB114	0.0005
2′,3,4,4′,5-Pentachlorobiphenyl	CB118	0.0001
2,3,3′,4,4′-Pentachlorobiphenyl	CB123	0.0001
2,3,3′,4,4′,5-Hexachlorobiphenyl	CB156	0.0005
2,3,3′,4,4′,5′-Hexachlorobiphenyl	CB157	0.0005
2,3′,4,4′,5,5′-Hexachlorobiphenyl	CB167	0.00001
2,3,3′,4,4′,5,5′-Heptachlorobiphenyl	CB189	0.0001

half-lives in humans. Within the European population, average dietary exposure (8–21 pg per kilogram body weight per week) frequently exceeds the tolerable weekly intake for dioxins and dioxin-like PCBs assigned by the European Union (EU) Scientific Committee on Food (14 pg WHO-TEQ per kilogram body weight) [6]. The European Commission (EC) has prescribed maximum and action levels for food, feed and feed ingredients [7–10]. To implement and enforce this legislation, many food and feed samples must be analysed for PCDD/Fs and WHO-PCBs each year, and thus there is a significant need for reliable, rapid and cost-efficient screening and confirmatory methods.

The determination of PCDD/Fs and WHO-PCBs in food and feed is challenging because the pollutants are present at ultratrace (parts per trillion) levels, in the presence of much higher levels of other chemical pollutants. Consequently, highly selective and sensitive analytical methods are required to reliably determine the PCDD/Fs and WHO-PCBs. Until now routine methods have generally included time-consuming extraction and cleanup and expensive gas chromatography (GC)–HRMS instruments. Therefore, the EC recognised a need to improve the cost efficiency of PCDD/F and WHO-PCB analysis in food and feed. Two research projects 'Dioxin analysis by using comprehensive gas chromatography' (DIAC) and 'Dioxins in food and feed—reference methods and new certified reference materials' (DIFFERENCE) were funded by the EC to evaluate options to improve cost-efficiency and develop alternative methods. In the present study one such technique was extensively characterised: comprehensive, 2D GC (GC×GC) coupled with micro electron capture detection (μECD).

GC×GC has evolved from heart-cut multidimensional GC (MDGC), which has been utilised for PCB and dioxin-like PCB analysis for a long time [11–13]. In MDGC, selected peaks from the first column can be transferred by pressure (Dean) switching to the second column. This second column must offer a different mode of separation [14]. MDGC is ideally suited for complex target analysis, e.g. the separation of coeluted isomers. However, MDGC is limited by the number of cuts that can be made in one analytical run. If too many heart cuts are made, compounds from consecutive injections will be coeluted in the second dimension, with consequent loss of resolution. MDGC will therefore not be suitable for the determination of PCDD/Fs and WHO-PCBs in environmental samples, for which 29 target analytes must be separated from numerous closely related, interfering compounds. In such cases, GC×GC is more appropriate.

In GC×GC, the principal separation is achieved on a long capillary column. All material that exits this first column is focused in a 'modulator' [14, 15] and then remobilised in narrow (time-compressed) bands for further separation using a fast (i.e. short, narrow, thin film) second-dimension column. The modulator is operated at such a frequency that each compound being eluted from the first column is sampled frequently enough to maintain the first-dimension separation. During data processing, the linear signal from the μECD instrument is converted to a series of secondary chromatograms and (generally) is stored in a data matrix format, which greatly facilitates quantification and visualisation as 2D contour plots [15, 16].

By a proper selection of stationary phases and dimensions an orthogonal separation is obtained [17]. Such a system delivers a peak capacity equal to the product of the peak capacity of the two individual columns. The peak compression caused by the modulator generally results in a 5–10-fold improvement in the limits of detection (LOD) compared with single-column GC [18]. Thus, in principle, GC×GC offers both exceptional separation power (peak capacity) and low LODs. When GC×GC is combined with μECD, sufficient selectivity and sensitivity can be achieved to allow PCDD/F and WHO-PCB measurements at levels close to or even below the EU maximum and action levels.

The results of an extensive performance evaluation of GCxGC-μECD for the determination of dioxins in food and feed are presented here and discussed in relation to the reference method (GC-HRMS), alternative techniques and to the requirements laid down in the European legislation [19, 20].

Materials and methods

Samples and experimental design

The performance evaluations were divided into three parts. First, the instrumental LOD and repeatability were assessed within the DIAC project using a standard solution and a salmon extract. Next, selected samples representing food items frequently reported to exceed the EU action limit were analysed to assess detectability, reproducibility, accuracy, and robustness. This part of the work was performed within the DIFFERENCE programme. A stepwise design in three rounds (DIFF 1–3) was used to make it possible to adjust the analytical protocol, if required. After each round the results were discussed in order to identify needs for improvements. Finally, the GC×GC-μECD procedure was used in a precertification exercise (CERT). The samples used in the various exercises are described in Table 2. Details on the sample preparation, homogeneity studies, etc. are given elsewhere [21]. Some of the materials (milk, chicken and pork feed, and chicken and pork tissue) were fortified (spiked) with additional PCDD/Fs and WHO-PCBs at levels close to the EU action limit to ensure a reasonable signal-to-noise ratio. The congener profile was adjusted to resemble the profile normally encountered in contaminated

Table 2 Description of samples, total tetrachlorodibenzo-*p*-dioxin equivalency concentration (*TEQ*) levels (picograms per gram of fat), contaminant profiles of spiked samples, and the methods used for extraction and cleanup

