CHAPTER 6

Environmental Applications

High-Resolution GC Coupled to High-Resolution MS in the Analysis of Dioxins and Related Substances, Principles and Applications

1. Introduction

Mass spectrometry is known to be the method of choice for the analysis of contaminants at trace levels. It provides not only a very specific quantification but also ensures the unambiguous identification of target compounds. A key figure of merit in trace analysis is the absence of interferences. It was quite rapidly achieved by a combination of high-resolution and high-mass accuracy using double-focusing magnetic sector instruments. The mass spectrometric instrumentation has considerably evolved during the last two decades with the apparition of robust quadrupoles, quadrupole ion traps, new time-of-flight, and Fourier transform instruments as well as powerful hybrid instruments. An alternative to high resolution has been introduced by quadrupole ion storage mass spectrometer in MS–MS mode. However, sectors instruments still find in that field, selected applications for which their performances are unmatched by any other techniques. A very illustrative example is the analysis of dioxins and related compounds that will be described here.

2. Dioxins, Furans, and PCBs

Polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and polychlorinated biphenyls (PCBs) are industrial substances that have been classified as persistent organic pollutants (POPs) by the United Nations Environment Programme (UNEP) (*1*). PCDD/PCDFs have never been produced deliberatively but are released as accidental by-products from combustion processes or industrial synthesis of other chlorinated chemicals. Although neither dioxins nor furans have ever had any commercial applications, PCBs have been heavily synthesized since 1930 for a variety of industrial uses such as, for example, dielectric fluids in transformers and capacitors. PCBs, which were supposed to be confined to the industrial setting can, however, like dioxins and furans, be found in virtually all global ecosystems. Dioxins and furans are planar tricyclic compounds that have similar chemical structures and properties (Fig. 1). PCBs are bicyclic compounds. The planarity depends on the chlorine substitution in positions 2,6,2′,6′ (*ortho* positions). Only non-*ortho*-chlorosubstituted PCB congeners adopt a planar geometry. These substitutional and geometrical parameters are of prime interest because they are closely related to the toxicity of these compounds.

All together, dioxins (75), furans (135), and PCBs (209) represent 419 congeners that can theoretically be found in environmental samples and have to be separated for individual quantification (Table 1). Fortunately, most of them do not bio-accumulate in living organisms. Furthermore, for PCDDs and PCDFs, a 2,3,7,8-chlorosubstitution pattern is required for efficient binding to a specific cellular receptor responsible for toxic effects. This represents a group of 7 PCDDs, 10 PCDFs, to which 12 so-called "dioxin-like" (DL) non-*ortho* and mono-*ortho* PCB congeners are added because of their structural similarity. A toxicity level

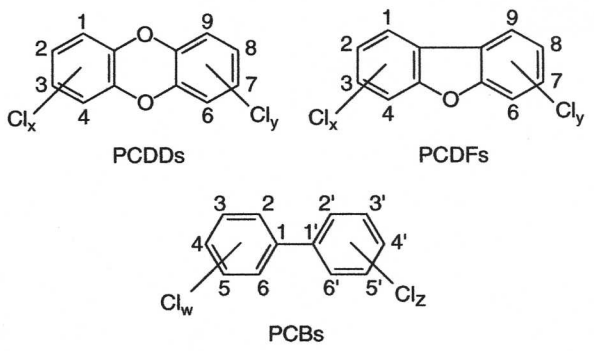

Figure 1
Chemical structures of PCDDs, PCDFs, and PCBs.

Table 1
Number of possible PCDD, PCDF, and PCB congeners

Number of chlorine atoms	Number of isomers		
	PCDDs	PCDFs	PCBs
1	2	4	3
2	10	16	12
3	14	28	24
4	22	38	42
5	14	28	46
6	10	16	42
7	2	4	24
8	1	1	12
9	—	—	3
10	—	—	1
Total number of congeners	75	135	209

has been assigned to each of those 29 compounds: the *toxic equivalency factor* (TEF). The TEF (Table 2) is a number that permits to translate the toxicity of a congener relatively to 2,3,7,8-TCDD, the most toxic congener (*2*). The concept of those relative toxicities permits to assess the total burden of a sample containing a mixture of PCDDs, PCDFs, and DL-PCBs in terms of 2,3,7,8-TCDD concentration. The global toxicity, or the *toxic equivalents* (TEQs), for a sample containing several different congeners is obtained using the following formula:

$$\mathrm{TEQ} = \sum_{n_1}(\mathrm{PCDD}_i \times \mathrm{TEF}_i) + \sum_{n_2}(\mathrm{PCDDF}_i \times \mathrm{TEF}_i) + \sum_{n_3}(\mathrm{PCB}_i \times \mathrm{TEF}_i) \quad (1)$$

This translation of individual concentrations, issued from a physicochemical analysis, in terms of TEQ facilitates risk assessment and regulatory control of levels and exposure to these compounds. Details concerning physical properties, formation, sources, toxicity, and structure–activity relationship can be found in the literature (*3*).

3. *Sample Preparation*

Environmental measurement of PCDDs, PCDFs, and PCBs occurs at the ultra-trace level (e.g., nano- to femtogram per gram of sample). Therefore, extremely large amounts of matrix-related interferences have to be removed before one can even think about measurement. Extraction of the analytes from the matrix and purification of the target compounds from undesirable interferences take place through an expensive and time-consuming multistep approach. Although Soxhlet and liquid–liquid extraction (LLE) are still used for solid and fluid matrices, respectively, more recent and specific extraction methods exist. The major ones are supercritical fluid extraction (SFE), microwave-assisted extraction (MAE), pressurized liquid extraction (PLE), and solid-phase extraction (SPE). Several comparative studies and reviews are available in the literature for the user to elaborate an objective opinion on method characteristics (*4,5*).

