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PCDD, PCDF, AND DL-PCB analysis in food: performance evaluation of the high-resolution gas chromatography/low-resolution tandem mass spectrometry technique using consensus-based samples

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Due to safety concerns regarding dietary exposure to POPs, regulatory bodies are issuing detailed guidelines for testing for polychlorodibenzodioxins (PCDDs) and polychlorodibenzofurans (PCDFs) ('dioxins') and dioxin-like (DL)-PCBs in foods of animal origin. Determination of the aforesaid chemicals at regulatory levels requires highly selective and sensitive testing techniques. The new generation of low-resolution mass spectrometers (triple quadrupoles) allows very low levels of quantification to be reached (in the order of tens of femtograms), thus suggesting a potential for their application in food and feed analysis. The performance of the low-resolution tandem mass spectrometry (LRMS/MS) approach with triple quadrupoles was assessed on a qualified set of food samples from proficiency tests (PTs) and defense analysis. Accuracy was tested comparing the results with data from high-resolution mass spectrometry (HRMS) and with consensus values from PTs. The cumulative TEQ results were characterized by deviations not exceeding 15% of PCDD + PCDF, DL-PCB, and PCDD + PCDF + DL-PCB (TEQ_{TOT}) reference consensus values (sample TEQ_{TOT} range, 2.29–25.1 pgWHO-TEQ_{97}/g fat). Congener analytical variabilities did not influence significantly the WHO-TEQ_{97} outcome of the corresponding sample. This preliminary performance evaluation highlights the potential of LRMS/MS as a routine technique for quantitative analysis of PCDDs, PCDFs, and DL-PCBs in food.

Due to bioaccumulation in the lipid component of animal organisms, food of animal origin is the predominant route of human exposure to lipophilic polychlorodibenzodioxins (PCDDs), polychlorodibenzofurans (PCDFs), and dioxin-like polychlorobiphenyls (DL-PCBs); PCDDs and PCDFs are also commonly referred to as 'dioxins'. Approximately 90% of the general population's aggregate exposure is due to consumption of the fats present in animal products, such as fish, milk and dairy products, meat, and eggs.[1] For this reason, international bodies (such as US FDA,[2] EU Commission,[3,4] and FAO/WHO[5]) have defined control strategies aimed at: (a) obtaining data on background levels of PCDDs, PCDFs, and DL-PCBs in animal food and feed; (b) identifying sources of contamination that can be eliminated or significantly reduced; (c) estimating the dietary exposure to the chemicals. The main objective of control strategies is to lower the dietary exposure of populations to the aforesaid contaminants – at present partly exceeding JECFA's provisional tolerable monthly intake (PTMI) of 70 pgWHO-TEQ_{97} per kg of body weight (kg-bw),[6] or the EU Commission's tolerable weekly intake (TWI) of 14 pgWHO-TEQ_{97}/kg-bw[7] – thus preventing possible health problems linked to excess exposure.

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The reliability of the mentioned control strategies calls for strict feed and food monitoring programs based on analytical methods capable of routinely reaching limits of quantitation (LOQs) for PCDD and PCDF congeners in the upper femtogram range and for non-*ortho* DL-PCBs in the low picogram range. The methods referred to in general rely on the combined techniques of high-resolution gas chromatography (HRGC) and a form of mass spectrometry (MS), the latter being the main subject of this paper. Detections greater than a critical concentration would trigger investigations and management measures to minimize or eliminate the contaminant source(s) and mitigate the exposure.

High-resolution mass spectrometry (HRMS), identifiable with magnetic sector instruments, has been and is the reference measurement technique for PCDDs, PCDFs, and DL-PCBs due to high sensitivity, selectivity, and reliability (US EPA Methods 1613-B (1994) and 1668-B (2008)): for these reasons, it is recognized as a confirmatory method par excellence. However, HRMS is an expensive technology, requiring highly qualified infrastructures and operators.

For routine food analysis, semiquantitative, rapid screening bioanalytical methods are currently proposed.[8] Less expensive than HRMS, they have a reduced probability (≤5%) to give both false positive and false negative results relative to regulatory maximum levels (MLs).[3,8] However, their intrinsic features entail that false negative results be verified at a rate of 2 to 10% with duplicate HRMS analyses of

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those samples screened as compliant: this will affect the overall cost-effectiveness of the screening approach.[9] In the light of the above, there seems to be a need for an alternative approach to the analysis of PCDDs, PCDFs, and DL-PCBs in food and feed: in particular, to identify the non-compliant samples with a tool less expensive than HRMS and more reliable than bioanalytical methods, requiring a relatively short performance time, and yielding a negligible number of false positive outcomes to reduce the need for HRMS confirmation.