Matrix	Amount	Origin	Total TEQ	"Spike profile"	Extraction		Cleanup				Exercise
					Column	Liquid–liquid	Multilayer silica	Carbon or PGC	PYE	H_2SO_4 silica	
Standard		Institut Quimic de Sarriá, Ramon Llull University, Spain	16[a]								DIAC
Salmon extract	4 ml (from 24 g fat)	Market, Barcelona, Spain; origin Norway. Extracted with 1:1 hexane/dichloromethane, and cleaned over sulfuric acid	4.0				X	X	X		DIAC
Eel extract	5 ml (from 5 g fat)	Pooled eels from several Dutch freshwater locations. Extracted with binary solvent mixture and cleaned over sulfuric acid silica	21				X	X		X	DIFF 1
Herring oil	7 ml	North Sea, west of Shetland Islands (60.40 N, 03.00 E)	9.9				X	X		X	DIFF 1
Spiked milk	250 ml	Sterilised whole milk, Deka Markt, IJmuiden, The Netherlands	14	Dutch milk		X	X	X		X	DIFF 1
Vegetable oil	5 g	Corn oil, Deka Markt, IJmuiden, The Netherlands	0.4–12[c]	Herring			X	X		X	DIFF 2
Vegetable oil	5 g	Corn oil, Deka Markt, IJmuiden, The Netherlands	6	Herring + PCB or PCN or PCDE[d]			X	X		X	DIFF 2
Chicken feed	100 g	Contaminated chicken feed	1.6	Herring	X		X	X		X	DIFF 3
Chicken tissue	70 g	Chicken meat from hens fed the contaminated feed	4.4		X		X	X		X	DIFF 3
Egg	100 g	Eggs from the abovementioned hens	6.4		X		X	X		X	DIFF 3
Pork tissue	70 g	Pork from background contaminated meat (Heemskerk, The Netherlands) mixed with meat from pigs fed contaminated feed (10% by weight)	1.4	Pork	X		X	X		X	DIFF 3
Herring tissue	70 g	North Sea (52.30 N, 02.00 E)	1.9[b]		X		X	X		X	DIFF 3
QA oil	5 g	Corn oil, Deka Markt, IJmuiden, The Netherlands	6	Herring			X	X		X	DIFF 1–3
Compound feed	100 g	Typical pig feed (including fortified salmon oil)	1.5	Salmon	X		X	X		X	CERT
Fish oil	5 ml	North Sea, west of Shetland Islands (60.40 N, 03.00 E)	12				X	X		X	CERT

PGC porous graphitised carbon, *PYE* 2-(1-pyrenyl)ethyldimethyl-silylated silica, *PCN* polychlorinated naphthalene, *PCDE* polychlorinated diphenyl ether

[a] Amount injected

[b] Concentration on product basis (in accordance with EU directive)

[c] Equal contributions from PCDDs/PCDFs and WHO-PCBs. The samples were used to determine detectability.

[d] Material spiked with 3 pg dioxin and 3 pg WHO-PCB-TEQ, and additional PCB (200 ng/compound/g oil) or PCN (10 ng/compound/g oil) or PCDE (20 ng/compound/g oil)

materials. Finally, corn oil samples were spiked at various levels of PCDD/F and WHO-PCBs to determine the LOD for actual samples. Portions of the spiked corn oil samples were also fortified with PCB, polychlorinated naphthalene (PCN) or polychlorinated diphenyl ether (PCDE) standards to test the robustness of the method.

The samples were extracted and cleaned up using well-validated methods [22]. Generally, the lipids were extracted using bipolar solvent mixtures and the lipid weights were determined gravimetrically. The extracts were reconstituted in a nonpolar solvent and lipids were removed using multilayer silica columns, which were packed with layers of silica and acid- and base-modified silica [23]. The remaining chemically persistent nonpolar compounds were then fractionated on the basis of planarity using open charcoal columns [23, 24] or high-performance liquid chromatography with porous graphitised carbon [25, 26] or 2-(1-pyrenyl)ethyldimethyl-silylated silica [27–29] as the stationary phase. Finally, polar residues from solvents and adsorbents were removed by filtration through miniaturised multilayer silica columns [23]. Further information on the extraction and cleanup techniques used for the various samples is compiled in Table 2.

Prior to extraction, CB 79, 1,2,3,4-TCDD, and 1,2,3,4,6,7,9-heptachlorodibenzo-*p*-dioxin (each at 200 pg) were added as internal standards for the non-*ortho* PCBs and PCDD/Fs, respectively, and CB 159 (4 ng) was added as an internal standard for the mono-*ortho* PCBs; and prior to instrumental analysis, 1,2,3,4-tetrachloronaphthalene and octachloronaphthalene (about 500 pg each) were added as recovery standards.

Two-dimensional gas chromatography–micro electron capture detection

The GC×GC measurements were performed at two laboratories (laboratories A and B). Both laboratories used Agilent HP6890 gas chromatographs (Palo Alto, CA, USA) equipped with μECDs. Laboratory A used a longitudinally modulating cryogenic system (Everest LMCS; Chromatography Concepts, Doncaster, Australia) for the GC×GC modulation, while laboratory B used a loop-type jet modulator (KT2002; Zoex, Lincoln, NE, USA). The flow of carbon dioxide cryogen to the modulators was adjusted using needle valves to obtain a modulator temperature 60–90 °C below that of the GC oven. A hot air pulse duration of 200 ms was used to desorb trapped material from the KT2002 modulator. The modulation period varied between 5 and 8 s, depending on the experiment. A high detector temperature (300 °C) and the highest possible make-up gas flow (150 mL/min) and data collection (50 Hz) rates were used to avoid unnecessary extra-column band broadening [15, 30]. However, it should be noted that there is a compromise between resolution (band width) and sensitivity. Thus, the sensitivity was reduced by the high make-up gas flow and data collection rates used.

Data evaluation and visualisation

Most of the quantification was performed using the HP ChemStation software. The individual peaks of each compound were identified using first and second dimension retention information (from analyses of authentic reference standards). Their peak areas were manually summed and the concentrations were calculated using relative response factors derived from the analyses of authentic reference standards. Three commercially available GC×GC software packages, i.e. ChromaTOF (Leco, St Joseph, MI, USA), HyperChrom (Thermo Finnigan, Milan, Italy) and GC Image (Zoex, Lincoln, NE, USA), were also tested to evaluate their potential for (semi-)automatic data evaluation. Transform software (Fortner Research, Sterling, VA, USA) was used to produce 2D chromatograms.