Independently of the extraction method used, highly efficient clean-up procedures are required to purify samples issued from the extraction step prior the final analysis and quantification. Automated solid–liquid adsorption chromatographic separations, based on sorbents such as silica, alumina, Florisil, and activated carbon, are often used to ensure high sample throughput (*6*). Another important aspect of the clean-up is the separation of the planar dioxins, furans, and PCBs from the nonplanar species. In practice, this fractionation results in a simplification of the gas chromatographic (GC) separation requirement prior to mass spectrometric (MS) analysis. Classically, a first fraction contains 17 PCDD/Fs and 4 non-*ortho*-PCBs, and a second fraction contains the 8 mono-*ortho*-PCBs, as well as a group of 6 indicator PCBs (Aroclor 1260) that have also to be monitored because of their significance. The two fractions are subjected to GC–MS separation and analysis separately.

4. *Gas Chromatographic Separation*

Once extracted samples have been cleaned-up from matrix interferences and fractionated, the chromatographic separation of the target compounds has to take place before analysis. Due to the semivolatility of dioxins and related compounds, GC is used to separate the different congeners and to allow nonambiguous identifications. Capillary high-resolution GC (HRGC) columns ensure the required selectivity, especially for congeners of the same chlorination level. The separation characteristics and elution profiles on GC columns have been studied. An appropriate combination of column length, internal diameter and stationary phase polarity is needed.

For the analysis of human or animal-based food samples, nonpolar GC columns are usually used. They allow separation between homologue groups and can also separate 2,3,7,8-substituted congeners (the ones that bioaccumulate) from each other (*7*). Usually 40–60 m columns with 0.18–0.25 mm internal diameter and 0.15–0.25 μm film thickness are selected.

The 5% diphenyl/95% dimethyl polysiloxane stationary phase is a thermally stable (350°C) and low bleed stationary phase that conducts to a good separation of the 2,3,7,8 toxic congeners from others,

Table 2
World Health Organization TEFs for humans

Congeners	WHO TEF	Congeners	IUPAC	WHO TEF
Dioxins		*Non-ortho PCBs*		
2,3,7,8-TCDD	1	3,3′,4,4′-TCB	77[a]	0.0001
1,2,3,7,8-PeCDD	1	3,4,4′,5-TCB	81	0.0001
1,2,3,4,7,8-HxCDD	0.1	3,3′,4,4′,5-PeCB	126	0.1
1,2,3,6,7,8-HxCDD	0.1	3,3′,4,4′,5,5′-HxCB	169	0.01
1,2,3,7,8,9-HxCDD	0.1			
1,2,3,4,6,7,8-HpCDD	0.01	*Mono-ortho PCBs*		
OCDD	0.0001	2,3,3′,4,4′-PeCB	105	0.0001
		2,3,4,4′,5-PeCB	114	0.0005
Furans		3,3′,4,4′,5-PeCB	118	0.0001
2,3,7,8-TCDF	0.1	2,3′,4,4′,5-PeCB	123	0.0001
1,2,3,7,8-PeCDF	0.05	2,3,3′,4,4′,5-HxCB	156	0.0005
2,3,4,7,8-PeCDF	0.5	2,3,3′,4,4′,5′-HxCB	157	0.0005
1,2,3,4,7,8-HxCDF	0.1	2,3′,4,4′,5,5′-HxCB	167	0.00001
1,2,3,6,7,8-HxCDF	0.1	2,3,3′,4,4′,5,5′-HpCB	189	0.0001
1,2,3,7,8,9-HxCDF	0.1			
2,3,4,6,7,8-HxCDF	0.1			
1,2,3,4,6,7,8-HpCDF	0.01			
1,2,3,4,7,8,9-HpCDF	0.01			
OCDF	0.0001			

[a] According to Ballschmiter and Zell (*24*).

especially for 2,3,7,8-TCDF for which 38 different substitution congeners are possible. The separation is however not always complete for hexachlorinated dioxin and furan congeners. Minimal separation requirements as defined, for example, in the Directives 2002/69/EC or 2002/70/EC, are nevertheless fulfilled and accurate measurement is possible. Planar non-*ortho*-PCBs, presents in the same fraction, are also separated using such a phase.

A little more polar stationary phase, the 8% phenyl polycarborane–siloxane, is often used for the separation of the PCBs contained in the second clean-up fraction. The carborane group has a high affinity for PCBs with a low degree of *ortho*-substitution. Although this phase does not allow the separation of all the 209 PCB congeners, it separates some critical pairs of co-elutions present with other phases (*8*). For example, indicator trichlorinated PCB-28 and PCB-31 (not followed), pentachlorinated mono-*ortho* PCB-123 and PCB-118, as well as hexachlorinated PCB-163 (not followed) and the indicator hexachlorinated PCB-138 are separated. In practice, the separation of the mono-*ortho*-PCBs and the indicator PCBs can be performed in 30 min on a 25 m × 0.25 mm × 0.25 μm column, a good compromise between the required resolving power and the GC run time. One should mention that to date, none of the existing stationary phases is capable of the separation of all the PCB congeners. Even emerging hyphenated methods such as comprehensive two-dimensional gas chromatography (GC×GC) coupled to time-of-flight (TOF) MS can at the most separate 192 congeners (*9*).

The use of more polar phases is required when considering environmental matrices (e.g., fly ash, air, soils, sediments) because those samples may contain other congeners than 2,3,7,8-substituted, which represent a large number of compounds that are not separated using a nonpolar phase. Environmental extracts still have to be analyzed by regular nonpolar column to have a separation depending on homolog series, but a 60 m long highly polar substituted cyanopropyl stationary phases (e.g., nonbonded 90% biscyanopropyl/10% phenylcyanopropyl polysiloxane) is also used as a confirmatory column to ensure proper separation of TCDD and TCDF isomers. The major drawbacks of the polar column are the short column lifetimes when thermally cycled and the low thermal stability of the nonlinked or semibonded phase (275°C), which tends to produce significant bleed.