Attempts have been made to carry out quantitative analysis of PCDDs, PCDFs, and DL-PCBs in food by highly specific techniques alternative to HRMS, such as low-resolution (LR) ion trap MS[10,11] and two-dimensional gas chromatography (GC) coupled with time-of-flight MS.[12] These attempts were also triggered by the need to face the challenge of a high analysis throughput in a rather short time,[13] requested in the case of emergencies or accidents, e.g. the recent contamination incidents related to dioxins[14] in which the involved countries were required to increase their analytical capabilities through the involvement of a broad number of laboratories. However, the analytical sensitivity of the aforesaid instruments does not meet the criteria requested for food and feed control analysis, despite the possibility offered by the GC/MS combination to yield congener-specific results, an added value in identifying contamination sources.

In the last years, a new generation of triple quadrupoles seems to offer promising candidates to be used in the first analysis on food, in a fashion not dissimilar from HRMS. When operating in the selected reaction monitoring (SRM) mode (LRMS/MS), the analytical selectivity of a triple quadrupole can be compared to that of a HRMS instrument used in the selected ion monitoring (SIM) mode, a feature that meets a selectivity criterion based on the desired number of identification points while providing a sensitivity in the upper femtogram range requested for control analysis of food and feed. The application of a selectivity criterion has been extended from the analysis of veterinary drug and pesticide residues to include the determination of PCBs in food,[15] as a general tool within a performance-based measurement system.[16] In 2011, Fürst *et al*. reported the achievement of comparable results by LRMS/MS and HRMS on food and feed samples available in the laboratory, with contamination levels in the range of 0.5–3 pgWHO-TEQ$_{97}$/g.[17]

In this paper, we present the results – in terms of sensitivity, selectivity, and accuracy – from the application of LRMS/MS to the determination of PCDD, PCDF, and DL-PCB congeners in a set of samples qualified with a consensus value and in samples of intercalibration studies and proficiency tests (PTs).

EXPERIMENTAL

Samples

The samples used to test the method were aliquots of PT samples from the European Reference Laboratory for Dioxins and PCBs in Food and Feed (EU-RL, Freiburg, Germany) and the Norwegian Institute of Public Health (Folkehelseinstituttet, Oslo, Norway). On the whole, the following consensus matrices were available for testing: breast milk, eggs, milk fat, mozzarella cheese, pig fat, pork meat, salmon, and trout fillet. In addition, aliquots of non-compliant samples of buffalo milk, chicken meat, eel fillet, lamb meat, and olein coming from food monitoring plans and delivered to our laboratory for HRMS defense analysis were also available.

Sample preparation

Samples were extensively homogenized, especially if not liquid. Of each sample (meat and milk), an amount was weighed to have some 5 g of lipids in the extract. Samples were spiked with as many internal standards (ISs) – ^{13}C-labelled PCDDs, PCDFs, and DL-PCBs – as the congeners to be quantified; fortification levels were approximately five times the congener LOQs. The fortified samples were allowed to stand overnight at 4 °C. Before extraction, samples were fully lyophilized. Each freeze-dried matrix was mixed with anhydrous sodium sulfate (1:1 by weight) and processed with an accelerated solvent extractor (ASE 200, Dionex Italia, Milan, Italy), applying two 60% flushing cycles with *n*-hexane at 100 °C and 10 mPa. A 120–360-mL extract solution was collected, of which a 5% aliquot was used for a gravimetric determination of the lipid content. The extract volume was reduced to a final 50-mL volume, and subjected to lipid removal by elution on a glass column containing Extrelut™ (VWR International, Milan, Italy) impregnated with concentrated sulfuric acid.[18] The pre-purified extract was reduced to a small volume and cleaned up with an automatic Power-Prep™ system (Labservice Analytica, Bologna, Italy): three different pre-packed columns (multilayer silica, alumina, and graphitized carbon) were used, as previously described.[18] Extracts were concentrated to a final volume of 20 µL adding 1 µL *n*-tetradecane as keeper and the appropriate recovery standard(s) for the subsequent instrumental analysis.