Results and discussion

Selection of column combinations

The complete GC separation of all PCBs (n=209) and PCDD/Fs (n=210) is a formidable task. However, to obtain reliable TEQs it is sufficient to separate the 12 WHO-PCBs and the 17 2,3,7,8-substituted PCDD/Fs from each other and from other congeners present in the purified sample extracts. The task is further simplified by the fact that usually only 2,3,7,8-substituted PCDD/Fs are found in biota, owing to their high persistence and biomagnification in comparison with non-2,3,7,8-substituted congeners. However, a complete separation is impossible using single-column GC. There are many columns available that can separate all WHO-PCBs or all 2,3,7,8-PCDD/Fs from each other, and one column (SGE HT-5) can, at least partially, separate all WHO-PCBs and 2,3,7,8-PCDD/Fs [31]. Unfortunately, there is, to the authors' best knowledge, no column available that can separate all 29 target analytes from the remaining 197 PCBs.

To overcome this problem, the PCBs and PCDD/Fs were fractionated according to their planarity using activated charcoal, porous graphitised carbon or 2-(1-pyrenyl)ethyldimethyl-silylated silica into three fractions comprising (1) the bulk of PCBs, (2) primarily mono-*ortho* PCBs, and (3) non-*ortho* PCBs and PCDD/Fs (the dioxin fraction). Consequently, the GC×GC column combination must be able to separate all non-*ortho* PCBs and PCDD/Fs, and all WHO mono-*ortho* PCBs from each other and from other PCBs that are eluted in fraction 2. If possible, all marker

PCBs (PCBs 28, 52, 101, 118, 138, 153 and 180) should also be separated from each other and from other PCBs that are eluted in the same fraction.

A large number of column combinations have been evaluated for their ability to resolve PCDD/Fs and/or PCBs (Table 3). In most cases, nonpolar stationary phases were used for the first-dimension separation. More polar phases, especially those with a high cyanopropyl content, may offer a better isomer separation but often bleed excessively. The bleeding has little effect when such phases are used as the short, thin-film secondary columns [31].

A wide array of stationary phases have been tested for use in the second dimension column. Most of the phases separate primarily on the basis of polarity, but their retention properties differ considerably depending on the type and proportion of polymethylsiloxane side chain groups. The interaction of PCDD/Fs and of WHO-PCBs with, and their retention on, the stationary phase increase with the phase polarity, i.e. in the order trifluoropropyl (column set 7, Table 3), phenyl (column sets 1, 2, 9, 11, 17 and 20), phenyl/carborane (column sets 3, 18 and 19), cyanopropyl (CyP; column sets 4, 5, 13, 14 and 15) and poly(ethylene glycol) (column set 6). The liquid-crystalline LC-50 (column sets 8, 12, 16) phase separates the target analytes on the basis of planarity, and exhibits a strong shape selectivity [32]. Columns coated with high contents of phenyl (007-65HT), CyP (VF-23ms) and liquid-crystalline (LC-50) phases were found to be particularly efficient. This is thought to be related to their interactions through strong intermolecular forces, which better exploit the small differences in physical properties between the PCB and PCDD/F isomers [21, 33].

Pure methylsilicone columns (DB-1) do not separate non-*ortho* PCBs and PCDD/Fs well [31]. Six analyte pairs are coeluted from these columns—PCBs 123/118, PCBs 169/5D1, 6F1/6F2, 6D1/6D2, 6D3, 6F4, and 8D/8F, and even second-dimension columns with high CyP or liquid-crystal contents are unable to separate more than two of the critical pairs (See electronic supplementary material, Table S1). HT-5

Table 3 Column sets evaluated for PCDD/PCDF and PCB separations

Set	First column[a]	Phase[b]	Second column[a]	Phase[b]	Targets separated	References
PCDDs/PCDFs and WHO-PCBs						
1	DB-1[c]	100% methyl	BPX-50[d]	50% phenyl	25 of 29	[31]
2			007-65HT[f]	65% phenyl	21 of 29	[31]
3			HT-8[d]	8 % phenyl PC	27 of 29	[31]
4			OV-1701[f]	14% vyanopropyl	25 of 29	[31]
5			VF-23 ms[g]	70–90% cyanopropyl	21 of 29	[31]
6			SupelcoWax-10[h]	Poly (ethylene glycol)	19 of 29	[31]
7			007-210[f]	50% trifluoropropyl	25 of 29	[31]
8			LC-50[i]	50% liquid crystal	21 of 29	[31]
9	HT-5[d]	5 % phenyl PC	007-65HT[f]	65% phenyl	24 of 29	[31]
10	Rtx-dioxin 2[e]	Proprietary, nonpolar	Rtx-500[e]	Proprietary PC	27 of 29	[38]
11			007-65HT[f]	65% phenyl	29 of 29	[31]
12	DB-XLB[c]	Proprietary, nonpolar	LC-50[i]	50% liquid crystal	29 of 29	[23, 31, 40]
13			VF-23 ms[g]	70-90% cyanopropyl	29 of 29	[40]
209 PCBs						
14	DB-XLB[c]	Proprietary, nonpolar	BPX-70[d]	70% cyanopropyl	11 of 12	[40]
15	–″ –	–″ –	SP-2340[h]	100% cyanopropyl	10 of 12	[40]
16	–″ –	–″ –	LC-50[i]	50% liquid crystal	11 of 12	[40]
17	–″ –	–″ –	BPX-50[d]	50% phenyl	11 of 12	[41]
18	–″ –	–″ –	HT-8[d]	8% phenyl PC	11 of 12	[41]
19	DB-1[c]	100% methyl	HT-8[d]	8% phenyl PC	11 of 12	[41]
20	HT-8[d]	8% phenyl PC	BPX-50[d]	50% phenyl	12 of 12	[41]

PC polycarborane methylsiloxane (backbone)

[a] The first-dimension columns were 30 or 60 m long with 0.25- or 0.32-mm diameters, and 0.18 μm or 0.25 μm phases, and the second-dimension columns were 1–3 m long with 0.1- or 0.15-mm diameters and 0.1 μm phases.

[b] The percentage of methyl side-chain groups was omitted to enhance the presentation.