An alternative approach is the recombination of the two clean-up fractions (or the two injections) to reduce the number of GC–MS injections. Capillary columns can be coupled either in parallel or in series to achieve the separation. The dual column strategy (parallel column coupling) consists in making both injections in the same time into two separate injectors, connected to two separate columns that are both connected to the same HRMS instrument (*10*). Fine

adjustment of the selected-ion monitoring (SIM) windows, dwell times, and delay times of the MS is necessary to ensure proper ion recording. The GC×GC strategy consists in connecting two GC columns in series and to use a fast sampling device (the modulator) to create sharp injection pulses with the first column eluants into the second column [see Chapter 2 (this volume): *Comprehensive Two-Dimensional Gas Chromatography Mass Spectrometry*]. Coupling GC×GC to fast scanning MS such as TOF-MS allows accurate description of the complex tri-dimensional chromatogram (*11*) [see Chapter 6 (this volume): *Human Biomonitoring of Persistent Toxicants Using Comprehensive Two-Dimensional Gas Chromatography and Time-of-Flight Mass Spectrometry*]. Finally, a new proprietary phase appeared recently as a single column for stand-alone PCDD/F analyses but it is yet too early to present it as the total solution (*12*).

5. *High-Resolution Mass Spectrometry*

Mass spectrometry is known to be the method of choice to quantify and identify trace level of organic compounds. Baughman and Meselson detected for the first time TCDD by HRMS in samples from Vietnam (*13*). But it was during the mid-1980s and spurred on by Patterson and coworkers that sector instruments took on a new lease of life with dioxin applications (*14*). The very particular nature of the analysis puts strong requirements on the detection method, leading to a less usual scanning technique, which is described hereafter.

Let us first summarize the specific aspects of the analysis having an impact on the mass spectrometric detection method. As mentioned above, the dioxin "family" is made of congeners corresponding to different chlorination levels (from four to eight chlorine atoms for toxic ones). Within the same chlorination level, one has to deal with a different number of isomers of different toxicity, which must be specifically identified and quantified. To comply with regulations, the results of the analysis will reflect not only the quantity of individual congeners but also the global toxicity of the sample as described above in TEQ. Levels to be detected with confidence lie in the sub parts-per-trillion levels (ppt), leading to injected amounts in the picogram range down or even below to 0.1 pg for low contamination levels. Starting sample amounts can be high (e.g., several grams of fat for biological samples) which often will not allow more than two re-injections in case of technical problems. The method has to be very reliable.

Even after an extensive clean up and a high-resolution chromatographic separation, the risk of interferences is still high. For that reason, the resolution ($M/\Delta M$) of the mass spectrometer should be set at least at 10 000 (10% valley definition). This allows mass discrimination at the 0.03–0.05 mass unit (dalton, Da) level in the tetra- to octa-substituted congeners mass range. Figure 2 shows some organic contaminants that could interfere with 2,3,7,8-TCDD at m/z 322 within a range of 0.08 Da. For instance, it indicates that a resolution of 18 000 is necessary to separate 2,3,7,8-TCCD [M + 2] mass from xanthene or benzylphenylether masses.

To reach the required resolution, only double focusing sector instruments were historically available. Currently, the very high degree of analytical performances achieved by the HRGC–HRMS method is unmatched by any other techniques and standards (*15–17*) require its use as the reference method for dioxin analysis. Fourier transform mass spectrometry could compete in terms of resolution but with higher limits of detection and at higher cost. Time of flight mass spectrometers still have to compromise between resolution and acquisition speed, which can be useful for fast GC monitoring (*18*). Bench-top low-resolution quadrupole ion storage mass spectrometry is a useful alternative to HRMS (*19,20*). The loss of mass resolution is counter-balanced by a gain in specificity in MS–MS mode [see Chapter 6 (this volume): *Dioxins in Environmental Samples Using GC-Ion-Trap MS*].

With sector instruments, full scan spectra acquisition is not possible at such low levels. The instrumental limit of detection with that scanning mode is in the nanogram range. To gain in signal-to-noise ratio by a higher counting rate, the classical SIM method is used. The spectrometer is rapidly switched between the apexes of peaks of interest, avoiding wasting time and sample running in the chromatography while measuring between masses. Thus, the time spent (dwell time) on each target ion in the acquisition list is longer (in the 10–150 ms range, see Tables 3 and 4). The number of ions which can be measured at any one time is generally limited because at least ten sampling points for each GC peak are needed in order to get a Gaussian peak for accurate integration and quantification. Selected ions are therefore grouped in various segments. It is not the only reason of grouping ions in segments. Indeed, a high mass resolution means narrow peaks. A precise setting of the masses is mandatory. To control the mass accuracy, a lock mass is measured in each cycle and a lock mass check is often included (e.g., perfluorokerosene, PFK). The lock mass is ideally located within the measured mass range. A feedback loop compensates for any deviation in the mass setting. A further difficulty arises from the magnet hysteresis. The required mass accuracy to set the detection on the top of the peaks is too high to cope with fast magnet positioning. The magnet is therefore set at a fixed nominal mass transmission (nominal source acceleration). Changing simultaneously the source acceleration voltage and analyzer deflection voltage performs the scanning (HV scan, see equations in