Each fat or oil sample (approximately 5 g each) was dissolved in 50-mL *n*-hexane, spiked with ISs, allowed to rest overnight, and eluted on the Extrelut™ column described above. Pre-purification was followed by Power-Prep™ clean-up. When deemed necessary, the amount of lipids dissolved in the *n*-hexane solution was verified.

Native and ^{13}C-labelled PCDD, PCDF and DL-PCB standards were purchased from Wellington Laboratories (Guelph, Ontario, Canada). Solvents were of the highest purity grade (Merck, Darmstadt, Germany).

Instrumentation

The HRGC-triple quadrupole system TSQ Quantum XLS™ (Thermo-Scientific Italia, Milan, Italy) and the HRGC-HRMS VG Autospec™ (Waters Italia, Milan, Italy) were used for this study. The HRGC-HRMS working conditions for quantification of PCDDs, PCDFs, and DL-PCBs were derived from US EPA Methods 1613-B (1994) and 1668-B (2008) and outlined elsewhere.[18]

The HRGC-triple quadrupole system comprised a Trace GC Ultra gas chromatograph equipped with a TriPlus™ autosampler, a PTV injector, and a TSQ Quantum XLS™ triple quadrupole detector. The instrument was operated in the electron impact SRM mode, using a highly inert ion volume, easily replaceable. The GC unit was equipped with a BPX-DXN column (60 m length × 0.25 mm $\varnothing_i$, 0.25 µm film thickness; SGE Analytical Science, Melbourne, Australia). The following working conditions were set for the HRGC-triple quadrupole analysis.

A programmed PTV injection in solvent split was used, optimized for an 8-µL injection; a 0.12-min injection time was selected with the following initial parameters: temperature,

110 °C; pressure, 200 kPa; vent flow of 30 mL/min. The transfer temperature rate, transfer line temperature, and transfer pressure were set at 14.5 °C/s, 290 °C, and 300 kPa, respectively; transfer time and splitless time were both 1.5 min. The GC oven was programmed from an initial temperature of 60 °C (isothermal for 1.10 min) to 230 °C at 40 °C/min, and then up to 310 °C at 2.0 °C/min, the end temperature being held for 5 min. He was used as a carrier gas at a constant flow of 1 mL/min.

The MS conditions were: electron energy, 37 eV; emission current, 50 μA; source temperature, 270 °C; chrom filter peak width, 10 s; collision gas, Ar; collision gas pressure, 1.5 mTorr. To guarantee the reproducibility of results, a substitution of both liner and ion volume was carried out on a regular basis after each analytical session of some 30 extract injections.

The LRMS/MS working conditions allowed maximum sensitivity to be achieved. The precursor ions $[M]^+$ or $[M+2]^+$ were selected from the molecular cluster ions for quantification of the chemicals. For DL-PCBs, the loss of two chlorine atoms from the precursor ion was the relevant transition: the most intense ion $[M–2Cl^{\bullet}]^+$ corresponded to the loss of two $^{35}Cl^{\bullet}$ atoms (mass, 70 Da) or a $^{35}Cl^{\bullet}$ and a $^{37}Cl^{\bullet}$ atom (mass, 72 Da). For PCDDs and PCDFs, the loss of the $COCl^{\bullet}$ fragment from the precursor ion was identified as the transition of interest: the most intense ion $[M–COCl^{\bullet}]^+$ corresponded to the loss of $CO^{35}Cl^{\bullet}$ (mass, 63 Da) or $CO^{37}Cl^{\bullet}$ (mass, 65 Da). For each congener, the selected quantifier and qualifier precursors and their products are reported in the Supporting Information, and are in agreement with those described elsewhere.[17] Collision energies varied in the ranges 21–23 V for PCDDs and PCDFs and 28–30 V for DL-PCBs. Precursor ion peak width was set at 0.7 Da.

Limits of quantification

According to Regulation 1883/2006/EC,[8] the confirmatory method LOQs (WHO-TEQ_{97}) for cumulative values of PCDDs + PCDFs and PCDDs + PCDFs + DL-PCBs (TEQ_{TOT}) must be approximately 1/5 of the regulatory MLs of interest, while a congener LOQ must be determined as the congener concentration in the sample extract that produces an instrumental response of the two different monitored ions with a signal-to-noise (S/N) ratio of 3:1 for the less sensitive signal. This approach could not be successfully applied in the case of MS/MS acquisitions due to the very low noise generated by the instrument, determining a high S/N ratio even when very weak signals were detected and generally high fluctuations of signals and background noise were expected. Therefore, congener LOQs were identified as the lowest concentrations associated with a relative standard deviation (RSD) of the relative response factor (RRF) from repeated measurements not exceeding $|\pm 30\%|$. Congener LOQs ranged from (injected) 30 to 80 fg for tetra- to hexachlorosubstituted congeners and from 80 to 320 fg for hepta- and octachlorinated congeners.