[c] J&W Scientific

[d] SGE International, Ringwood, Australia

[e] Restek, Bellefonte, PA, USA

[f] Quadrex, New Haven, CT, USA

[g] Varian, Middleburg, The Netherlands

[h] Supelco, Bellefonte, PA, USA

[i] J & K Scientific, Milton, ON, Canada

seems to provide a slightly better resolution, but the Rtx-dioxin 2 and DB-XLB phases are superior, as they separate all the target compounds except TCDD (4D1) and PCB 126. If the DB-XLB column is combined with a high-percentage CyP column (such as VF-23 ms), or with the LC-50 column, all 29 target compounds can be separated (sets 12 and 13). Such column combinations can also be used to separate marker and mono-*ortho* PCBs from multi-*ortho* PCBs (See electronic supplementary material, Table S2). With use of the DB-XLB×LC-50 column combination, all mono-*ortho* PCBs except PCB 167, and all marker PCBs except PCBs 101 and 153, are eluted as single components. Since none of the coeluted PCBs are present in any of the technical PCB formulations in excess of 0.05% [34], they would not significantly bias the measurements. However, if necessary, these coelutions can be avoided by using column set 20 (HT-8×BPX-50).

Thus, using a properly optimised GC×GC column assembly such as DB-XLB×LC-50, one can completely resolve all WHO-PCBs and 2,3,7,8-PCDD/Fs from each other and from all other PCBs that are present in the same cleanup fraction. In addition, this column combination also seems to be less affected by coextracted (potentially interfering) matrix components, most probably owing to the high selectivity of the LC-50 stationary phase for planar compounds. This can clearly be seen in Fig. 1, which presents 2D contour plots from the analysis of tetra-CDD/Fs and penta-CDD/Fs in a purified fish extract. TCDD, 4F and 5D were coeluted with other sample components in the lower chromatogram, obtained using column set 13 (DB-XLB×VF-23 ms), but were eluted as discrete peaks in the upper chromatogram, which was obtained using column set 12 (DB-XLB×LC-50). The DB-XLB×LC-50 column combination was therefore selected for subsequent experiments.

Detectability

Considering the peak compression obtained via the GC×GC modulation process, exceptional sensitivity would be expected from GC×GC systems equipped with μECD instruments. However, such sensitivity is not always

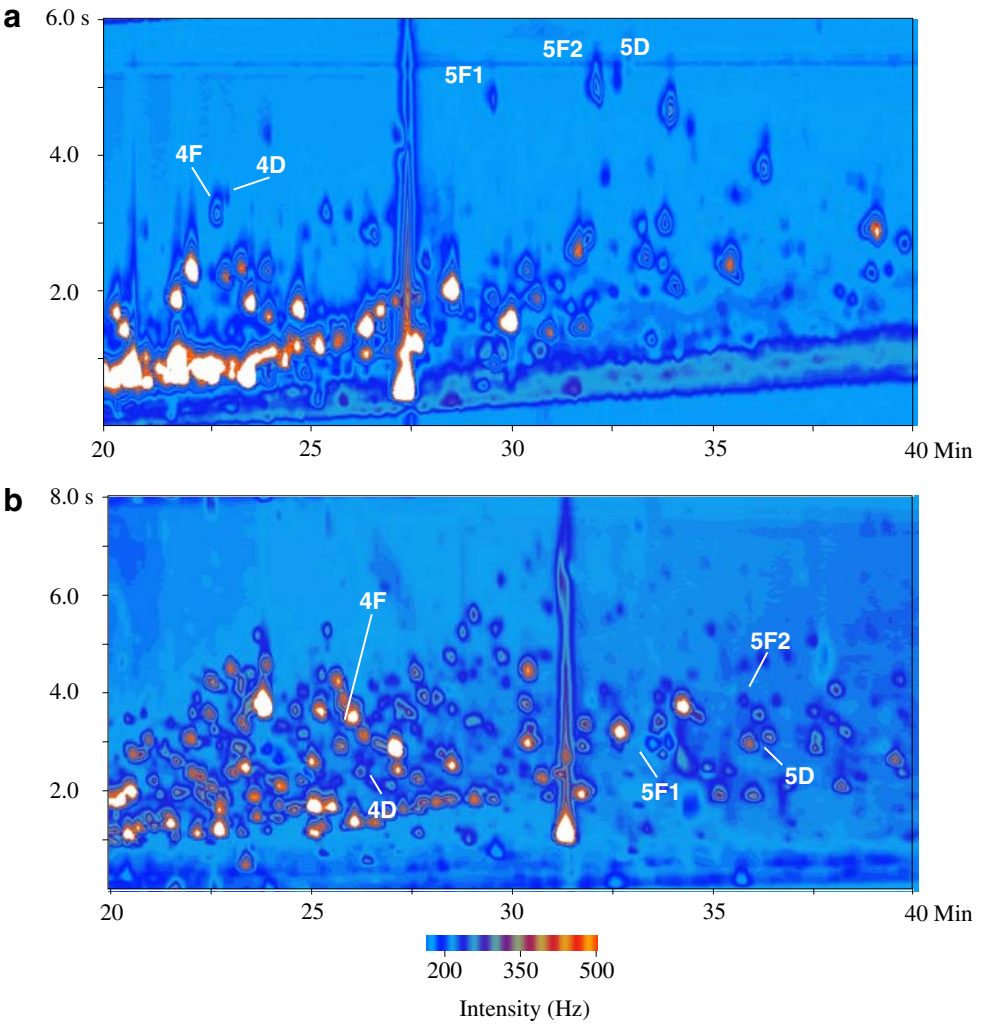

Fig. 1 2D gas chromatography (GC) micro electron capture detection (GC×GC-μECD) contour plots of the tetrachlorinated dibenzo-*p*-dioxins and dibenzofurans and pentachlorinated dibenzo-*p*-dioxins and dibenzofurans region from the analysis of the dioxin fraction of a fish sample. **a** Obtained using DB-XLB×LC-50 (column set 12) and **b** obtained using DB-XLB×VF-23ms (column set 13)