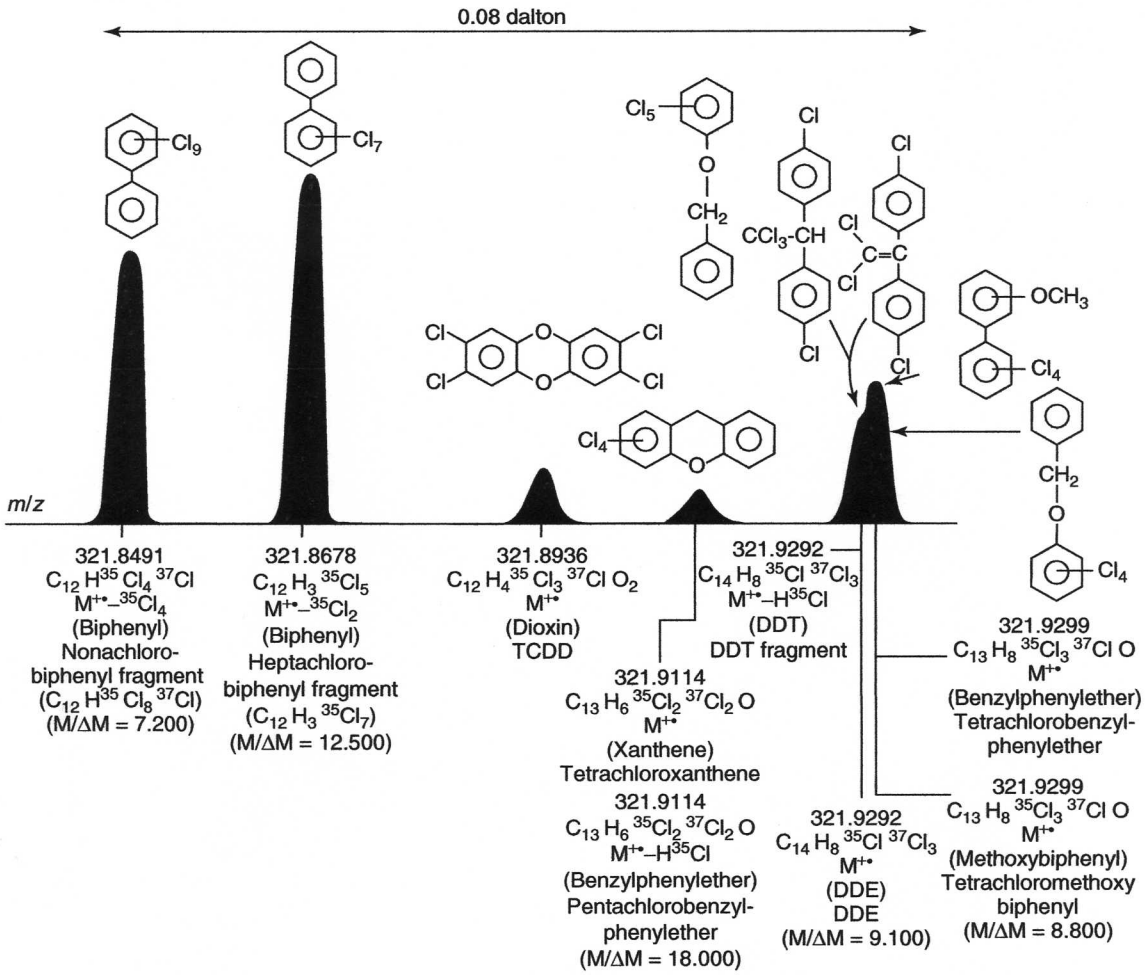

Figure 2
Possible interferences with 2,3,7,8 TCDD at *m/z* 322 (Micromass source).

Table 5). Lowering the acceleration voltage leads to source defocusing and loss of transmission. Only a few percent of the nominal mass are then accessible. To reach the full mass range required by the analysis from tetra- to octa-chlorinated congeners, the parking position of the magnet is changed during acquisition according to mass segments corresponding to groups.

The chromatographic challenge is then to bring the compound by groups (chromatographic windows) with no overlap, which would result in loss of congener measurement. Tables 3 and 4 give an overview of the HRMS acquisition parameters such as the time windows, the ions monitored, the dwell times and the isotopic ratios in SIM mode for non-*ortho* PCBs, PCDD/Fs and mono-ortho PCBs, respectively. The sensitivity of the technique is considerably improved and HRMS manufacturers can guarantee a typical specification of 100 fg injected with a signal-to-noise ratio of 100/1 for TCDD at 321.8936 *m/z* in SIM mode with a mass resolution of 10 000 (10% valley definition).

6. *Identification of PCDDs, PCDFs, and PCBs*

With such a scan, the identification of the compounds in terms of mass spectra is lost but can be confirmed by two other types of available information: the retention time (identification) and the measurement of the isotopic composition of the ions (identification and confirmation of the absence of interference). The presence of congeners affected by different TEFs leads to severe requirements for isomeric separation, which relies on HRGC (*15–17*). The retention time of native and labeled standard peaks must be within a range of 2 s. To control the chlorination level and therefore the identity and the absence of interfering compounds, the measurement of the isotopic