RESULTS AND DISCUSSION

The analysis of PCDDs, PCDFs, and DL-PCBs in food and feed requires high analytical sensitivity (low LOQs) as well as high selectivity and accuracy. As a consequence, the EU legislation acknowledges exclusively HRMS as a confirmatory technique. Moreover, such a technique must be capable of quantifying a total TEQ value at a level of at least 1/5 of

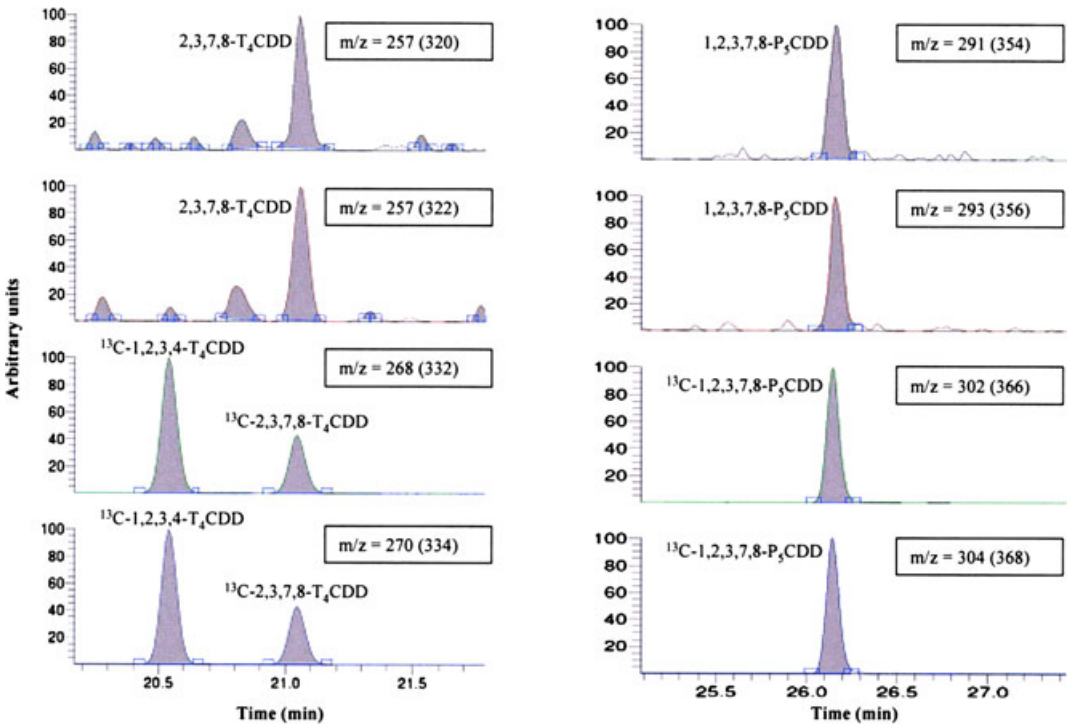

Figure 1. The selectivity and sensitivity provided by the LRMS/MS technique proved to be good, as exemplified by the mass chromatograms of 2,3,7,8-T_4CDD and 1,2,3,7,8-P_5CDD in a buffalo milk sample (estimated 40 and 200 fg injected, respectively). The ^{13}C-1,2,3,4-T_4CDD was added prior to instrumental analysis as injection standard. Each SRM transition is identified in the pictures by the monitored product ion mass and its precursor ion (in parentheses).