observed, because sensitivity is limited by the chemical noise arising from column bleed, septum bleed and carrier gas impurities, rather than by electronic noise. Impurities entering the system prior to the modulator are especially detrimental to the sensitivity because they are focused in the modulator. Therefore, low-bleed, nonpolar phases like DB-XLB are used for the first-dimension separation, as discussed in the previous section. Such columns generally release low amounts of nonpolar bleed components, which can easily be separated from the target analytes in the second-dimension column. The sensitivity and linearity (r^2>0.998) of such a GC×GC system are almost as good as for GC-HRMS systems [23, 31]. LODs of 40–150 and 30–60 have been reported for standard solutions of 2,3,7,8-PCDD/Fs and WHO-PCBs, respectively [31]. LODs were assessed within the DIFFERENCE project by the analysis of vegetable oil spiked at levels close to the EU maximum levels (0.75 pg WHO-PCDD/F-TEQ/g oil). The oil was spiked with a PCDD/F mixture that resembled a typical fish sample in profile. The LOD of the GC×GC-μECD method was found to be 0.5 pg WHO-PCDD/F-TEQ/g oil.

The current EU requirements for analytical suitability include the criterion that the LOD must be at least one fifth of the level of interest, i.e. of the maximum or action level; thus, it may be concluded that GC×GC-μECD can, in principle, be applied. With a reasonable sample mass for analysis (more than 5 g), it is possible to accurately measure the levels of all 2,3,7,8-PCDD/Fs and WHO-PCBs and determine whether the TEQ of vegetable oil is above or below the maximum level and, thus, to assess whether it complies with the regulation. It is also possible to determine whether the TEQ of a lot of oil is above or below the action level of 0.5 pg WHO-PCDD/F-TEQ/g. Assuming a similar PCDD/F pattern to that in the vegetable oil, one can calculate the minimum sample size required for other types of food and feed. Realistic sample quantities (more than 5 g) for regulatory compliance testing are required, while the amounts needed to assess compliance with the action levels are larger, 50 g for lean meat and eggs, and 250 g for lean fish.

Repeatability and reproducibility

The repeatability and reproducibility of GC×GC-μECD were assessed in both the DIAC and DIFFERENCE projects. Ten replicate determinations of a standard solution were performed, and the results were compared with those from a reference GC-HRMS laboratory. The best results were reported by the GC-HRMS laboratory, with coefficients of variation (CV) in the range 1.2–7.3% for the individual PCDD/F congeners, and 0.7–1.9% for the individual WHO-PCBs. A laboratory experienced in both dioxin and GC×GC analysis (laboratory A) reported a comparable repeatability (0.8–7.4% for PCDD/Fs and 0.8–4.9% for WHO-PCBs), while a laboratory experienced in PCB, organochlorine pesticide and GC×GC analysis (laboratory B) reported slightly wider ranges (3.2–24% for PCDD/Fs and 1.4–3.8% for WHO-PCBs).

Repeatability and reproducibility were then assessed using various food and feed samples. The results obtained by laboratory A are compiled in Table 4. Acceptable median coefficients of variation were obtained for both PCDD/F (generally less than 20%) and WHO-PCB congeners (less than 10%). As expected, higher variance was occasionally observed for congeners present at very low concentrations, e.g. PCB 189 and some hexachlorinated and heptachlorinated dibenzo-*p*-dioxins and dibenzofurans. The variance in the total WHO-TEQs was also low (less than 10%) for the GC×GC-μECD method. This was significantly lower than the maximum variance stipulated for screening methods (less than 30%) and for confirmatory methods (less than 15%) for official control of dioxins in food and feed [19, 20].

Accuracy

A number of samples contaminated with PCDD/Fs and WHO-PCBs at levels close to the EU maximum limits were analysed using both GC×GC-μECD and GC-HRMS. An example of a set of results from a PCDD/F and non-*ortho* PCB analysis is given in Fig. 2. It was clearly possible to

Table 4 Repeatability and reproducibility of two-dimensional gas chromatography–micro electron capture detection determinations (laboratory A) of PCDDs/PCDFs, WHO-PCBs and total WHO-TEQs calculated as coefficients of variation and expressed as percentages

Matrix	*n*	Type	Individual PCDDs/PCDFs	Median	Individual WHO-PCBs	Median	Total TEQ
Standard	10	Repeatability	1–7	2	1–5	3	3
Salmon	10	Repeatability	4–45	18	NA	NA	6
Chicken feed	5	Reproducibility	1–15	8	3–77	6	5
Milk	6	Reproducibility	5–42	14	5–36	10	8
Pig feed	6	Reproducibility	8–42	25	3-11	8	4
Pork	5	Reproducibility	8–32	12	4–57	10	8
Herring oil 1	6	Reproducibility	7–40	18	5–18	10	5
Herring oil 2	6	Reproducibility	5–35	18	4–44	8	3