Table 3
Target masses for PCDD/Fs and non-*ortho* PCBs in SIM mode for HRMS

	Window (min)	Monitored ions		Ion dwell time (ms)	Interscan time (ms)	Theoretical isotopic ratios	15% for isotopic ratios
		Quantitation ion	Confirmation ion				
TCB		291.9194 [M + 2]	289.9224 [M]	110		0.77	0.65–0.88
TCB $^{13}C_{12}$	20–26	303.9597 [M + 2]	301.9626 [M]	40	10	0.77	0.65–0.88
Lock mass		316.9824 [l]	316.9824 [l]	50			
TCDF		305.8987 [M + 2]	303.9016 [M]	100		0.77	0.65–0.88
TCDF $^{13}C_{12}$		317.9389 [M + 2]	315.9419 [M]	15		0.77	0.65–0.88
TCDD		321.8936 [M + 2]	319.8965 [M]	100		0.77	0.65–0.88
TCDD $^{13}C_{12}$	26–30	333.9339 [M + 2]	331.9368 [M]	15	10	0.77	0.65–0.88
TCDD $^{13}C_6$[a]		331.9078 [M + 6]		85			
PeCB		325.8804 [M + 2]	327.8775 [M + 4]	100		0.64	0.56–0.75
PeCB $^{13}C_{12}$		337.9207 [M + 2]	339.9177 [M + 4]	15		0.64	0.56–0.75
Lock mass		330.9792 [l]	330.9792 [l]	50			
PeCDF		339.8597 [M + 2]	337.8627 [M]	120		0.61	0.53–0.71
PeCDF $^{13}C_{12}$		351.9000 [M + 2]	349.9029 [M]	15		0.61	0.53–0.71
PeCDD		355.8546 [M + 2]	353.8576 [M]	150		0.61	0.53–0.71
PeCDD $^{13}C_{12}$	30–35	367.8949 [M + 2]	365.8978 [M]	15	10	0.61	0.53–0.71
HxCB		359.8415 [M + 2]	361.8385 [M + 4]	100		0.61	0.69–0.94
HxCB $^{13}C_{12}$		371.8817 [M + 2]	373.8788 [M + 4]	15		0.81	0.69–0.94
Lock mass		380.9760 [l]	380.9760 [l]	50		0.81	
HxCDF		373.8207 [M + 2]	375.8178 [M + 4]	150		0.81	0.69–0.94
HxCDF $^{13}C_{12}$		385.8610 [M + 2]	387.8580 [M + 4]	15		0.81	0.69–0.94
HxCDD	35–42	389.8156 [M + 2]	391.8127 [M + 4]	150	10	0.81	0.69–0.94
HxCDD $^{13}C_{12}$		401.8559 [M + 2]	403.8530 [M + 4]	15		0.81	0.69–0.94
Lock mass		380.9760 [l]	380.9760 [l]	50			
HpCDF		407.7818 [M + 2]	409.7788 [M + 4]	150		1.04	0.88–1.20
HpCDF $^{13}C_{12}$		419.8220 [M + 2]	421.8190 [M + 4]	15		1.04	0.88–1.20
HpCDD	42–47	423.7767 [M + 2]	425.7737 [M + 4]	150	10	1.04	0.88–1.20
HpCDD $^{13}C_{12}$		435.8169 [M + 2]	437.8140 [M + 4]	15		1.04	0.88–1.20
Lock mass		430.9728 [l]	430.9728 [l]	50			
OCDD		459.7348 [M + 4]	457.7377 [M + 2]	150		0.89	0.75–1.01
OCDD $^{13}C_{12}$		471.7750 [M + 4]	469.7780 [M + 2]	15		0.89	0.75–1.01
OCDF	47–52	443.7398 [M + 4]	441.7428 [M + 2]	150	10	0.89	0.75–1.01
OCDF $^{13}C_{12}$		455.7801 [M + 4]	453.7830 [M + 2]	15		0.89	0.75–1.01
Lock mass		466.9728 [l]	466.9728 [l]	50			

[a] Syringe standard added prior to GC–HRMS analysis and used for recovery.

composition of the two most intense ions of both native and ^{13}C-labeled ion clusters must be $\pm 15\%$ around the theoretical ion abundance ratio (Tables 3 and 4). Any deviation out of this range will cause rejection of the congener's result. Figure 3 shows a typical chromatogram for TCDD. In practice, four ion traces (two native and two ^{13}C-labeled ions) per congener are plotted separately for integration and quantification. In the case of TCDD, an additional 1,2,3,4-TCCD (internal standard) is used to calculate recovery rates. This standard, also called the syringe standard, is added prior to HRGC–HRMS analysis. It enables to calculate the percentage of recovery standard (used for quantification) added prior to extraction. EPA 1613 recommends the use of the labeled $^{13}C_{12}$ 1,2,3,4-TCCD but other possibilities exist. In this example, $^{13}C_6$ 1,2,3,4-TCCD can be used to calculate recovery rates and to check the mass resolution at the same time (*21*). For this purpose, the mass 331.9078 m/z ($^{13}C_6$ 1,2,3,4-TCCD [M + 6]) is monitored and a resolution of 11 400 is required to completely resolve in mass 331.9368 m/z ($^{13}C_{12}$ 2,3,7,8-TCCD [M]) from

Table 4
Target masses for mono-*ortho* PCBs in SIM mode for HRMS

	Window (min)	Monitored ions		Ion dwell time (ms)	Interscan time (ms)	Theoretical isotopic ratios	15% for isotopic ratios
		Quantitation ion	Confirmation ion				
TCB $^{13}C_{12}$[a]		303.9597 [M + 2]	301.9626 [M]	26		0.77	0.65–0.88
PeCB		325.8804 [M + 2]	327.8775 [M + 4]	26		0.64	0.56–0.75
PeCB $^{13}C_{12}$	9–22	337.9207 [M + 2]	339.9177 [M + 4]	26	10	0.64	0.56–0.75
HxCB		359.8415 [M + 4]	361.8385 [M + 2]	26		0.81	0.69–0.94
HxCB $^{13}C_{12}$		371.8817 [M + 4]	373.8788 [M + 2]	26		0.81	0.69–0.94
Lock mass		316.9824 [l]	366.9792 [l]	1.3			
HpCB		393.8024 [M + 2]	395.7994 [M + 4]	96		1.04	0.88–1.20
HpCB $^{13}C_{12}$	22–28	405.8432 [M + 2]	407.8402 [M + 4]	96	10	1.04	0.88–1.20
Lock mass		366.9792 [l]	404.9760 [l]	4.1			

[a] Syringe standard added prior to GC–HRMS analysis and used for recovery.

Table 5
Equations for HV scanning in SIM mode for sector instruments

The electrostatic sector discriminates ions according to their kinetic energy $\frac{1}{2}mv^2$, which is fixed by the source acceleration voltage $\frac{1}{2}mv^2 = eV$.

The magnetic sector discriminates ions according to their momentum $mv = \sqrt{2meV}$ according to the relation $eB = mv/R$, where R is the curvature radius of the magnet.