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the regulatory ML of interest, as already recalled, with a maximum 20% difference between the upperbound (UB) and lowerbound (LB) estimates when the contamination level is in the order of, or greater than, 1 pg WHO-TEQ_{97}/g fat, and in the range of 25–40% for somewhat lower contamination levels. Also, the HRMS accuracy is expected to be high, as expressed by a trueness within a $|\pm 20\%|$ difference between a measured value and an assigned one for a certified material, and a precision expressed as a reproducibility relative standard deviation (RSD_R) of less than $|\pm 15\%|$. On an analytical basis, an acceptable variation in congener determination should not affect the cumulative TEQ value more than $|\pm 10\%|$.[8] The selectivity provided by the LRMS/MS technique proved to be very good, as exemplified by the mass chromatograms of 2,3,7,8-T_4CDD and 1,2,3,7,8-P_5CDD in a buffalo milk sample (Fig. 1). This is in agreement with the general identification criteria set for residue analysis by the EU Commission, which score with 2 points each of the two diagnostic ions usually monitored by HRMS and score with 2.5 points each pair of ions (precursor and product) monitored by LRMS/MS. Taking into account the two precursor-product transitions considered for each congener, in our case the final score is 5, against 4 of the HRMS approach.

Table 1 summarizes the cumulative results (pgWHO-TEQ_{97}/g) from determinations carried out on non-compliant samples coming from food monitoring plans: all LRMS/MS results are well within less than a 15% deviation ($|\pm\Delta\%|$) from the corresponding HRMS data; an underestimation pattern is prevailing. The results delivered by LRMS/MS in PTs are also reported together with the corresponding consensus data: in general, z-scores qualify from good to very good. The LRMS/MS approach appears to meet the regulatory requirements of trueness and UB/LB ratio for samples from PTs with consensus values around the pertinent MLs. A PT sample (pork meat) had a PCDD + PCDF TEQ level and a TEQ_{TOT} value less than 1/5 of the pertinent MLs when computed on a 13% fat content: nevertheless, with the exception of DL-PCB results ($2 < \text{z-score} < 3$), the analytical congener concentrations (Table 2) and the cumulative PCDD + PCDF and TEQ_{TOT} values met the regulatory requirements of trueness and UB/LB ratio. It can be pointed out that the TEQ_{TOT}

Table 1. Performance comparison of LRMS/MS vs. HRMS using reference routine samples and of LRMS/MS vs. consensus values (CVs) for different matrices from the participation to proficiency tests

	Routine samples				Proficiency test samples			
Chemical	Matrix	LRMS/MS	HRMS	$\|\pm\Delta\%\|$	Matrix	LRMS/MS	CV	z-score
PCDDs + PCDFs	Olein[a]	1.01	1.06	4.7	Milk fat[a]	3.6	3.3	1.0
DL-PCBs		1.06	1.22	13.1		3.7	3.6	0.3
TEQ_{TOT}		2.06	2.29	10.0		7.4	7.0	0.6
PCDDs + PCDFs	Buffalo milk[a]	2.64	2.63	0.4	Fat of pigs[a]	0.84	0.77	0.9
DL-PCBs		1.08	1.20	10.0		0.70	0.69	0.1
TEQ_{TOT}		3.72	3.84	3.1		1.5	1.5	0.5
PCDDs + PCDFs	Chicken[a]	7.11	7.96	10.7	Breast milk[b]	0.18	0.14	1.3
DL-PCBs		16.8	17.1	1.8		0.07–0.09[c]	0.07–0.08[c]	0.2–0.4[c]
TEQ_{TOT}		23.9	25.0	4.4		0.34	0.30	0.7
PCDDs + PCDFs	Lamb[a]	10.7	11.6	7.8	Pork meat[b]	0.025	0.020	1.1
DL-PCBs		6.95	7.90	12.0		0.009–0.006[c]	0.006–0.004[c]	2.5–2.8[c]
TEQ_{TOT}		17.6	19.5	9.7		0.039	0.030	1.6
PCDDs + PCDFs	Eel[b]	3.54	3.23	9.6	Trout[b]	0.86	0.78	0.57
DL-PCBs		21.5	21.8	1.4		2.33–0.90[c]	2.30–0.84[c]	0.11–0.31[c]
TEQ_{TOT}		25.0	25.1	0.4		4.1	3.9	0.24
PCDDs + PCDFs					Mozzarella[b]	0.79	0.76	0.2
DL-PCBs						0.34–0.05[c]	0.35–0.05[c]	−0.1–0.0[c]
TEQ_{TOT}						1.19	1.20	−0.1
PCDDs + PCDFs					Salmon[b]	3.20	3.10	0.2
DL-PCBs						3.89–1.12[c]	3.90–1.20[c]	0.0– −0.3[c]
TEQ_{TOT}						8.21	8.10	0.1
PCDDs + PCDFs					Egg[b]	0.43	0.35	1.1
DL-PCBs						0.41–0.05[c]	0.37–0.05[c]	0.5–1.0[c]
TEQ_{TOT}						0.89	0.76	0.9

[a]Values expressed in pgWHO-TEQ_{97}/g fat.
[b]Values expressed in pgWHO-TEQ_{97}/g fresh weight.
[c]First entry: non-*ortho* PCBs; second entry: mono-*ortho* PCBs.