NA not available

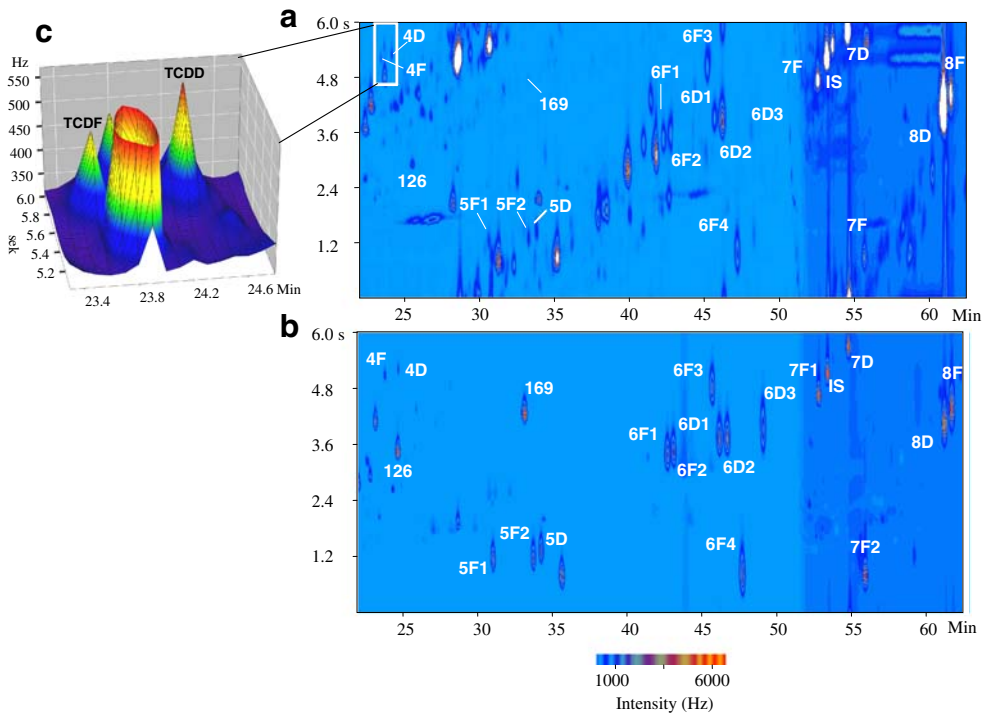

Fig. 2 GC×GC-μECD contour plots (DB-XLB×LC-50) of non-*ortho* polychlorinated biphenyls (PCBs) and polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) in the dioxin fraction of a compound feed sample (**a**) and a standard solution (**b**). The internal standard, 1,2,3,4,6,7,9-heptachlorodibenzofuran, is denoted *IS* and the intensity scales of the contour plots were truncated at 6,000 Hz to enhance the presentation of minor PCDD/Fs. The intensities of 7F1, IS, 7D, 8D and 8F were 20,000, 220,000, 280,000, 630,000 and 12,000 Hz, respectively. Details of the chromatographic conditions are given in [21]. The 3D zoom in on the tetrachlorodibenzo-*p*-dioxin (*TCDD*) and tetrachlorodibenzofuran (*TCDF*) region of the sample (**c**) illustrates the enhanced separation obtained by the use of a second-dimension separation. The large truncated peak (approximately 4,000 Hz) preceding that of TCDF would confound accurate single-column GC analysis

separate all target analytes from each other, from matrix constituents and from system peaks. Many minor components, like tetrachlorodibenzofuran, were accurately determined owing to the second-dimension separation (Fig. 2c).

The total WHO-TEQ values obtained from the analyses are compiled in Fig. 3. Generally, there was good agreement between the GC×GC-μECD and GC-HRMS results, although a slight tendency towards overestimation was observed for GC×GC-μECD, especially for laboratory B. For laboratory A the difference between the GC×GC-μECD and GC-HRMS results was generally less than the variability in the two methods, as demonstrated by overlapping confidence intervals. All data from this laboratory (except those for the eel extract) were also within ±20% of the GC-HRMS results, which is the EC requirement for the trueness of confirmatory (mass-spectrometric) methods for dioxin analysis. In summary, laboratory A produced data with quality comparable to GC-HRMS data, while laboratory B generally overestimated the levels slightly. It should be noted, however, that if GC×GC-μECD was to be applied as a screening method, a slight overestimation would not be very problematic since false-positive results are of less concern than false negatives. The quality of the data also improved over time, especially between test round DIFF 1 (eel extract, herring oil and spiked milk) and later rounds (DIFF 2–3 and CERT), despite the fact that the PCDD/F and PCB levels in the materials tested in the later rounds were generally lower than in DIFF 1. Thus, the experience gained through the stepwise experimental design clearly improved the quality of analysis.

In contrast to other screening methods such as bioassays, the GC×GC-μECD technique not only produces TEQ data, but also provides a full congener profile. Congener-specific data for a compound feed and fish oil are shown in Fig. 4. The congener patterns provided by GC×GC-μECD faithfully reproduce the profiles obtained by GC-HRMS. Almost without exception, the confidence limits of the two techniques overlap. Thus, GC×GC-μECD could also be used as a routine method for the congener-specific analysis of 2,3,7,8-PCDD/Fs and WHO-PCBs in food and feed. Unfortunately, the current EC legislation does not allow the use of techniques other than GC-HRMS as confirmatory methods for official control of dioxins in food. This could change in the future if further evidence is accumulated to demonstrate that GC×GC-μECD produces data of the same quality as, or very similar quality to, GC-HRMS.

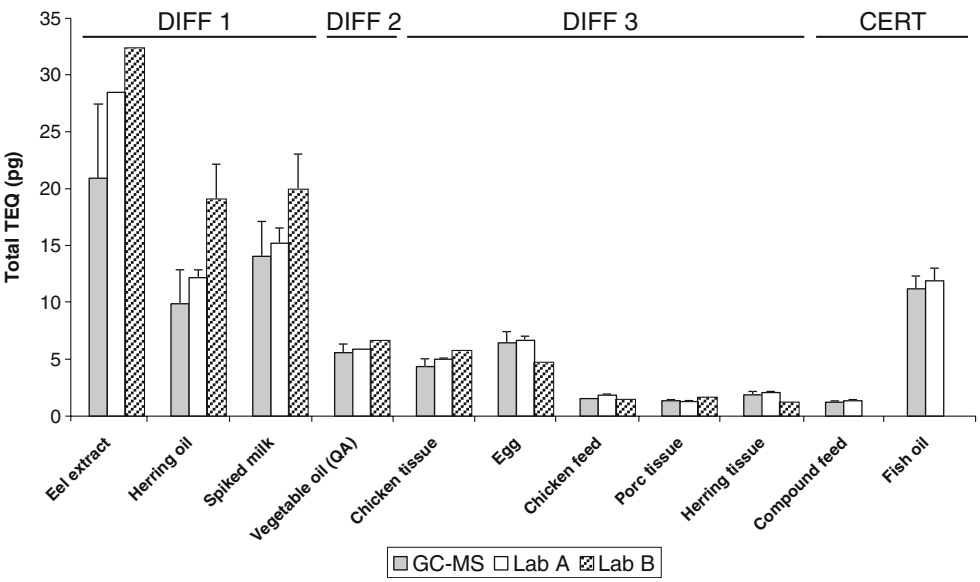

Fig. 3 Comparisons of total TCDD equivalency concentration (*TEQ*) values produced by two GC×GC-μECD laboratories and a number of GC–high-resolution mass spectrometry (*MS*) reference laboratories for 11 sample materials evaluated within the DIFFERENCE project. The *error bars* correspond to 95% confidence intervals