Combining those equations leads to the magnetic sector equation $\frac{m}{e} = \frac{B^2R^2}{2V}$. Any change in the source acceleration voltage will change the ion's velocity. A mass will be transmitted by the parking magnet only if it fits with the above relations. To allow that new mass to be detected, it passes the electrostatic sector. As the momentum should be constant (B fixed), a higher mass means a lower velocity and therefore a lower kinetic energy. To allow focusing of higher masses, the electrostatic voltage and the source voltage should be lower according to the relation

$$\frac{E_x}{E_0} = \frac{V_x}{V_0} = \frac{m_x v_x^2}{m_0 v_0^2} = \frac{B^2}{m_x}\frac{m_0}{B^2} = \frac{m_0}{m_x}$$

331.9078 m/z. By measuring the small peak that appears at the retention time of the recovery standard in the internal standard trace window (i.e., 331.9078 m/z) and by calculating the ratio with the peak 331.9368 m/z, one can check if the resolution is at least 10 000 during the GC run.

7. *Isotopic Dilution Technique for Quantification by HRMS*

The power of mass spectrometry in quantitative analysis can be further enhanced by the isotopic dilution technique. This technique consists of spiking samples with an ideal recovery standard, which is an isotopically labeled standard (e.g., $^{13}C_{12}$ 2,3,7,8-TCDD), showing almost identical characteristics to the compound of interest (the native compound, e.g., $^{12}C_{12}$ 2,3,7,8-TCDD) during the extraction, the clean-up, the GC separation (almost the same retention time). The small mass difference (e.g., 12 m/z) enables the discrimination between the compound of interest and its recovery standard (Fig. 3). However, a discrepancy between native and labeled standards can be observed during electron ionization (EI). A corrective factor has to be taken into account for accurate quantification. This factor is calculated with a minimum of five calibration points performed for all the PCDD/F and DL-PCB congeners with known amounts of native and recovery standards encompassed within the working range. By plotting the concentration ratio of native to analogue ^{13}C-labeled against the area ratio of native to analogue standard ^{13}C-labeled, the slope of the calibration curve gives

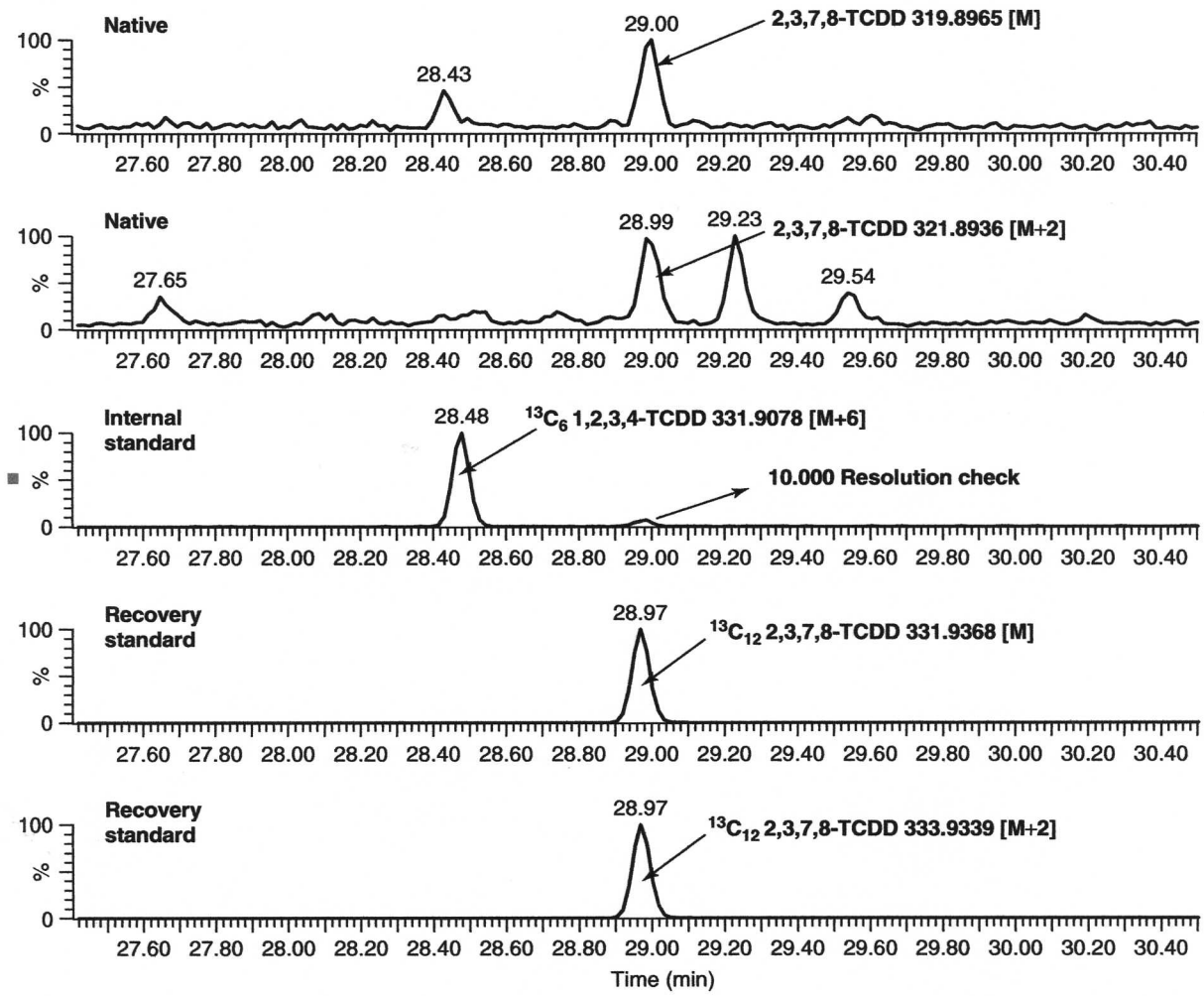

Figure 3
Isotopic dilution technique, SIM windows for TCDD including native, internal standard, and recovery standard traces.