Table 2. PCDD, PCDF, and DL-PCB congener-specific results and their z-score ratings obtained from the assay of different matrices in proficiency tests. Analytical values rounded off to three figures and to a maximum of two decimal places

	Milk fat		Fat of pigs		Breast milk		Pork meat		Trout		Mozzarella		Salmon		Egg	
Chemical	pg/g fat	z-score	pg/g fat	z-score	pg/g fw	z-score	pg/g fw	z-score	pg/g fw	z-score	pg/g fw	z-score	pg/g fw	z-score	pg/g fw	z-score
PCDDs and PCDFs																
2,3,7,8-T_4CDD	0.53	1.2	0.09	—	0.02	−0.1	<0.01	2.6	0.14	0.6	0.10	0.3	0.27	−0.2	0.04	3.9
1,2,3,7,8-P_5CDD	0.89	−0.3	0.11	−2.7	0.05	0.8	<0.01	0.1	0.18	−0.1	0.24	0.0	0.52	0.2	0.10	1.2
1,2,3,4,7,8-H_6CDD	1.19	2.3	0.35	2.7	0.02	1.9	<0.01	4.0	0.01	3.8	0.08	−0.2	0.04	1.7	0.05	−0.7
1,2,3,6,7,8-H_6CDD	1.44	−1.2	0.32	−0.8	0.12	1.5	<0.01	1.1	0.07	1.6	0.35	0.7	0.25	0.9	0.21	1.2
1,2,3,7,8,9-H_6CDD	0.82	−0.2	0.32	2.2	0.03	1.3	<0.01	4.0	<0.01	−5.0	0.10	0.6	0.02	−0.4	0.09	1.5
1,2,3,4,6,7,8-H_7CDD	4.17	0.5	0.86	−0.3	0.17	1.3	0.02	−0.7	0.07	2.3	0.17	−0.6	0.07	1.5	0.28	−0.5
O_8CDD	9.73	1.4	4.49	1.9	1.16	0.9	0.11	−1.0	0.25	1.0	<0.05	−3.1	0.41	3.8	0.63	0.1
2,3,7,8-T_4CDF	0.50	1.0	0.11	1.3	0.02	2.3	0.01	1.9	2.25	0.6	0.02	−0.8	5.27	0.4	0.51	1.0
1,2,3,7,8-P_5CDF	0.80	2.7	0.22	0.8	0.02	7.6	0.00	−3.3	0.22	1.0	0.04	−0.5	0.64	0.0	0.21	0.4
2,3,4,7,8-P_5CDF	2.71	1.0	0.72	2.9	0.16	1.5	0.01	−0.9	0.57	0.8	0.53	0.3	3.58	0.3	0.32	1.3
1,2,3,4,7,8-H_6CDF	1.31	0.8	0.69	3.7	0.04	1.5	0.01	1.8	0.03	2.2	0.46	0.3	0.09	−0.1	0.13	0.7
1,2,3,6,7,8-H_6CDF	1.22	0.9	0.29	0.2	0.05	3.1	0.01	2.9	0.04	2.3	0.42	0.4	0.13	0.1	0.10	0.3
1,2,3,7,8,9-H_6CDF	0.61	−0.4	0.20	−0.9	<0.01	6.6	<0.01	3.8	<0.01	3.8	0.01	−0.1	<0.01	−1.4	0.02	0.8
2,3,4,6,7,8-H_6CDF	0.38	−1.1	0.16	—	0.03	3.3	0.02	8.5	0.04	2.2	0.38	0.9	0.10	−0.2	0.08	−0.4
1,2,3,4,6,7,8-H_7CDF	1.62	1.7	1.12	1.2	0.04	1.4	0.02	−0.3	<0.01	−2.9	0.18	−1.1	0.02	0.4	0.15	0.4
1,2,3,4,7,8,9-H_7CDF	0.69	1.4	0.34	3.7	0.01	4.4	<0.01	1.7	<0.01	2.9	0.02	−0.6	<0.01	−0.4	0.01	−1.8
O_8CDF	3.97	5.2	1.47	1.6	<0.01	−0.6	0.01	−2.2	0.03	−2.9	<0.04	−0.1	0.05	2.0	0.06	0.4
DL-PCBs																
T_4CB 77	489	−0.4	130	−0.1	0.06	−2.4	1.82	27.4	29.5	0.7	0.20	−3.3	82.5	−0.9	3.37	1.0
T_4CB 81	26.2	0.1	7.39	0.2	0.03	0.4	0.11	20.0	3.06	0.9	0.45	0.4	1.84	−0.8	0.32	1.6
P_5CB 105	4690	0.5	804	0.3	43.9	0.4	7.67	10.3	1250	0.3	65.3	−0.2	1650	−0.2	47.4	0.5
P_5CB 114	260	−0.8	24.1	−2.9	11.4	0.7	<0.2	−3.0	67.4	−0.4	8.88	0.4	103	0.2	2.77	1.3
P_5CB 118	9100	0.1	1500	−0.3	180	0.1	28.7	5.3	3350	0.4	222	0.0	4130	−0.7	142	0.7
P_5CB 123	208	0.9	55.3	3.9	2.42	0.8	<0.2	1.8	69.9	0.6	3.76	−0.1	55.7	0.7	2.49	1.2
P_5CB 126	15.7	0.2	3.02	0.3	0.68	0.1	0.08	2.6	21.9	0.2	3.30	−0.1	37.9	0.0	4.02	0.6
H_6CB 156	952	0.1	244	1.0	104	0.6	4.42	1.1	654	0.5	30.7	0.0	759	0.2	54.2	1.0
H_6CB 157	224	0.6	27.4	−1.8	19.0	0.0	0.84	0.2	121.4	−0.1	9.03	0.5	191	0.8	11.1	2.0
H_6CB 167	245	−1.0	37.7	−1.9	25.3	0.5	<0.2	−4.2	339	−0.2	13.6	−0.2	383	0.2	24.7	−0.1
H_6CB 169	0.74	0.1	0.06	—	0.57	0.8	0.05	−0.1	14.7	0.2	1.27	0.3	8.90	0.4	0.57	0.7
H_7CB 189	13.4	−3.2	11.9	0.0	7.47	0.1	0.34	−0.3	58.7	−0.1	4.78	−0.5	74.1	0.2	8.26	1.0