Robustness

Because other classes of halogenated organic compounds could interfere in the determination of PCDD/Fs and WHO-PCBs spiked portions of a vegetable oil were analysed. All oils were spiked with a realistic mixture of PCDD/Fs at 3 pg WHO-TEQ/g and most oils were also fortified with potentially interfering compounds at much higher levels (total concentration 200 ng/g). The potential interferences included 31 PCBs, 12 PCNs and a mixture of monochloro- through decachlorodiphenyl ethers (PCDEs). No significant bias was observed in the PCDD/F or WHO-PCB results for oils that were spiked with PCDEs, PCNs or poly-*ortho* PCBs, compared with the unspiked oils.

Data evaluation

The quantification process using GC×GC data is essentially the same as for conventional GC, with one important distinction. Each component eluted from the first column is split into several second-dimension chromatograms. In addition, the baseline of the ECD may be affected by residual matrix. Thus, quantification is challenging in GC×GC analyses and highly efficient sample cleanup, utilising ultrapure solvents and adsorbents, is essential. Even with very efficient cleanup, the quantification of low levels of PCDD/Fs and non-*ortho* PCBs is time-consuming because manual correction of the peak integrals is often required. Consequently, the time required for manual peak integration and data processing for one sample may exceed 1 h.

There is thus a need for reliable and automatic data evaluation software that can accurately identify the second-dimension peaks of all compounds, bundle the corresponding peak areas, quantify the compounds, and report and visualise the results. At present, three such software packages are available: ChromaTOF by Leco, HyperChrom by Thermo Electron and GC Image by Zoex. The Leco and Thermo Electron software packages are only sold with GC×GC hardware from the same company, while GC Image is sold separately. The packages perform quantification in different ways: ChromaTOF and HyperChrom integrate the peaks of the individual second-dimension chromatograms in the same way as standard software packages for GC, while GC Image handles the 2D chromatograms as pictures, determines the boundaries of each peak and *sums* the individual values of all data points ('pixels').

All three programs seem to perform well for intense, isolated peaks, but all perform less well for assigning and integrating low-level components, components that are partially coeluted in the first dimension and components with first-dimension retention time shifts (compared with the calibration standard). A major advantage of using automatic peak integration is the strong reduction in time needed for integration, down to 10 min per sample for all software packages. Even after manual reintegration of some components, the time saving is substantial compared with fully manual integration. The problems associated with low signal-to-noise ratios could be addressed through enhanced detector robustness, reduced detector volume and improved peak-integration algorithms and editing tools. Retention time markers (e.g. PCB or PCDD/F congeners that are not present in the food or feed under study) may help to accommodate retention time shifts. Improving the resolution of components that are partially coeluted in the first dimension is a more difficult task because the first-dimension peaks are generally undersampled. Only four to five second-dimension chromatograms are usually collected across each first-dimension peak in order to achieve the required sensitivity and speed. Four or five data points are insufficient to accurately reconstruct the