the corrective factor also called the relative response factor (RRF) (Fig. 4). The RRF_i is calculated by the following equation:

$$\mathrm{RRF} = \frac{(A^1_{\mathrm{native},i} + A^2_{\mathrm{native},i}) \times C_{\mathrm{l},i}}{(A^1_{\mathrm{labeled},i} + A^2_{\mathrm{labeled},i}) \times C_{\mathrm{n},i}} \qquad (2)$$

where $A^1_{\mathrm{native},i}$ and $A^2_{\mathrm{native},i}$ are the areas of the quantitation and confirmation ions for the native congener i; $A^1_{\mathrm{labeled},i}$ and $A^2_{\mathrm{labeled},i}$ are the areas of the quantitation and confirmation ions for its corresponding labeled compound i; $C_{\mathrm{n},i}$ is the concentration of the native compound i in the calibration solution and $C_{\mathrm{l},i}$ is the concentration of the labeled compound i in the calibration solution.

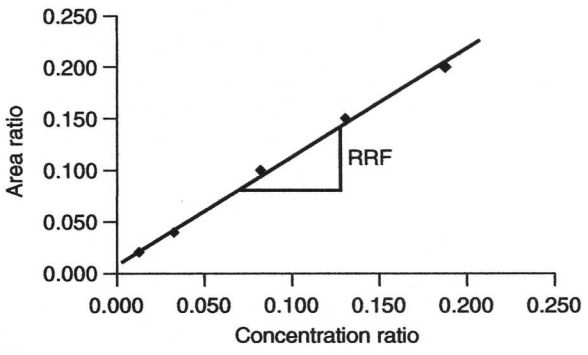

Figure 4
Five calibration points for 2,3,4,7,8 PeCDF.

The mean RRF value over the five-point calibration range has to fulfill precision criteria according to the requirements of the standards (*15–17*). Thus, the RRF value directly affects the congener quantification as indicated in the following equation:

$$[\text{congener}]_i = \frac{(A^1_{\text{native},i} \times A^2_{\text{native},i}) \times Q_i}{(A^1_{\text{labeled},i} \times A^2_{\text{labeled},i}) \times \text{RRF}_i \times m} \quad (3)$$

where $[\text{congener}]_i$ is the concentration of the congener i (e.g., ng kg^{-1}); areas are defined in Eqn. (2); Q_i is the amount of the corresponding recovery standard i spiked (e.g., ng) in the sample; RRF_i is the relative response factor of the congener i and m is the weight of the sample (e.g., kg).

Finally, the quantification expressed in TEQ is calculated according to Eqn. (1).

8. *Quality Assurance and Quality Control*

The objective of quality assurance and quality control (QA/QC) programs is to control analytical measurement errors at levels acceptable to the end-user of the data and to assure that the analytical results have a high probability of acceptable quality. It is generally evaluated on the basis of its measurement uncertainty associated with the final result which should match the end-user requirements. Accreditation according to the Standard ISO/CEN 17025 is an adequate way to implement QA/QC programs in a routine laboratory. Dioxin analysis does not escape from the rule. We already mentioned some quality criteria for identification and quantification for those compounds by HRGC–HRMS and standards (*15–17*) provide guidelines and a comprehensive list of requirements that have to be fulfilled. We purely mention some of them which are of primary importance when carrying out ultra trace level analysis.

One of the most important features of a QA/QC program is the use of procedure blank and internal quality control (IQC) samples. They have to be implemented with the series of real samples in the daily routine work. Both have to follow as much as possible the entire analytical procedure. IQC have to be characterized by sufficient homogeneity and long-term stability to ensure that the analytical system is under control. When available, they should as much as possible match analyte, level, and matrix of real samples tested. Control charts (plots of the data from blank or IQC vs. time) provide the most effective mechanism for interpreting data. Blanks results and IQC data are both reported graphically on QC charts. These graphs provide the performances of the analytical system. Limits and decision rules have to be implemented in order to maintain the system stabilized and under control (Fig. 5). The strategy of the decision process, the corrective and preventive actions to be taken when a lack of control is observed, should be clearly identified and followed.

The use of certified reference materials (CRMs) and the participation at relevant interlaboratory studies complete the requirements for assessing the accuracy of the analytical method (*22*). Statistics and chemometrics are tools that are more and more involved in the extraction of the information from QA/QC data with the ultimate aim of improving the quality of the analytical results.

9. *Recent Advances and Future Perspectives*

High-resolution gas chromatography coupled with high-resolution mass spectrometry is the basis of a very mature method for ultra trace levels analysis. In the case of dioxins analysis, the knowledge accumulated since two decades makes it one of the most described analytical methods. It will probably remain the standard in that field. However, difficulties begin when more compounds are added to the monitoring list. The SIM method will find its limits due to dwell time requirements. As the responses are given in toxic equivalents (TEQ), biological tests measuring the global toxicity are attractive solutions. However many compounds interfere with the biological activity or are not yet known. Large discrepancies may occur, which makes bioassays mostly useful as screening tests. Confirmation and random analysis using HRGC–HRMS remains mandatory. In addition, congener-specific analysis brings additional useful information on patterns and sources of contamination.