consensus value reported for the pork sample (0.030 pgWHO-TEQ$_{97}$/g fw or about 0.23 pgWHO-TEQ$_{97}$/g fat) was approximately 1/2 of the pertinent action level (AL),[19] and appears to qualify as an extreme situation basically outlying the application field defined by the regulatory framework for the contaminants dealt with. On an analytical basis, the congener variabilities observed in PT samples (Table 2), as suggested by z-scores, meet the aforementioned acceptance criteria in that they do not affect in a relevant way the final cumulative TEQ estimates (Table 1), in spite of a few z-scores exceeding $|\pm 2|$. However, when analytical results from LRMS/MS were compared with internal HRMS results, the aforesaid variability was reduced by approximately one z-score point (data not reported). This may be taken as an indication that the source of variability reflects uncertainties in the laboratory procedure, irrespective of the differences in the MS detection/measurement technique.

The congeners' analytical LOQs – when computed on the default amount of extracted lipids (ca. 5 g) and an 8-μL injection (40% of the final extract volume), and applied to PCDD + PCDF, DL-PCB, and TEQ$_{TOT}$ congener arrays after TEQ conversion (cumulative TEQ 'thresholds') – determined conservatively estimated contributions to TEQ concentrations in the order of 0.14, <0.01, and 0.14 pgWHO-TEQ$_{97}$/g fat, respectively. In Table 3, the influence of the aforesaid thresholds is evaluated against the 25th percentiles (Q$_{.25}$) of PCDD + PCDF, DL-PCB, and TEQ$_{TOT}$ cumulative TEQ value distributions, as inventoried by EFSA,[20] and against the EU WHO-TEQ$_{97}$ ALs and MLs in foods of animal origin.[3,19] The aforesaid cumulative TEQ thresholds would in general contribute to AL values less than 10%, with the exception of pork meat (PCDD + PCDF threshold contribution, 23%): however, according to Regulation 1883/2006/EC,[8] a difference between UB and LB estimates in the 25–40% range is still considered to be an acceptable uncertainty when TEQ$_{TOT}$ values are quite lower than 1 pgWHO-TEQ$_{97}$/g fat.