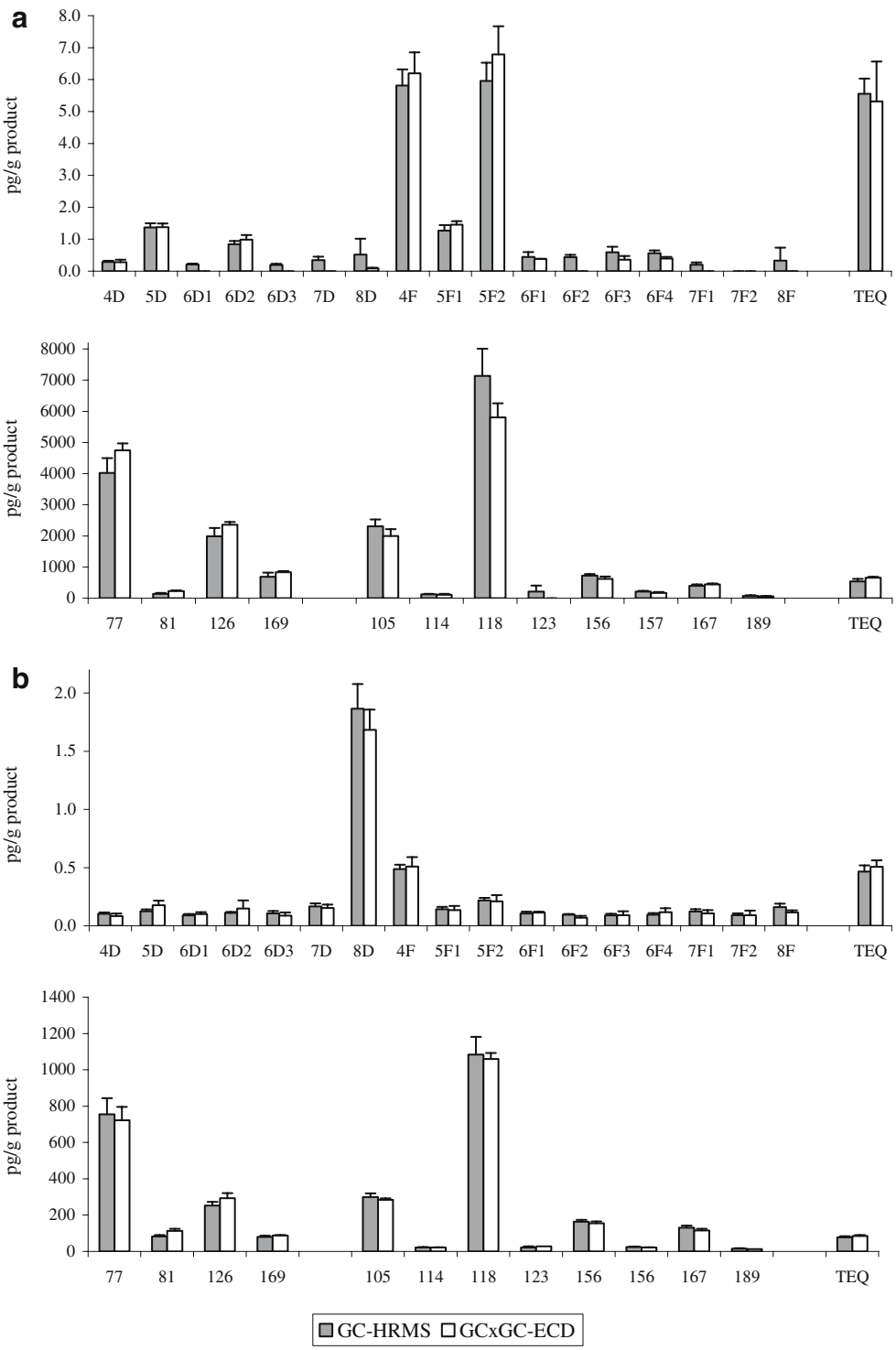

Fig. 4 Levels of PCDD/Fs and World Health Organisation PCBs in **a** fish oil and **b** compound feed as determined by GC– high-resolution MS (*HRMS*) (*left bars*) and GC×GC-μECD (laboratory A, *right bars*). The non-*ortho* PCB and PCB-TEQ levels have been magnified 50-fold and 100-fold, respectively. The *error bars* correspond to 95% confidence intervals

first-dimension peak profile; therefore, a peak-fitting procedure is required to quantify such partially resolved peaks. This should be implemented into the GC×GC software.

Cost of analysis

The cost of analysis was estimated for GC×GC-μECD and compared with that of GC-HRMS. Calculations were based on the analysis of 100 samples per month under routine conditions, and included the costs of labour (in person-hours) for all steps of analysis, consumables used, instrument investment and depreciation, and service costs. Labour costs were calculated on the basis of an estimated rate of €75/h for a technician.

The total costs of analysis were estimated to be about €1,000 for GCxGC-ECD and €500 for GC-HRMS; thus,

GC×GC-μECD is currently about twice as expensive as the reference (GC-HRMS) method. The distribution of costs differs between the methods. The costs associated with extraction, cleanup and fractionation are roughly the same for both methods, approximately €350 per sample. The remaining costs for GC×GC-μECD are dominated by labour costs related to data evaluation (€500 or more per sample), while the remaining costs for GC-HRMS are mainly for instrument operation (€100 per sample). The instrument depreciation costs of GC×GC-μECD are, on the other hand, very low (less than €25 per sample).

The costs of GC×GC-μECD analysis must be greatly reduced to make the technique economically competitive. Automation of the data evaluation process would provide the most significant saving. It should be stated that for higher concentrations, e.g. in case of more highly contaminated samples or in case of other contaminants, the manual integration is often not needed and much shorter times for integration and data evaluation are required. Fortunately, manufacturers are rapidly developing software that should lead to simpler automated integration of chromatograms of less contaminated samples and a reduction of the labour involved. Further cost reductions could be obtained through the use of a cryogen recovery system [35] or by changing to air cooling instead of the more costly carbon dioxide cooling, which was recently demonstrated to be feasible for semivolatile compounds [36]. Alternative sample extraction and cleanup procedures should also be explored. A selective pressurised liquid extraction procedure has recently been developed for simultaneous sample extraction and cleanup [37]. Using this technique, one can obtain ready-to-inject extracts for around €120, which would reduce the total cost of analysis by around €200. If selective pressurised liquid extraction was used for extraction and purification and the amount of time needed for GC×GC-ECD analysis and data evaluation could be decreased to 0.5 h, the overall cost of analysis could be reduced to €200.

Conclusions and outlook

GC×GC-μECD is a promising technique for the analysis of dioxins and dioxin-like PCBs. However, postcolumn band broadening in the μECD causes deterioration of the chromatographic resolution. Further technical development and commercialisation of μECDs is predicted that will fully exploit the potential of the technique. The increased use of mass spectrometry (MS) for detection is also anticipated, since sensitive and robust fast-scanning instruments are now commercially available. Focant et al. [38] recently showed that good chromatographic resolution, acceptable sensitivity and accuracy can be achieved by a GC×GC time-of-flight (ToF) MS system The LODs reported were, however, too high for the analysis of food and feed samples with low levels of dioxins, such as butter fat and lean fish. Korytár et al. [39] showed that electron-capture negative ion chemical ionisation (ECNI) might be used as an alternative ionisation technique to electron impact (EI) to enhance the response to the target analytes. With a few exceptions, lower LODs were obtained using GC×GC-ECNI quadrupole MS in comparison with GC×GC-EI ToF-MS [38] or GC×GC-μECD [39]. Unfortunately, ECNI has a major drawback—low responsiveness to TCDD. The LOD for TCDD (710 fg) was 1–2 orders of magnitude higher than the LODs for other PCDD/Fs, except octachlorodibenzo-*p*-dioxin (400 fg). ToF-MS, in general, and ECNI-ToF-MS, in particular, might still be good alternative detection techniques for samples with moderate or high dioxin or PCB levels and containing significant concentrations of potential interferences. It is important to note that the use of an MS detector significantly increases the instrument costs and the need for skilled operators, thus reducing cost-efficiency.

Probably the most important development is to improve software for instrument control, quantification and data visualisation. Ideally, the same software should be able to perform all tasks. Two of the commercially available software packages, ChromaTOF and HyperChrom, can handle all stages from sample injection to data reporting. However, the tools for visualisation and, in particular, peak detection and quantification require development to handle peaks with low signal-to-noise ratios and peaks that partially overlap in the first dimension. Such improvements would increase the sample throughput and the quality of data for ultratrace analysis of environmental contaminants like PCDD/Fs, and would facilitate routine, cost-efficient analysis of PCDD/Fs and WHO-PCBs by GC×GC-μECD.

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