Arguments only based on the cost of analysis do not hold, as the instrument part (38%) in the cost is largely compensated by the stability and the robustness of the method when compared to maybe less expensive instruments (*11*). Another reason is linked to the required sensitivity. Since several years, background levels of dioxins and related compounds in the environment and in serum samples have declined to the point that their measurement has become increasingly difficult. This is the result of all the measures and efforts that have been undertaken to reduce the occurrence of dioxin in the environment as well as in the food chain. Increasing the sample size to solve the problem is not the right way to do it as a lot of energy and money were invested in the past few years to reduce the clean-up steps by automation and integrated approaches. Additionally, increasing sample size for certain matrices like human serum is not feasible in practice. Reducing the sample size by increasing the sensitivity of the analysis method is thus an area of considerable interest. A comprehensive two-dimensional modulation device GC coupled to a HRMS is a promising technique to solve this problem. A recent paper indicates that 540 ag (ag = attogram) of 2,3,7,8-TCDD from human serum extract injected by

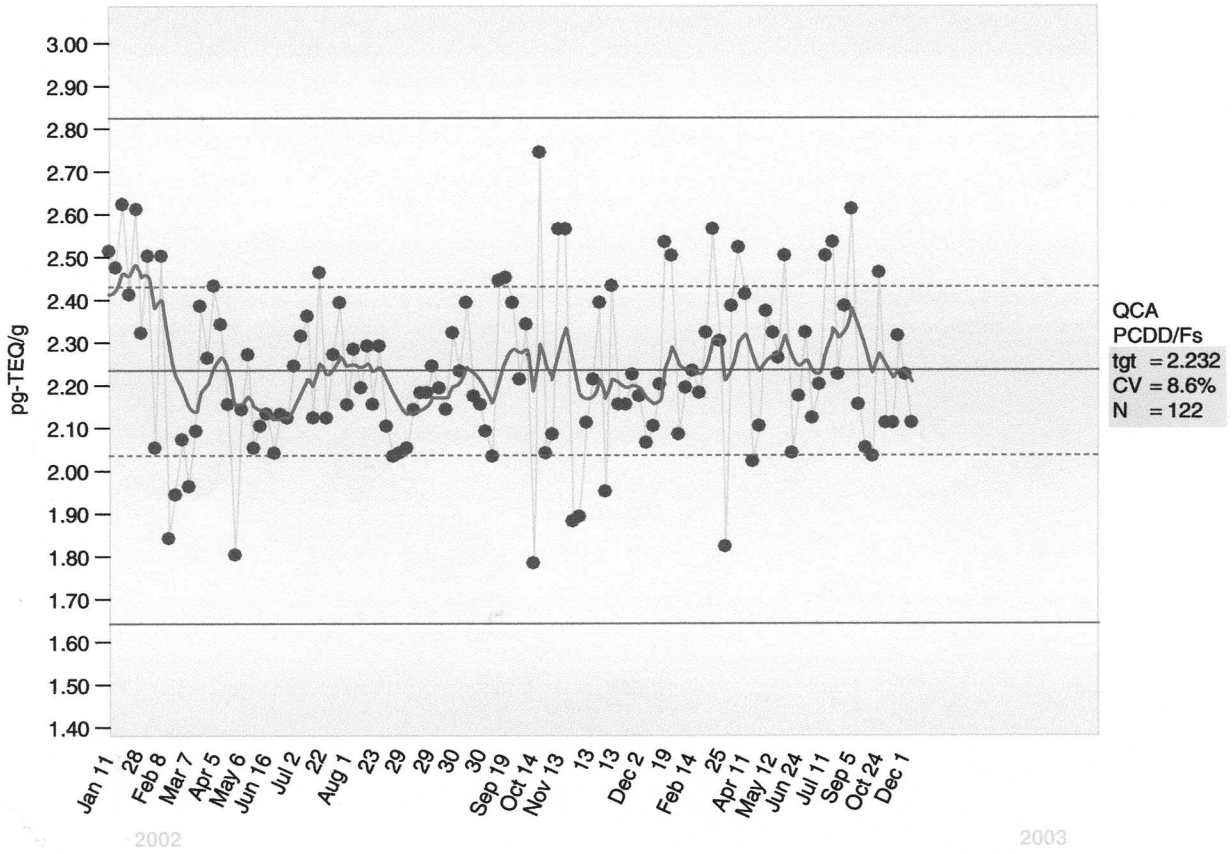

Figure 5
Quality control chart of PCDD/Fs expressed in TEQ in animal feeding stuffs.

this technique, display a signal-to-noise ratio of 479 to 1 (*23*).

Operation at 10 000 resolution is not always sufficient to remove an interference from the target compound as already mentioned in Section 5 and shown in Fig. 2. The problem is generally remedied by increasing the resolution, resulting in a reduction in ion transmission and hence, a drop in sensitivity. New detection systems have been designed to solve this problem. An interference that would normally require 25 000 resolution can easily be removed with such detectors when operating at 10 000 resolution.

The general trend to develop faster analytical methods is also present in the dioxins field. A perspective is the total integrated approach between sample preparation and GC–MS analysis. By coupling extraction technique, clean-up steps and GC–MS detection, an alternative to rapid screening bioassays is conceivable. In that case, the bottleneck of a congener-specific method will be the data processing and reporting.

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Dioxins in Environmental Samples Using GC-Ion-Trap MS

1. Introduction

Among the thousands of existing anthropogenic compounds, some are persistent and remain in the environment for years once generated. An important family of persistent organic contaminants is the one known as "dioxins," constituted by polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs), which are compounds of great interest because of their extreme toxicity and high persistence in the environment (*1*). PCDD/Fs are formed as by-products during chemical and combustion processes and tend to bio-accumulate towards the food chain up to humans. Monitoring of their levels in various types of matrices, such as environmental, foodstuffs, and biological fluids, is therefore required in order to ensure human health protection.

Many aspects of the toxicity of dioxins have been reported, and generally these are related to the chemical structure of the individual compounds. In organisms, dioxins act through binding to the aryl hydrocarbon receptor, and this action gives rise to a cascade of effects, including liver damage, weight loss, atrophy of thymus gland, and immunosuppression. The affinity to the receptor is highest for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), which has been also classified as carcinogenic to humans by the International Agency for Research on Cancer (IARC) (*2*). In addition, all 2,3,7,8-substituted compounds (17 PCDD/Fs from the 210 possible congeners) are toxic, but their potency differs widely between homologues and/or within congeners. To