With regard to the possibility to determine background levels – as those that may tentatively be identified with concentrations below the Q$_{.25}$ values of contaminant level distributions for foods inventoried in Europe – the LRMS/MS congener LOQs and related cumulative TEQ thresholds may be suitable to monitor background levels in bovine meat, dairy products, eggs, and fish if it is acceptable that cumulative results (TEQ$_{TOT}$) below about 0.5 pgWHO-TEQ$_{97}$/g be influenced from thresholds up to some 50% of the measured value. A specific case is pork meat, as its TEQ concentrations may be very low (Tables 1 and 2): in our study, pork meat seems to represent an extreme situation, with contamination levels too low relative to the reference cumulative values shown in Table 3.

Table 3. PCDD + PCDF, DL-PCB, and PCDD + PCDF + DL-PCB (TEQ$_{TOT}$) in foods of animal origin: influence of upper TEQ threshold estimates on some cumulative TEQ values relevant to risk management

Chemicals and parameters[a]	Dairy milk[b]	Pork meat[b]	Bovine meat[b]	Poultry meat[b]	Eggs[b]	Fish[c]	Eel[d]
	Estimated cumulative TEQ thresholds						
PCDDs + PCDFs			0.11–0.14			0.014	0.055
DL-PCBs			<0.01			<0.001	<0.003
TEQ$_{TOT}$			0.11–0.14			0.014	0.058
	Values of interest in pgWHO-TEQ$_{97}$/g fat						
PCDDs + PCDFs, Q$_{.25}$	0.26	0.17	0.26	0.25	0.28	0.13	0.98
DL-PCBs, Q$_{.25}$	0.25	0.02	0.34	0.12	0.16	0.24	0.05
TEQ$_{TOT}$, Q$_{.25}$	0.67	0.24	0.75	0.42	0.50	0.38	3.2
PCDDs + PCDFs, AL	2	0.6	1.5	1.5	2	3	3
DL-PCBs, AL	2	0.5	1	1.5	2	3	6
PCDDs + PCDFs, ML	3	1	3	2	3	4	4
TEQ$_{TOT}$, ML	6	1.5	4.5	4	6	8	12
	Threshold contributions (%) on the corresponding reference level						
PCDDs + PCDFs, Q$_{.25}$	54	82	54	56	50	11	5.6
DL-PCBs, Q$_{.25}$	<4	<50	<3	<8	<6	<0.4	<6
TEQ$_{TOT}$, Q$_{.25}$	21	58	19	33	28	3.7	1.8
PCDDs + PCDFs, AL	7.0	23	9.3	9.3	7.0	0.47	1.8
DL-PCBs, AL	<0.5	<2	<1	<0.7	<0.5	<0.03	<0.05
PCDDs + PCDFs, ML	4.7	14	4.7	7.0	4.7	0.35	1.4
TEQ$_{TOT}$, ML	2.3	9.3	3.1	3.5	2.3	0.18	0.48

[a]Q$_{.25}$, 25th percentile; AL, action level from Recommendation 2006/88/EC; ML, maximum level from Regulation 1881/2006/EC.
[b]Values expressed in pgWHO-TEQ$_{97}$/g fat.
[c]Values expressed in pgWHO-TEQ$_{97}$/g fresh weight (fat content conservatively estimated at 10 % for threshold conversion from fat basis to fresh weight basis).
[d]Values expressed in pgWHO-TEQ$_{97}$/g fresh weight (fat content conservatively estimated at 40 % for threshold conversion from fat basis to fresh weight basis).

CONCLUSIONS

Based on this first performance evaluation, the new generation triple quadrupoles in HRGC/LRMS/MS systems seem to be capable of the quantitative analysis of PCDDs, PCDFs, and DL-PCBs in food and feed on a routine basis and with a high-performance output, likely offering an innovative alternative to the classical HRMS method. The triple quadrupole approach could benefit from a broader practicability, as the analytical costs would be abated by a less expensive and more flexible instrumentation, still requiring, however, experienced staff and well-equipped laboratories. Lastly, triple quadrupole performances appear to be adequate for a use of the LRMS/MS technique not only for a first sample analysis within the EU regulatory framework, but also to collect data for intake assessment on contaminant occurrence in foods present in European countries, as long as performances allow detection of contaminant concentrations near the $Q_{.25}$ s of occurrence distributions, in particular for those food matrices most contributing to intake (i.e. fish, milk, and eggs).[19]

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article.

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